Asbestos Related Pleural Disease

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ABSTRACT

Introduction

Asbestos exposure is known to cause a number of pleural pathologies. Despite a ban of asbestos use over 30 years ago, the incidence of asbestos related pleural disease continues to increase due to the prolonged lag period from exposure to disease development. In this thesis I explore a number of gaps in the literature relating to asbestos related benign and malignant pleural pathology.

Methods

The first study is a prospective case series of diffuse pleural thickening. Full respiratory function of patients with different distributions of pleural thickening on CT were compared against a matched control population. The results confirm a significant reduction in lung function in patients with all forms of diffuse pleural thickening by size criteria, including those with no costo-phrenic angle involvement.

The second study assesses the role of MRI as an exploratory tool in those with equivocal pleural thickening on CT. Early differentiation of malignant from benign pleural thickening is crucial to offer patients the best clinical management. MRI was investigated as a potential non-invasive method of diagnosing patients with equivocal CT findings.

The third study is a multi-centre randomised controlled study (RCT) to investigate whether a Positron Emission Tomography (PET)-CT targeted biopsy is superior to a standard CT guided biopsy, for patients with suspected pleural malignancy, who have had one non-diagnostic biopsy. 52 patients have been recruited to date and the trial will finish recruitment in September 2018.

The fourth study is a multi-centre RCT investigating the feasibility of delivering zoledronic acid or placebo concurrently with chemotherapy for patients with Mesothelioma. Whilst we did not meet our primary feasibility outcome of randomising 50 patients across 3 sites in 13 months, we did obtain valuable information that would help us in designing a full phase-III trial.

The final study is a prospective cohort study investigating the role of serial mesothelin biomarker monitoring of patients with MPM, who are receiving best supportive care. A 10% rise in mesothelin can reliably predict progressive disease.

Discussion

The findings discussed as a part of this thesis add to our current knowledge on asbestos related pleural disease and hopefully will inform clinicians managing patients with asbestos related pleural disease.

AUTHOR'S DECLARATION

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's *Regulations and Code of Practice for Research Degree Programmes* and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

Signed …….. Date …….

DEDICATION

I would like to dedicate this thesis to my parents who enabled me to realise my dreams and always supported me in every possible way, even from 5000 miles away….

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ADC	Apparent diffusion coefficient
AE	Adverse Event
ARSAC	Administration of Radioactive Substances Advisory Committee
AUC60	Area under curve at 60 seconds
AUROC	Area under receiver operating curve
BAPE	Benign asbestos related effusion
BAP1	Breast cancer associated protein 1
BHOC	Bristol Haematology and Oncology Centre
BMI	body mass index
BRI	Bristol Royal Infirmary
BSC	Best supportive care
CABG	coronary artery by-pass graft
CMR	Complete metabolic response
CONSORT	Consolidated Standards of Reporting Trials
COPD	chronic obstructive pulmonary disease
CPA	costo-phrenic angle
CR	complete response
CRC	Clinical Research Centre
CRF	case record form
CRP	C reactive protein
CT	Computed tomography
CTEU	Clinical Trials and Evaluation Unit
CXR	Chest radiograph
DCE	Dynamic contrast enhanced
DPT	Diffuse pleural thickening
DWI	Diffusion weighted imaging
eGFR	estimated glomerular filtration rate
ELISA	Enzyme linked immunosorbent assay
EPD	Extended pleurectomy and decortication

EPP	Extra pleural pneumonectomy
FBC	full blood count
FDG	Fluorodeoxyglucose
FEV1	Forced Expiratory Volume at 1 second
FVC	forced vital capacity
HRA	Health Research Authority
IASLC	International association for the study of lung cancer
IIDB	Industrial Injuries Disablement Benefit
ILO	International Labour Organisation
IPC	Indwelling pleural catheter
IQR	interquartile range
KCO	diffusion co-efficient
LAT	Local anaesthetic thoracoscopy
MARS	Mesothelioma and Radical surgery
MDT	Multi-disciplinary team
MPM	Malignant Pleural Mesothelioma
MRC	Medical Research Council
mRECIST	modified-response criteria in solid tumours
MRI	Magnetic resonance imaging
NBT	North Bristol NHS Trust
NHS	National Health Service
NPV	negative predictive value
P16-FISH	p16 - fluorescence in-situ hybridization
PA	Postero-anterior
PACS	picture archiving and communication system
PD	pleurectomy/decortication
PD	progressive disease
PET	Positron emission tomography
PI	Principal Investigator
PMR	partial metabolic response
PPV	positive predictive value

PR	partial response
PS	Performance status
QA	Qualitative Analysis
RCT	randomised controlled trial
REC	Research Ethics Committee
RECIST	Response criteria in solid tumours
RfPB	Research for Patient Benefit
ROI	Region of interest
RUH	Royal United Hospital
SAE	serious adverse event
SD	standard deviation
SD	stable disease
SMD	Stable metabolic response
SSI	Semi Structured Interviews
SUV	Standard Uptake Value
TGV	Total Glycolytic Volume
TLC	Total lung capacity
TNM	Tumour node metastases
TSC	Trial Steering Committee
UK	United Kingdom
VATS	Video assisted thoracoscopic surgery
WHO	World Health Organisation
WT-1	Wilm's Tumour -1
ZA	Zoledronic acid

CHAPTER 1 INTRODUCTION

1.1 Asbestos

Asbestos refers to a group of fibrous silicate materials, well known for their properties of high tensile strength, thermal and electrical resistance. The term 'asbestos' is somewhat commercially coined rather than a mineralogical term and is derived from the Greek words for 'unquenchable' or 'inextinguishable', due to the unique properties exhibited by the material [1]. Once known as the 'magic mineral', the history of asbestos spans a number of centuries. The earliest documented reports of its use date back to 2500 BC when it was used in the Finnish pottery industry[1]. Widespread mining and commercial use started in the 19th century, with the discovery of asbestos deposits in Quebec and Ural mountains of Russia [2]. Asbestos fibres are of varying length, diameter and shape. They are broadly sub-classified into 2 groups; chrysotile and amphibole [3]. The amphibole group consists of 5 sub-types: crocidolite, amosite, anthophyllite, tremolite and actinolite [1, 3].

Chrysotile has an approximate chemical composition of $Mg_3Si_2O_5(OH)_4$. Chrysotile fibres are curvy cylindrical fibrils with a central hollow, and splayed end [4]. They are extremely heat resistant, being able to withstand temperatures up to 575 Centigrade [1]. Due to its length, curvy, linear structure and natural tendency to clump in bundles, these fibres are often intercepted at the carina of the bronchial tree when inhaled, and a majority are therefore cleared within hours to weeks[3].

Amosite and Crocidolite, also widely known as brown and blue asbestos, are the 2-main amphibole types of asbestos used commercially. These shorter needle shaped structures penetrate the lung tissue more readily than chrysotile fibres and, due to their relative insolubility and increased bio persistence tend to last decades before being cleared from the

lungs [3]. Fibres can often be found in post-mortem lung tissue as asbestos bodies in the lung parenchyma [5, 6]. Tremolite, actinolite and anthophyllite were less widely used for commercial purposes.

Chrysotile was the commonest commercially used from of asbestos with approximately 90% of the industries using this type [7]. At its peak in the mid to late 20th century, a number of industries including shipbuilding, manufacturing, insulation and construction, relied heavily on asbestos incorporated products, which led to an increase in the number of deaths attributed to asbestos in those working within these industries [8].

Asbestos has also been used as a major component of a number of day-to-day products such as flooring, roofing, textiles, plastics and cement [7]. Asbestos is most dangerous as a dust when it is readily inhaled. If the asbestos fibres are held within a matrix of another material (eg. within cement or plastic), the risk of inhalation is lower, unless the material is damaged allowing the fibres to be released [3].

1.2 The Pleura

The pleura is the thin membranous covering of the lung and the chest cavity, comprising of the visceral and parietal pleurae, which in reality are one contiguous layer [9]. The visceral pleura encases the lung parenchyma including the fissures, while the parietal pleura covers the inside of the thoracic cavity, pericardium, thoracic surface of the diaphragm, and the mediastinal structures. Composed of a single layer of mesothelial cells, the pleura is rich in microvilli, while a layer of connecting tissue rich in blood vessels and lymphatics sits beneath the layer of mesothelial cells [9]. The space between the visceral and parietal pleurae (known as the pleural space) contains a small volume of fluid (approximately 0.26ml/kg in each hemi-thorax) [10]. This fluid is constantly produced by the pleural capillaries in the parietal pleura

and reabsorbed via the pleural lymphatics [9]. Pleural fluid is thought to lubricate the pleural surfaces, allowing them to glide smoothly over each other during respiration [11].

Animal studies investigating the pleura in an attempt to understand the composition and functions have discovered that the visceral pleura is thicker than parietal pleura, measuring 25-83 µm vs 10-25 µm for parietal pleura, in sheep [9]. Injuries and insults can lead to thickening of the pleura, particularly the parietal pleura, which is more susceptible to the effects of injury. The mesothelial cell layer is fragile and when injured will be repaired by migration of neighbouring cells and secretion of growth factors, analogous to epithelial cells [12]. Mesothelial cells can be easily dislodged from the pleura and can often be found in fluid aspirated from the pleural cavity.

1.3 Biological aspects of asbestos related disease

Several factors are closely interlinked in the pathogenicity of inhaled asbestos dust particles [13]. The main factors responsible for the biological consequences and bio-persistence of asbestos particles are fibre dimension (diameter and length), chemical composition and the surface state of the fibres [13, 14]. Large inhaled particles/fibres are cleared by the muco-ciliary system in the upper airways and the larger bronchioles [4]. Smaller fibres, often if less than 20µm, can migrate to the alveoli [4]. Once in the alveoli several processes can take place that may decide the fate of the fibres. They could remain in the alveoli indefinitely or be ingested by macrophages (if the fibres are smaller than 5µm). Some fibres can be partially or completely dissolved over a prolonged period of time. This is rare in amphibole structures due to their relative insolubility but chrysotile fibres which have a higher Magnesium content, may succumb to the gradual leaching process [4]. Finally, some fibres may migrate further from the alveoli – on their own or via macrophages – to the pulmonary interstitium or to the pleura [13, 15, 16].

The parietal pleura's increased susceptibility to the carcinogenic effects of asbestos fibres is not completely understood but their bio-persistence and a chronic inflammatory response may play a major role [15-17]. In addition, asbestos can cause DNA damage directly by interfering with the segregation of chromosomes during mitosis [18]. Asbestos fibres are ingested by macrophages but may not necessarily be completely dissolved. As the macrophage life span is only about 50 days, when they die, the undissolved fibres are re-ingested by other macrophages and this cycle repeats itself. Fibre size also has a role in carcinogenicity [19]. Smaller particles are ingested and dissolved by phagocytosis. Larger fibres are intercepted in the tracheo-bronchial tree by the muco-ciliary system and cleared this way. This then leaves the intermediate length fibres, which are particularly resistant to degradation. The intermediate fibres can penetrate the cell membranes of the macrophages when they are ingested, discharging lysozymes and other enzymes from macrophages leading to surface reactions on the asbestos fibres. The electrically charged surface states have a role in generating free radicals and biological responses [15]. Amphibole fibres in particular, have a higher content of iron which leads to the formation of reactive oxygen species causing mutagenic oxidative lesions which may also play a role in the development of malignant pleural mesothelioma (MPM) [16].

Approximately 60-70% of pleural mesothelioma cases are related to prior asbestos exposure [20], while the remainder maybe as a consequence of chest radiation, genetic predisposition or spontaneous occurrence [21]. Novel genomic analyses have defined a range of molecular alterations that drive pleural mesothelioma. BRCA associated protein 1, or BAP1 has been identified as one of the commonest genes, where deletion or truncation of BAP1 gene is commonly seen in pleural mesothelioma [22, 23]. Similarly, the absence of CDKN2A and NF2 genes also predispose more towards pleural mesothelioma, in comparison to mesothelioma

of other organs such as peritoneal, pericardial and tunica vaginalis [22, 24]. As in other malignancies it is the inactivation of tumour suppressor genes that appear to play a crucial role in the development of the disease [23]. Further work is ongoing to explore the genetic predilection and how this can be incorporated in the diagnostic and prognostic aspects of mesothelioma [24].

A study by Yang et al. demonstrated that asbestos fibres can lead to necrotic cell death resulting in the release of Highly Motile Group Box – 1 (HMGB1) which causes a chronic inflammatory response with the accumulation of macrophages and secretion of tumour necrosis factor alpha (TNFα) [25]. TNFα on one hand protects human mesothelial cells from asbestos induced cell-death but on the other hand promotes the growth of human mesothelial cells that have accumulated damaged-DNA from asbestos exposure [17].

The dose-response relationship between asbestos fibre exposure and development of related disease has been well recognised [2]. At the *International Meeting of Experts of Asbestos, Asbestosis and Cancer*, in January 1997 in Helsinki, the exposure criteria were standardised taking into consideration the evidence available at the time [26]. These criteria are now known as the 'Helsinki criteria' and in brief they state, 1 year of heavy exposure (such as manufacture of asbestos products, spraying asbestos, insulation work with asbestos lagging and demolition of old buildings) and 5-10 years of moderate exposure (for example construction and shipbuilding) is likely to equate to a greater than 25 fibre/ml/years of exposure. At this level of exposure, the risk of lung cancer is more than doubled [26]. This is also the minimal dose at which asbestosis – interstitial fibrosis of the lung parenchyma - can occur. MPM can occur at much lower exposure levels [27]. These criteria are now routinely used when attributing disease causation to asbestos[27] .

1.4 Asbestos related benign pleural disease

Asbestos exposure can cause several pathologies benign and malignant as shown in Table 1-1.

Benign pathologies	Malignant pathologies
Pleural plaques	Mesothelioma
Benign asbestos related pleural effusions	Lung cancer
Diffuse pleural thickening	
Asbestosis	

Table 1-1: Asbestos related pleuro-parenchymal diseases

As this thesis is concentrating on asbestos related pleural disease, the pleural pathologies are discussed in more detail below.

1.4.1 Pleural Plaques

Pleural plaques are focal areas of fibrosis limited to the parietal pleura (Figures 1-1 and 1-2). Pleural plaques are harmless and do not transform to a malignant process, however a 'large plaque load' signify heavy exposure, and can be associated with development of malignancy due to a similar dose-response relationship [28]. The presence of pleural plaques is pathognomonic of asbestos exposure. Pleural plaques appear on chest radiographs approximately 12 years after exposure and can take up to 20 years before calcifying [29, 30]. Pleural plaques are often found in the areas of greatest friction between the lung and chest wall. They tend to spare the apices and are commonly found on the diaphragm. They can be of varying sizes and shapes and, occasionally so extensive that they involve almost the entire hemi-thorax resulting in a restrictive respiratory impairment [31]. Often pleural plaques are an incidental finding on chest radiography and given their benign course they do not require on going monitoring or follow-up. However, their presence signifies previous asbestos

exposure and hence patients are at increased risk of other asbestos related pathologies, including malignancy.

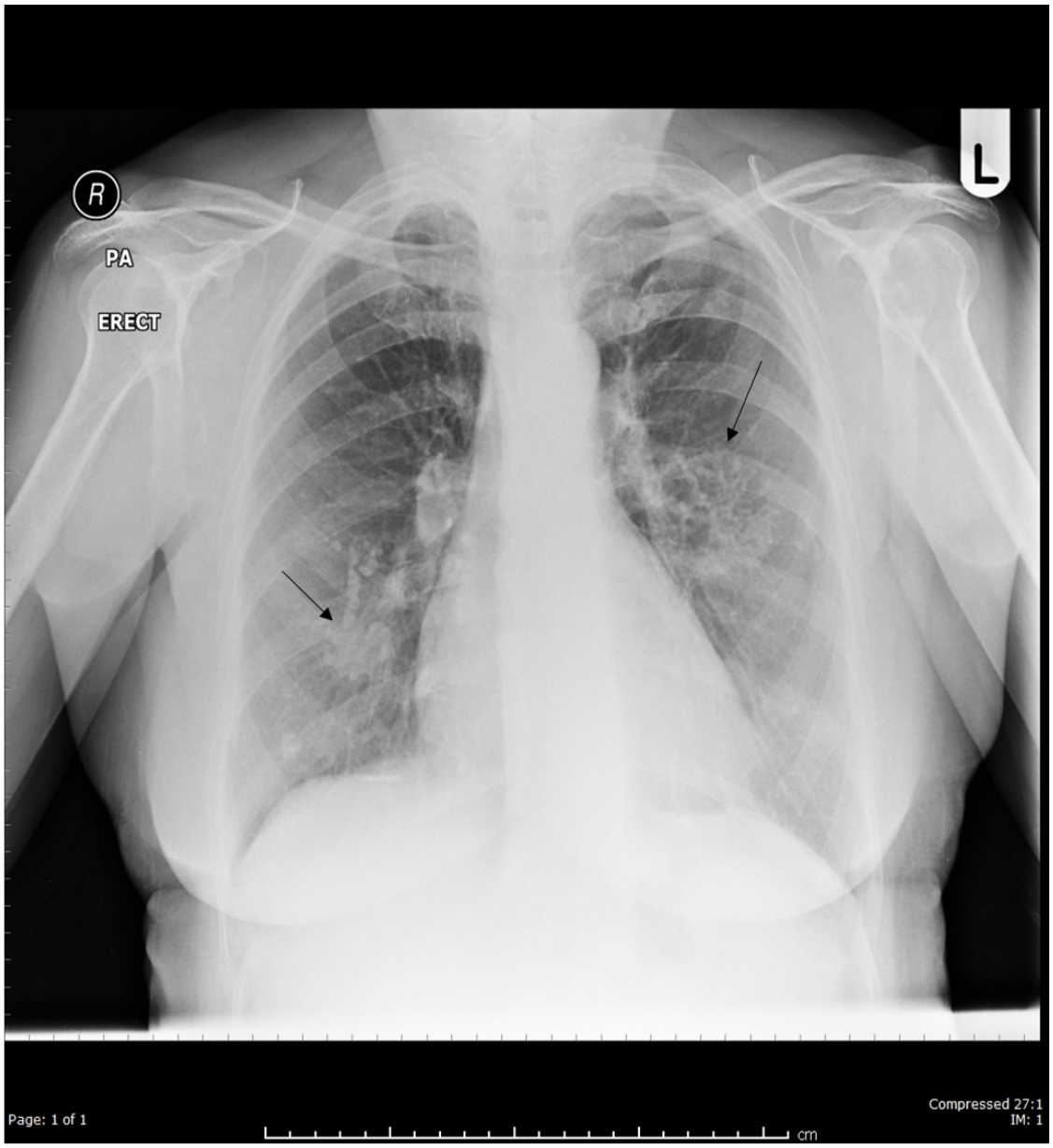

Figure 1-1: Pleural plaques (shown in black arrows) as seen on chest radiograph

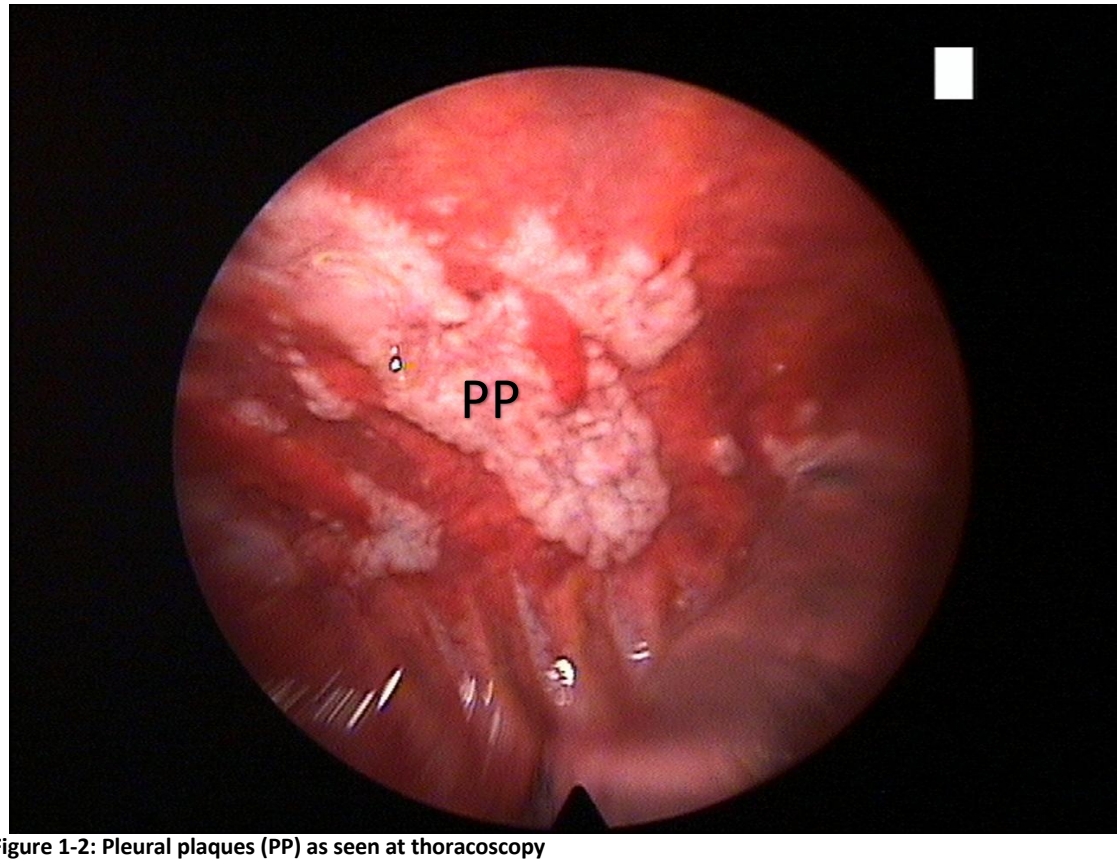

Figure 1-2: Pleural plaques (PP) as seen at thoracoscopy

1.4.2 Benign asbestos related pleural effusions

Benign asbestos pleural effusion (BAPE) is a diagnosis of exclusion in patients with pleural effusions and evidence of previous asbestos exposure. Presenting symptoms are variable with some patients being completely asymptomatic and the discovery of the effusions being incidental, or symptoms of an active inflammatory pleuritis with fever, breathlessness or pleuritic chest pain [16, 32]. The reasons behind the development of these effusions are poorly understood, however, the literature suggests an acute inflammatory reaction secondary to asbestos exposure as a potential cause [33]. The lag period for developing BAPE is much shorter (10 years from exposure) than that for other asbestos related disease such as mesothelioma or asbestosis (30-40 years) [32]. Due to the high risk of malignancy in this cohort of patients, this should be excluded by means of a pleural biopsy and sufficient follow up. Depending on the size of the effusion, BAPE can be debilitating, requiring drainage of the

effusion for symptom relief. BAPE also has been cited as the trigger for visceral and parietal pleural fibrosis which can lead to diffuse pleural thickening (discussed below) [32, 33]. It is prudent to observe these patients for a number of years to ensure that the diagnosis is in fact BAPE and not a misclassification of an early stage MPM.

1.4.3 Diffuse pleural thickening (DPT)

DPT refers to extensive fibrosis of the visceral pleura, forming frequent adhesions to the parietal pleura, leading to obliteration of the pleural space [34]. As mentioned above, it is thought to be the sequel of an exudative effusion (BAPE) and develops in approximately 7% of those with historical asbestos exposure [35, 36]. In most cases DPT is an incidental finding on radiological investigations performed for other reasons. Historic literature used plain chest radiographic (CXR) appearances for confirming the presence of DPT, due to ease of access to CXR at the time. The international labour organisation (ILO) still relies on the CXR appearances for classifying pleural thickening as 'diffuse' [37] not taking into account the current gold standard imaging modality for assessing the pleura – computed tomography (CT) [38].

The '*American Thoracic Society (ATS) guidelines on diagnosis and initial management of non-malignant diseases related to asbestos*' use the definition set out by the International Labour Organisation (ILO) for diagnosing DPT; obliteration of the costophrenic angle (CPA) by pleural thickening on a postero-anterior chest radiograph [29]. There is still no consensus on a unified definition for DPT. Many authorities cite the definition of DPT on CT proposed by Lynch et al. based on size criteria; continuous thickening of the pleura measuring ≥5 cm wide, ≥8 cm long craniocaudally and 3 mm in thickness, with or without CPA involvement [39]. Regardless of the radiological definition, the important clinical implication of DPT is the significant respiratory disability [34, 40-42]. When DPT is related to asbestos exposure, it is currently

recognized as a compensatable disease under the Industrial Injuries Disablement Benefit (IIDB) scheme in the United Kingdom (UK) [43].

1.5 Malignant Pleural Mesothelioma

Malignant primary tumours of the pleura as an entity was somewhat controversial in the early 20th century when some pathologists denied their existence [44]. Scattered case reports of a primary malignant tumour of the pleura, 'mesothelioma', started surfacing towards the mid-20th century coinciding with the increasing use of asbestos [45, 46]. Not until 1960 when Wagner et al published a landmark study, explicitly describing the association between asbestos exposure and mesothelioma in a cohort of workers from the north west Cape of South Africa, did mesothelioma earn its reputation as a malignancy attributable to asbestos [6]. They described 33 cases of primary pleural tumours in patients with a definite asbestos history and characteristic pathological findings. They also reported the possibility of a prolonged lag period between 20 to 40 years or more, from exposure to development of mesothelioma, as observed in some of their cases [6]. Another observation at the time was those working predominantly with amphibole type asbestos had a much higher risk than those working with mixed asbestos or chrysotile alone.

Shortly after this publication, there was an increased interest around controlling inhaled asbestos exposure. The 1969 asbestos regulations came into force on the 14th of May 1970, which imposed stringent requirements around limiting asbestos inhalation in any industries that used asbestos in the UK [47]. In 1985 the import of crocidolite and amosite asbestos was banned in the UK [48]. Since then, several asbestos control regulations have come into effect and have been updated regularly [49]. Due to the long latency period the incidence of malignant pleural mesothelioma (MPM) is still on the rise and is expected to hit a peak in 2025 [50]. The reasons behind the prolonged latency period is still not completely understood.

MPM is an aggressive and universally fatal tumour, the incidence of which is still considerable in many parts of the world [51, 52]. In the UK it currently accounts for 1% of malignant disease, with a majority developing mesothelioma as a result of previous asbestos exposure [53]. At its early stages MPM is believed to follow an indolent course therefore, patients may not present with symptoms until their disease is advanced [54]. Common symptoms at presentation are non-specific such as chest pain, shortness of breath (in the presence of a pleural effusions), fatigue and weight loss [55]. Asbestos exposure and the presence of above symptoms warrant urgent further investigation.

There are 3 main histological sub-types in mesothelioma - epithelioid, sarcomatoid and biphasic - with further sub-classification within groups as shown in Table 1-2 [56]. As the histological subtype can provide prognostic information - sarcomatoid MPM has a much worse survival than epithelioid - histological subtyping at the time of diagnosis is essential [57, 58].

Histological sub-types and patterns of mesothelioma
Epithelioid mesothelioma Tubulopapillary Micropapillary Trabecular Acinar Adenomatoid Solid Clear cell Deciduoid Adenoid cystic Signet ring cell Small cell Rhabdoid Pleomorphic
Sarcomatoid mesothelioma Conventional, spindle cell Desmoplastic Heterologous differentiation (osteosarcomatous, chondrosarcomatous) Lymphohistiocytoid

Biphasic/mixed

Table 1-2: Histological sub-types and patterns of mesothelioma

1.5.1 Investigation and diagnosis

1.5.1.1 CT

Although simple and easily accessible, chest radiographs (CXR) are neither sensitive nor specific at diagnosing MPM. Early CT is recommended for detailed examination of the pleura [29]. Specific radiological criteria such as pleural nodularity, pleural thickening measuring > 10mm and thickening extending over the mediastinum on CT, can aid in the distinction between benign and malignant pleural thickening [59, 60]. In its early stages when pleural thickening is subtle, or in the absence of above features, the sensitivity of CT for diagnosing MPM is low [61, 62]. On morphological criteria alone, the sensitivity and specificity for detection of MPM is 72% and 83%, respectively [59]. Novel methods of analysing CT such as volumetric CT are currently being investigated [63].

1.5.1.2 Magnetic Resonance Imaging (MRI)

MRI is a safe, readily accessible radiation free imaging modality. Its superiority at delineating soft tissue, and spatial resolution makes MRI an attractive radiological investigation, but its role in pleural malignancy is yet to be fully defined. Historically movement from respiratory motion and cardiac impulses created significant artefact when analysing MRI images, but with modern breath holding techniques, respiratory and navigator gating this issue is largely overcome [64]. Different image acquisition methods providing qualitative and quantitative analyses can all help in obtaining a range of data that can be potentially useful in assessing the pleura.

Diffusion weighted imaging (DWI) MRI is one such technique that relies upon the random ('Brownian') motion of water molecules within a voxel (a region of tissue that corresponds to a pixel on an image), with the switching of the magnetic field [65]. The associated signal loss resulting from the restricted motion of water molecules can be quantified using the apparent diffusion coefficient (ADC), which in turn provides information regarding cellularity of neighbouring tissue [66]. In the last two decades a number of small studies have tried to evaluate the role of DWI-MRI in patients with established pleural malignancy [67, 68], yet its role in the diagnostic pathway of MPM is yet to be fully defined.

In dynamic contrast enhanced (DCE) MRI, sequential images are acquired during administration of a gadolinium-based contrast agent [69]. The uptake and washout of the contrast by tissue gives information about the micro vascularity and permeability of the tissue, which in turn can be mapped to provide information regarding the benign/malignant nature of the tissue under consideration [69, 70]. The persistent gradual uptake of contrast is suggestive of benign tissue, whilst the rapid uptake and washout type is suggestive of malignant tissue, reflecting the increased vascularity of malignant tissue. The plateau type represents an intermediate type [71].

A recent small study using visual assessment of the DWI scans suggested a technique called pleural pointillism may be useful in the setting of early pleural malignancy [72]. This technique involves assessing the pleura on b-0 and b-1000 DWI images. Multiple hyperintense areas seen on b-1000 images are suggestive of pleural pointillism. This technique to date has only been described in one study and further evidence is needed to clarify its role.

Early studies using MRI in pleural malignancy have demonstrated promising results, but these studies have not been easily reproducible, with low patient numbers, often retrospective with no adequate control populations [65, 67].

1.5.1.3 ^{18}Fluorodeoxyglucose – Positron emission tomography (FDG-PET)

Metabolically active cells such as malignant cells, foci of infection and acute inflammatory cells have a high affinity for glucose. PET scanning exploits this affinity of glucose to metabolically active areas. A radio-labelled 18-fluorodeoxyglucose (FDG) is administered intravenously and the PET scan is performed after a short period of sedentary waiting (usually 60 minutes) when the FDG can be detected on the PET scan highlighting areas of possible neoplastic tissue or infected/inflammatory tissue [73]. This imaging modality is increasingly used in other cancers to inform management. From a diagnostic perspective in MPM, a number of small studies have consistently shown that integrated PET-CT does perform well with accuracy levels for diagnosis consistently approaching 90% for radiological diagnosis [74, 75]. A systematic review of 16 studies of 745 patients by Treglia et al., found a pooled sensitivity of 95% and specificity of 82% for FDG-PET in differentiating benign from malignant pleural lesions [76]. Despite the encouraging results PET-CT is currently not routinely used for diagnostic purposes in pleural malignancy.

1.5.1.4 Staging of MPM

MPM staging provides crucial information for the management of patients, particularly those being considered for surgery, and for prognostication. Although radiological staging is least invasive and often used, the gold standard for MPM staging is surgery, reflecting the origins of the surgically derived Tumour, Node, metastases (TNM) classification system currently used in MPM [77]. The recently updated staging system currently in its 8th edition is shown in Table 1-3 [78]. Table 1-4 shows the stage groupings.

Stage	Definition
Primary tumor (T)	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
T1	Tumor limited to the ipsilateral parietal ± visceral ± mediastinal ± diaphragmatic pleura
T2	Tumor involving each of the ipsilateral pleural surfaces (parietal, mediastinal, diaphragmatic, and visceral pleura) with at least one of the following features: • involvement of diaphragmatic muscle • extension of tumor from visceral pleura into the underlying pulmonary parenchyma
T3	Describes locally advanced but *potentially resectable* tumor. Tumor involving all of the ipsilateral pleural surfaces (parietal, mediastinal, diaphragmatic, and visceral pleura) with at least one of the following features: • involvement of the endothoracic fascia • extension into the mediastinal fat • solitary, completely resectable focus of tumor extending into the soft tissues of the chest wall • nontransmural involvement of the pericardium
T4	Describes locally advanced *technically unresectable* tumor. Tumor involving all of the ipsilateral pleural surfaces (parietal, mediastinal, diaphragmatic, and visceral pleura) with at least one of the following features: • diffuse extension or multifocal masses of tumor in the chest wall, with or without associated rib destruction • direct transdiaphragmatic extension of tumor to the peritoneum • direct extension of tumor to the contralateral pleura • direct extension of tumor to mediastinal organs • direct extension of tumor into the spine • tumor extending through to the internal surface of the pericardium with or without a pericardial effusion, or tumor involving the myocardium
Regional lymph nodes (N)	
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastases
N1	Metastases in the ipsilateral bronchopulmonary, hilar, or mediastinal (including the internal mammary, peridiaphragmatic, pericardial fat pad, or intercostal lymph nodes) lymph nodes
N2	Metastases in the contralateral mediastinal, ipsilateral, or contralateral supraclavicular lymph nodes
Distant metastasis (M)	
M0	No distant metastasis
M1	Distant metastasis present

Table 1-3: The International Association for the Study of Lung Cancer (IASLC) 8th edition of the TNM classification for Mesothelioma

Stage	N0	N1	N2
T1	IA	II	IIIB
T2	IB	II	IIIB
T3	IB	IIIA	IIIB
T4	IIIB	IIIB	IIIB
M1	IV	IV	IV

Table 1-4: TNM staging groups

1.5.1.5 Pleural fluid cytology for diagnosis of MPM

Data from the Bristol Academic Respiratory UNIT (ARU) confirm over 80% of MPM patients have a pleural effusion at presentation, 40% of these are large (manuscript under review for publication). Other studies have quoted higher incidence of effusion at presentation, 91% in a study by Tanrikulu et al [79]. Pleural fluid cytology analysis is a minimally invasive option for pathological confirmation of malignancy, with reported sensitivities between 50-60% in all malignant pleural effusions [80-82]. Yet the diagnostic sensitivity for MPM with cytology alone remains low, 20% to 32% quoted in some studies [80, 83]. Therefore, most patients would require a pleural biopsy for confirmation of disease.

1.5.1.6 Pleural biopsy

For patients presenting with a pleural effusion the first choice of biopsy is usually local anaesthetic thoracoscopy (LAT), as this could be a diagnostic and therapeutic procedure in one sitting (Figure 1- 3), performed under conscious sedation. Once the effusion is drained and biopsies obtained, patients will be pleurodesed (artificial obliteration of the pleural space by adhesion of the visceral and parietal pleurae) to prevent recurrence of effusion [80]. For pleural malignancy LAT has a high diagnostic yield with sensitivities over 90% [80]. Pleural biopsy with video assisted thoracoscopic surgery (VATS) is the gold standard for diagnosing pleural malignancy with a diagnostic yield > 95% [84]. However, due to associated complications and invasiveness, VATS biopsy is usually only considered when other less invasiveness options have failed to give a definitive diagnosis.

In the absence of a pleural effusion most patients would have a percutaneous biopsy under image guidance. CT guided cutting needle biopsy is proven to be the most efficient biopsy technique for obtaining a pleural biopsy in this setting [85]. However, the yield is variable as only one small area of the pleural thickening is biopsied, leading to occasional false negative

results [86]. MPM is a heterogenous tumour where areas within the tumour may lack the level of cell differentiation or invasion required to distinguish benign from malignant mesothelial cell proliferation [56, 87]. Specific immunohistochemical markers commonly employed for differentiating MPM from other cancers (particularly lung cancer variants) such as Calretinin, Wilms Tumour 1 (WT-1) and Cytokeratin 5/6 are often dependant on the histological sub-type of MPM and the other differential cancers under consideration [56]. Breast cancer associated protein 1 (BAP-1) is a tumour suppressor gene, mutations of which is associated with hereditary cancer syndromes [88]. Recent literature suggests the absence of this gene increases the risk of MPM [88, 89]. Loss of BAP-1 can be tested immunohistochemically and can be useful as an adjunct in the diagnosis of MPM [90]. A meta-analysis by Wang et al. with 12 studies and 1824 patients demonstrated a pooled sensitivity of 56% and specificity of 100% for the loss of BAP-1, in diagnosing MPM [91]. Currently, some centres have access to and regularly use this test in the diagnostic pathway. Similar to BAP-1, the loss of p16 gene detected by Fluorescence in situ hybridization (P16 FISH) could help in the diagnosis of sarcomatoid mesothelioma, but larger studies are needed in this area before widespread use [92].

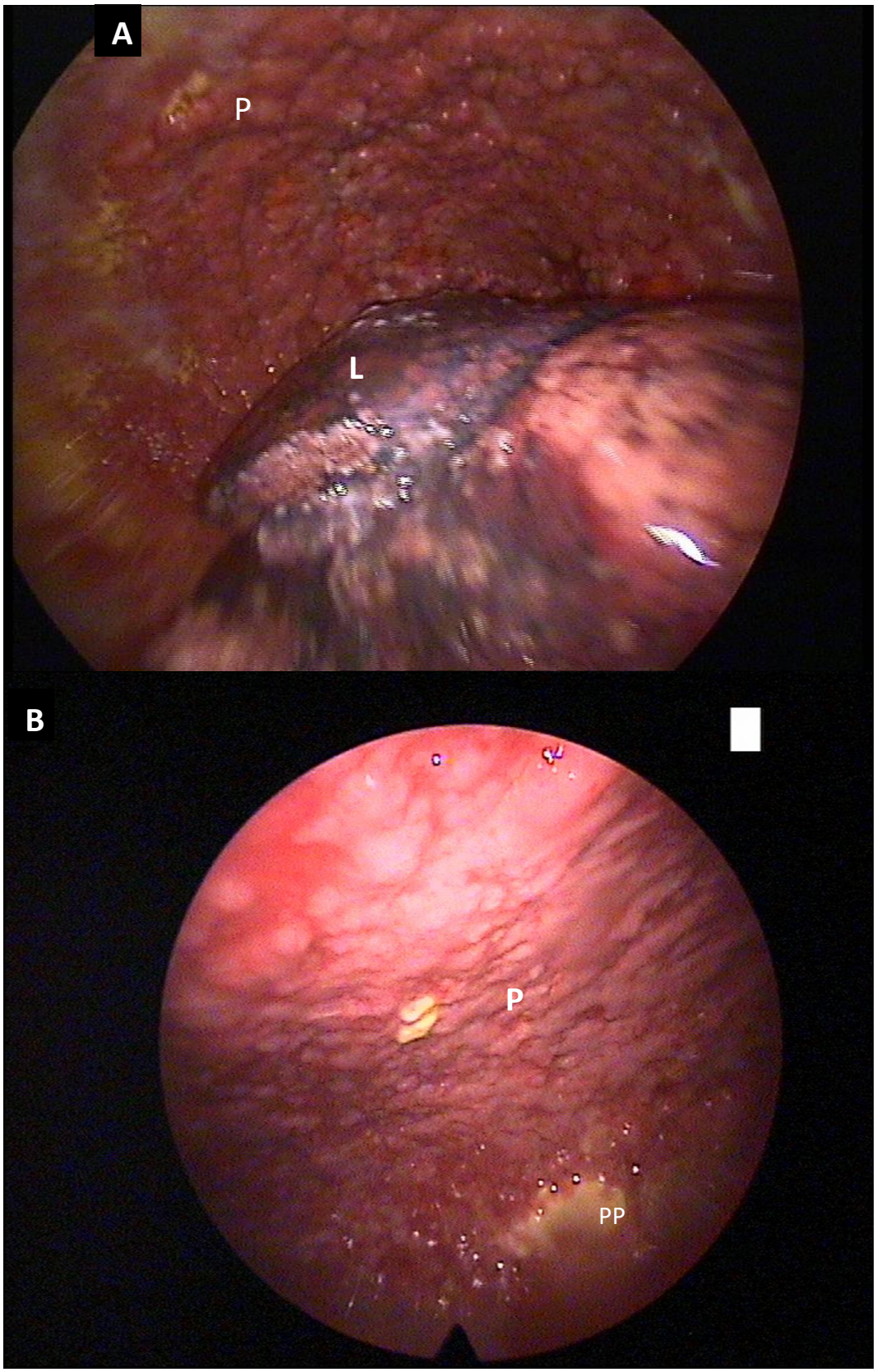

Figure 1-3: Diffusely abnormal parietal pleura as seen at thoracoscopy. Image A shows diffuse pleural nodularity (P) with partially collapsed lung (L). Image B shows widespread pleural irregularity (P) with an infiltrated appearance to the parietal pleura. Pleural plaque (PP) seen at the bottom right.

1.5.1.7 Mesothelin for diagnosis

Biomarkers are a non-invasive, reliable and efficient way of diagnosing and monitoring disease, and response to treatment. Biomarkers such as soluble mesothelin related peptide (SMRP) or mesothelin, Fibulin-3, megakaryocyte protein (MPF) and Osteopontin have all been evaluated for their role in the diagnosis, monitoring and screening of MPM [93, 94]. Mesothelin is a 40kDa membrane bound glycoprotein expressed by mesothelial cells that is detected in both serum and pleural fluid [95]. Tumours with epithelioid histological characteristics express mesothelin more readily compared to non-epithelioid sub-types and the level of serum mesothelin correlates to tumour bulk [96]. A review by Cui et al, of 28 publications including 7550 patients found a pooled sensitivity of 61% and specificity of 87% for serum mesothelin for diagnosis of MPM [97]. The same study reviewed performance indicators for pleural fluid mesothelin and found a pooled sensitivity of 75% and specificity of 85%, for diagnosing MPM. Given the lack of prospective validation, mesothelin is currently not used in isolation for the diagnosis of MPM. However, in the correct clinical and radiological context, it can however, support a diagnosis of MPM.

1.5.2 Management of mesothelioma

1.5.2.1 Surgery

Surgery for mesothelioma has been a controversial subject for decades. The role of curative surgery as a life prolonging treatment in MPM is yet to be established [98]. One of the earliest and widespread surgeries to be performed in MPM, extra-pleural pneumonectomy (EPP) involved en-bloc resection of the lung, ipsilateral parietal pleura, pericardium and hemidiaphragm [99, 100]. Due to high mortality and post-op complications the appropriateness of this surgery in MPM has been questioned repeatedly [101]. The mesothelioma and radical surgery (MARS) feasibility trial attempted to answer this question

by randomising patients to EPP versus no EPP [102]. In this feasibility study the no-EPP group did better with a median survival of 19.5 months compared to those who had EPP, 14.4 months. The feasibility trial did not progress to a full phase III trial due to concerns around safety of EPP. Despite compelling data from the MARS 1 study and a number of large surgical case series showing the detrimental effects of EPP, it continues to be performed in some centres in Europe, United States of America and Canada [98, 103-105]. Even more surprisingly, the recently published American Society of Clinical Oncology guidelines on treatment of mesothelioma made a strong recommendation that in select patients with early stage disease a maximal surgical cytoreduction (ie. EPP) should be performed [106]. On the contrary, the British Thoracic Society mesothelioma guideline on the investigation and management of MPM adopts a more conservative stance, recommending surgery only in a clinical trial setting [107]. To answer the important question regarding the role of surgery for extending survival, researchers in the UK are currently conducting the MARS-2 trial (NCT02040272) recruiting patients to surgery or no surgery (alongside chemotherapy) to assess if a less aggressive yet potentially equally effective form of surgery such as pleurectomy/decortication (PD) or extended pleurectomy/decortication (EPD) would prolong survival.

Apart from surgical cytoreduction to prolong survival, surgery is also performed for pleural fluid management. For patients with symptomatic pleural effusions, pleurodesis with talc slurry/poudrage was shown to be a better option over surgery with VATS partial pleurectomy, by the recently concluded MESO-VATS trial [108]. Another pilot and feasibility RCT (NCT03412357) is currently recruiting MPM patients with trapped lung, to indwelling pleural catheter (IPC) versus video assisted thoracoscopic surgery – pleurectomy/decortication

(VATS-P/D) to inform a full phase III study in the future which may shed light on whether there is any role for surgery in the trapped lung setting.

1.5.2.2 Chemotherapy

The current standard first line chemotherapy is with the combination of pemetrexed and cisplatin following the findings of a landmark study by Vogelzang et al., showing this combination conferred a 3-month additional survival benefit compared to Cisplatin alone [109]. However, only about 40% of patients responded to this treatment and as mentioned, the survival benefit was only 3 months. The addition of an anti-angiogenic agent such as Bevacizumab is shown to extend survival by a further 2 months, at the expense of significant side effects and toxicity [110]. Bevacizumab is currently not licensed in the UK and a search for other first line treatments in isolation or in combination with chemotherapy, continues. A range of phase I-III trials investigating cytotoxic chemotherapy treatments, anti-angiogenics and immunotherapy are currently underway both as first line, second line, combination and maintenance therapy [111].

1.5.2.3 Zoledronic acid

Zoledronic acid (ZA) is a nitrogen containing bisphosphonate which inhibits angiogenesis and apoptosis in certain cancers [112]. In vitro and animal studies have suggested a potential role for ZA in MPM [113, 114]. One human study investigating the effects of ZA in advanced MPM failed to show a significant advantage on disease control using ZA alone [115]. However, a synergistic role with existing chemotherapy treatment has not been examined.

1.5.3 Monitoring treatment response in MPM

1.5.3.1 CT

Given the low response rate to conventional treatment, and uncertainty around the efficacy of newer therapies, the ability to accurately monitor disease is essential for oncologists and trialists. The current standard for monitoring is, as for many tumours, serial CT scans. However, unlike many tumours, MPM does not grow spherically, instead growing as a 'rind' encasing the thoracic cavity, making sequential

comparisons more difficult. An adaptation of the Response Evaluation Criteria in Solid Tumours (RECIST), used for comparison of CTs in other cancers, to the modified RECIST (mRECIST) criteria has only partially allowed for this unique morphology [116]. Other issues including low sensitivity to predict progression, subjectivity in radiologists' choice of measurement sites and complications from the presence of pleural plaques or fluid, mean research into a more robust and reproducible radiological marker is ongoing [117]. In the absence of a better radiological or biomarker CT continues to be used for monitoring mesothelioma.

Response assessment by mRECIST involves identifying 2 target pleural lesions that are more than 1 cm in thickness perpendicular to the chest wall, that can be measured at three different levels of the hemi thorax. The sum of the 6 measurements gives a univariate diameter which can be compared with subsequent CTs and scores to assess response or progression. Table 1-5 summarises mRECIST definitions and response criteria [118].

mRECIST	Definitions
Target lesions	Tumour thickness measured in 2 sites at 3 different levels on transverse cuts of CT scan + up to 2 lymph nodes if short axis diameter measures ≥ 1.5cm
Complete response (CR)	Disappearance of all target lesions with no evidence of tumour elsewhere
Partial response (PR)	At least a 30% reduction in the total tumour measurement
Stable disease (SD)	Those who don't fulfill criteria for PR or PD
Progressive disease (PD)	At least a 20% increase in total tumour measurement

Table 1-5: mRECIST definitions and response criteria

1.5.3.2 Integrated PET-CT

Studies have shown that serial FDG-PET scanning can be used to predict response [119] but is significantly limited by false positive uptake in inflamed tissue from surgical intervention or pleurodesis, as well as restricted availability and expense [120]. An adaptation of the mRECIST criteria, PERCIST (PET response criteria in solid tumours) allows quantitative comparisons of PET characteristics

such as maximum standard uptake value (SUVmax), mean SUV and total glycolytic volume (TGV) which assesses the metabolic activity of the whole tumour, on sequential scans [121]. Early studies using PET-CT to determine disease response or progression have all been promising [122-124] and this imaging modality is often used in clinical trial settings to assess treatment response.

The optimal timing of when the PET-CT scan should be performed in the treatment cycle is unclear with some studies suggesting performing the scan after the first cycle, but after 10 days from treatment for best results [121, 122].

1.5.3.3 Mesothelin

In certain cancers such as prostate and colorectal, biomarkers have a role in monitoring treatment response and recurrence. A potential role as such for mesothelin was shown by Wheatley-Price and colleagues, in a small cohort of patients with MPM who had surgical cytoreductive surgery [125]. The elevated pre-surgery levels normalised after debulking, further strengthening the association between Mesothelin levels correlating to tumour bulk [125]. Another study by Grigoriu et al., with 40 patients who had treatment for MPM with best supportive care (BSC), palliative chemotherapy or an intrapleural adenovirus vector as a part of a Phase I trial, also demonstrated a falling level of Mesothelin correlated to CT and PET-CT findings and overall survival [126].

A serum or pleural fluid biomarker that could reflect disease activity is an attractive option for MPM given the challenges with radiological assessment. The most investigated biomarker as such in MPM is mesothelin [127]. Mesothelin is found in the blood and pleural fluid of patients with MPM and levels correlate with tumour stage and bulk [96]. It lacks the sensitivity to be used as a diagnostic marker given reduced expression in non-epithelioid MPM. A systematic review we performed found 8 studies that had assessed serum mesothelin's ability to monitor disease during chemotherapy, and found it correlated with radiological markers and survival [128]. No studies have assessed the utility of mesothelin in the longer-term monitoring of patients not receiving chemotherapy.

1.6 Research questions and thesis overview

As discussed in this chapter there are clinical uncertainties surrounding the diagnosis and management of asbestos related pleural disease. This doctoral thesis attempts to explore the following clinically relevant questions:

- What is the association between different distributions of diffuse pleural thickening on CT and respiratory impairment?
- What is the role of DWI and DCE-MRI in the assessment of equivocal pleural thickening on CT?
- Is a PET-CT targeted pleural biopsy better at diagnosing pleural malignancy than a standard CT guided pleural biopsy, in patients with suspected pleural malignancy who have already had one non-diagnostic biopsy?
- Is it feasible to randomise 50 patients across 3 centres in a 13-month period to have zoledronic acid alongside standard first line chemotherapy for mesothelioma?
- Is there a role for serial mesothelin in the monitoring of patients with mesothelioma, who are receiving best supportive care?

CHAPTER 2 THE PHYSIOLOGICAL CONSEQUENCES OF DIFFERENT DISTRIBUTIONS OF DIFFUSE PLEURAL THICKENING ON CT IMAGING.

2.1 Background

There is still no universally accepted radiological definition for diffuse pleural thickening (DPT). The International Labour Organization (ILO) [37] base their definition of DPT on chest radiographic (CXR) appearances, requiring obliteration of the costophrenic angle (CPA) to be present, not taking into consideration the extent of the thickening on CT, which is the current gold standard radiological investigation for imaging the pleura. We hypothesise that the radiographical changes on a postero-anterior (PA) chest X ray are insufficient to appreciate the full extent of DPT.

The aim of this study was to evaluate the impact of DPT defined by CT and correlate its distribution, specifically involvement of the costophrenic angle, with chest radiographic findings and contemporaneous lung function testing.

2.2 Methods

Patients with suspected pleural thickening who attended pleural clinic at North Bristol NHS Trust between 09/2011 and 10/2016 were screened for the study. Patients who were classed as DPT according to size criteria (>3mm thick, >5 cm axial width and >8cm craniocaudal length) [39] on CT and had contemporaneous CXR and lung function were included in the study. The study was approved by the Southwest Research Ethics Committee (Ref: 08/H0102/11).

Data collected included patient demographics, previous asbestos exposure history including duration and level of exposure, smoking history and other comorbidities. Patients with breathlessness had their degree of breathlessness documented against the Medical Research Council (MRC) dyspnoea score, where a score of 1 = not troubled by breathlessness except on strenuous exercise, 2= short of breath when hurrying on a level or when walking up a slight hill, 3 = walks slower than most people on the level, stops after a mile or so, or stops after 15 minutes walking at own pace, 4 = stops for breath after

walking 100 yards or after a few minutes on level ground, 5 = too breathless to leave the house or breathless when dressing/undressing [129].

2.2.1 CT scans

The CT scans were performed for clinical reasons using a range of multi-slice detector CT scanners (GE HD750 Freedom edition, GE Optima – General Electric, Boston, USA; Toshiba Aquilion CX – Toshiba, Tokyo, Japan; Philips Ingenuity – Phillips, Eindehoven, Netherlands). Standard departmental protocols were used with volumetric datasets acquired with or without contrast as indicated clinically. All datasets were available with orthogonal isotropic reconstructions at 1-1.25mm collimation using soft-tissue and lung algorithms.

2.2.2 Lung function tests

All patients included in the study had full lung function tests, which included spirometry, lung volumes (by helium dilution and body plethysmography) and gas transfer (single-breath carbon monoxide diffusing capacity). All patients had their height and weight recorded at the time of their lung function test. Lung function tests within 12 months of the CT scan were used for analysis. Lung function testing was performed using NSpire UK (www.nspirehealth.com) body plethysmograph and HD2000 fast gas analyser. Lung Function indices were expressed as a percentage of predicted and standardised residual (SR) values. Predicted values were European Reference values.

2.2.3 Control group

The control group comprised of patients with discrete pleural plaques only. Patients in this group were matched for age, body mass index (BMI) and smoking status, to the diffuse pleural thickening group.

2.2.4 Radiology review

Two experienced chest radiologists (with a collective experience of 34 years) independently reviewed all CT scans and chest radiography. CT images were reviewed with orthogonal reformatted data and electronic callipers on a soft tissue algorithm using standard mediastinal window settings (40/400).

Diffuse pleural thickening was assessed according to Lynch's size criteria whereby to qualify as DPT the thickening needed to be more than 3mm in thickness, 5 cm in width on axial CT slices and 8cm in length cranio-caudally [39]. In addition, to the size criteria, diffuse pleural thickening was characterised morphologically by its tapered margins and ancillary signs of visceral pleural fibrosis, namely the presence of adjacent folded lung or pleuroparenchymal bands [130, 131]. These additional CT criteria were applied in order to exclude patients with large areas of confluent discrete plaques. The plain chest radiographs were assessed for costophrenic angle obliteration and when present classified as unilateral (side specified) or bilateral.

2.2.5 Statistical analysis

Statistical analyses were carried out using the Stata statistical package, version 14.2 (Stata Corp, Texas USA). Patient demographics such as age, BMI, smoking history were expressed as means with standard deviations for each DPT group against the control group. Inter-observer agreement when assessing chest radiographs and CT imaging was assessed using a weighted kappa coefficient. A p value < 0.05 was considered statistically significant unless otherwise specified in the text. Distribution for normality was assessed using the Shapiro-Wilks test. Student t-test was used when comparing parametric data.

2.3 RESULTS

Eighty-five patients who were seen at the pleural clinic with suspected pleural thickening and or pleural plaques on their chest radiographs between 09/2011 and 10/2016 were included in the initial screening. Patients were excluded if they had significant pleural effusions at the time of the CT (n=16); co-existent interstitial pulmonary fibrosis (n=5); or a subsequent diagnosis of pleural malignancy (n=6). Fifty-eight patients (37 with DPT and 21 with pleural plaques alone) were then evaluated, including full pulmonary function testing (Figure 2-1).

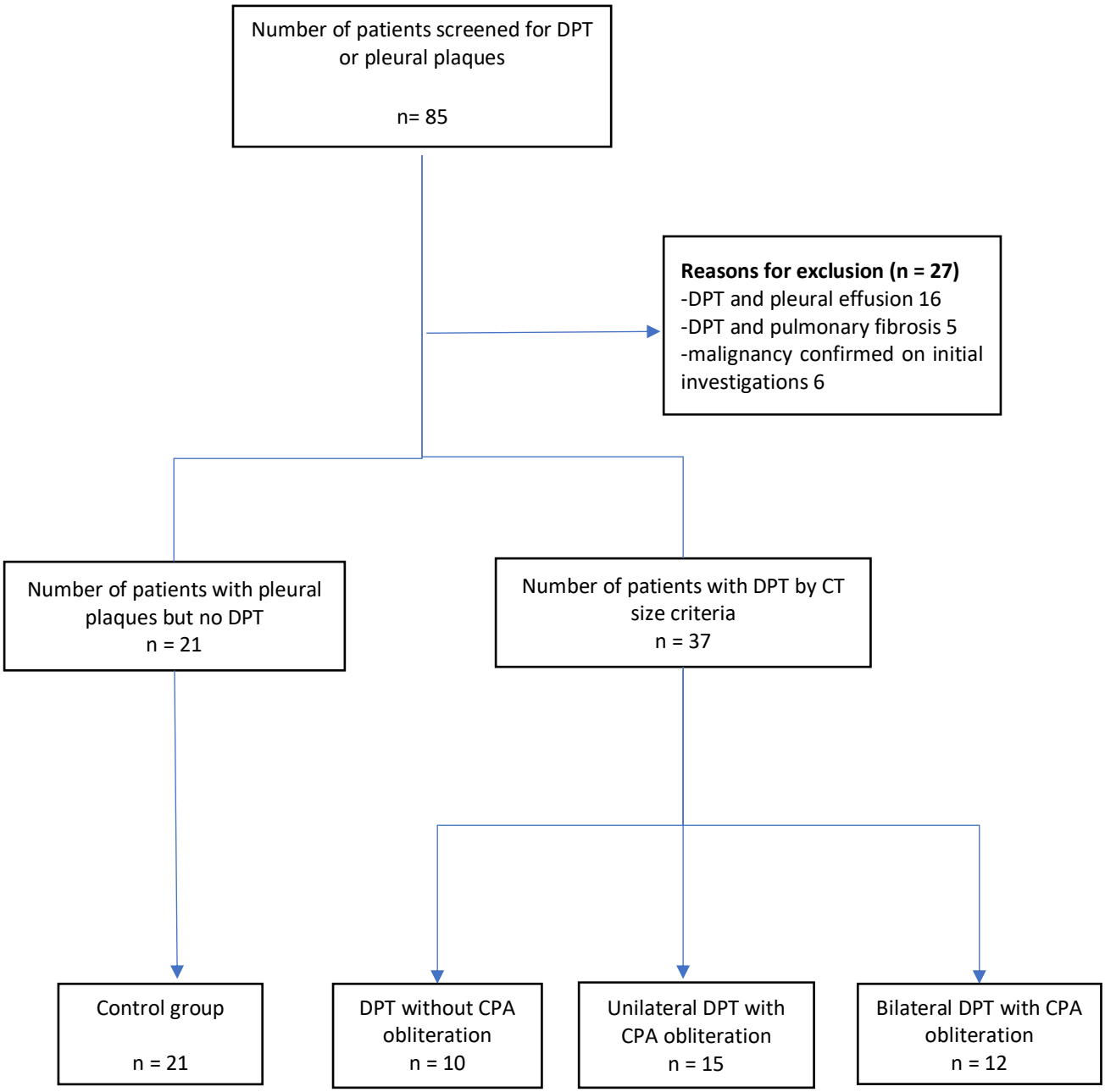

Figure 2-1: Flow diagram of included patients

The 37 patients who met diffuse pleural thickening by CT size criteria were classified into three separate groups;

Group 1: DPT by CT criteria with no CPA obliteration on chest radiograph,

Group 2: DPT by CT criteria with unilateral CPA obliteration on the chest radiograph,

Group 3: DPT by CT criteria with bilateral CPA obliteration on the chest radiograph.

An example chest radiograph and CT of a patient with right sided DPT but no CPA obliteration and bilateral DPT are shown in Figure 2-2.

Overall, there was good inter-observer agreement on radiological findings. There was moderate agreement for CPA obliteration on CXR with a weighted kappa value of 0.79 and good agreement for DPT by size criteria on CT, with a weighted kappa value of 0.82. Discrepancies were resolved by consensus review of images.

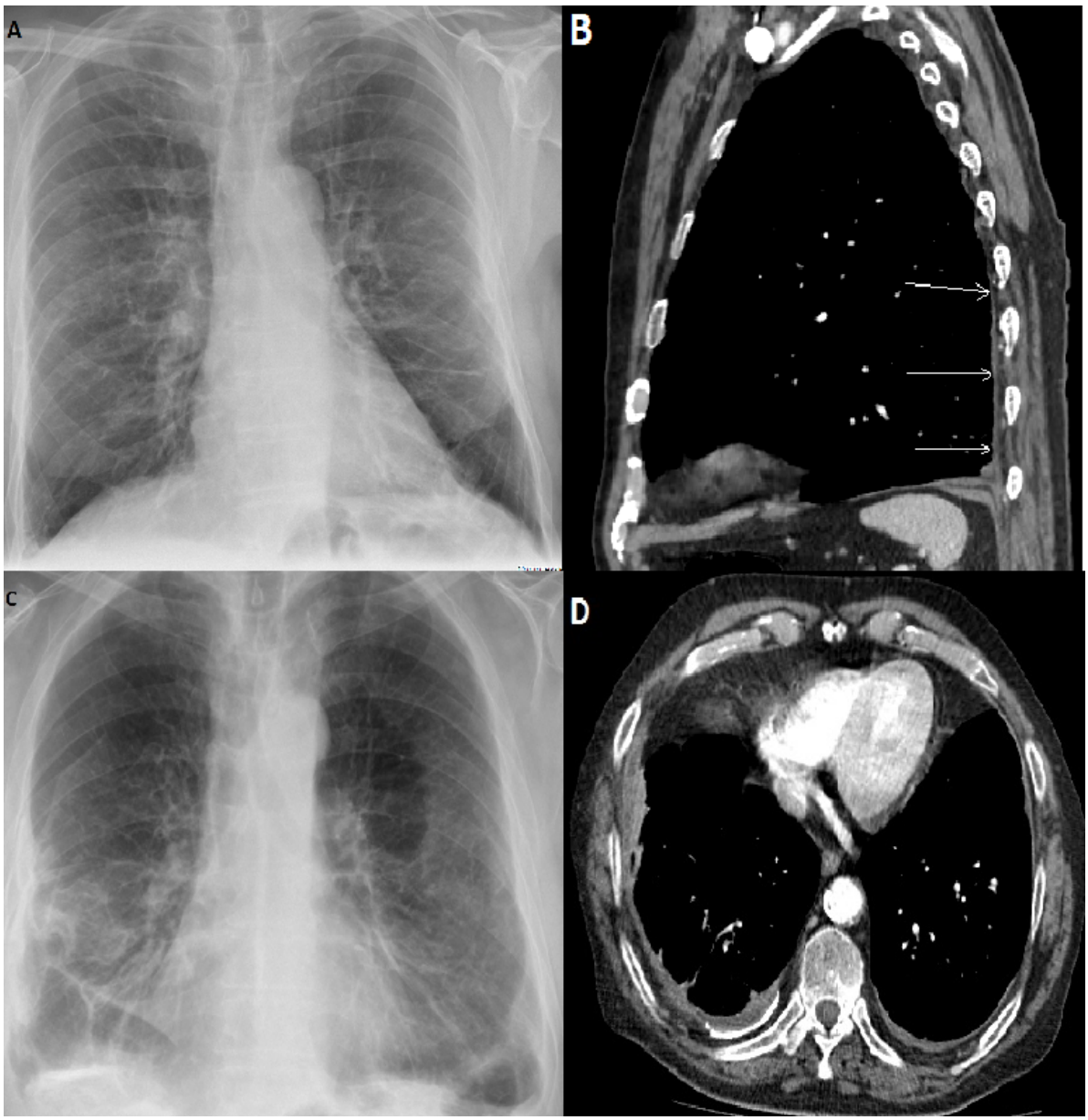

Figure 2-2:Images of DPT on chest radiograph and CT. Image A shows a postero-anterior chest radiograph of a patient with right sided DPT with no CPA obliteration. Image B is a sagittal CT view of the same patient showing DPT involving the posterior pleura. Image C is a patient with bilateral DPT and CPA obliteration. Image D is an axial image CT image confirming bilateral pleural thickening of same patient.

A summary demographics table, including the control group (pleural plaques only) is shown in Table 2-1. Fifty-six of the patients were male (97%), the mean age was 69.6 (CI: 67.3-71.9). The mean BMI was 29 (CI: 28-30) and the average pack year history was 22.9 (CI: 17.6-28.2). There was no significant difference between the ages, BMI, MRC grade or pack year history of smoking in the subgroups.

Demographic	Control Group	DPT with no CPA obliteration	Unilateral DPT	Bilateral DPT	P value
	n =21	n = 10	n = 15	n =12	
Age (years) (mean 95% CI)	69.4 (CI: 65.7-73.1)	69.5 (CI: 64.9-74.0)	70.2 (CI: 65.0-75.5)	69.0 (CI: 62.0-76.1)	0.99
BMI (kg/m2) (mean (95% CI)	28.2 (CI: 26.6-29.9)	30.5 (CI: 27.4-33.6)	28.7 (CI: 27.1-30.3)	29.4 (CI: 26.6-32.2)	0.45
MRC grade (mean (95% CI)	3.5 (CI: 2.9-4.2)	3.9 (CI:2.8-5.0)	3.4 (CI: 3.5-4.3)	4.6 (CI: 3.6-5.6)	0.20
Comorbidities (n (%))					
-COPD	4/21 (19%)	2/10 (20%)	3/15 (20%)	3/12 (25%)	
-CABG	1/21 (5%)	1/10 (10%)	4/15 (27%)	2/12 (17%)	
-Other	3/21 (14%)	0	3/15 (20%)	1/12 (8%)	
-nil	13/21 (62%)	8/10 (80%)	5/15 (33%)	6/12 (50%)	
Smoking history (pyrs) (mean (95% CI)	**20.5 (CI: 9.8-31.2)**	**24.2 (CI: 11.7-36.7)**	**22.4 (CI: 12.6-32.2)**	**27.0 (CI: 13.9-40.0)**	0.85
-Current smoker (n (%))	2/21 (9%)	1/10 (10%)	2/14 (14%)	4/11 (36%)	
-Ex-smoker (n (%))	13/21 (62%)	8/10 (80%)	10/14 (72%)	5/11 (46%)	
-Never smoker (n (%))	6/21 (29%)	1/10 (10%)	2/14 (14%)	2/11 (18%)	

Table 2-1: Baseline characteristics by patient group (BMI – body mass index; MRC - Medical research council; COPD – chronic obstructive pulmonary disease; CABG – coronary artery bypass graft; DPT – diffuse pleural thickening; pyrs – pack years) P values obtained by comparing the DPT groups against the control group.

Most patients (53/58) had clear documentation of the predominant occupation that exposed them to asbestos (Table 2-2). The largest proportions exposed were in construction work (12.1%), civil engineering (8.6%) or heating and insulation engineering (8.6%). The miscellaneous group 15/58 (25.9%), included factory workers, a dockworker, ship plater and a chemistry teacher.

Occupation	n	Percentage (%)
Construction worker	7	12.1
Civil engineer	5	8.6
Heating and Insulation engineer	5	8.6
Electrician	2	3.5
General labourer	2	3.5
Mechanical Engineer	2	3.5
Naval engineer	2	3.5
Painter/decorator	3	5.1
Para-exposure	3	5.1
Plumber	3	5.1
Miscellaneous	15	25.9
No known asbestos exposure	4	6.9
Not documented	5	8.6
Total	58	100%

Table 2-2: Occupation of exposure
Predominant occupation where exposure occurred listed by frequency

Seven (18.9%) patients in the DPT cohort had no pleural plaques on CT. Of these seven patients, 2 patients have had a previous coronary artery bypass graft and 1 patient had a history of an eosinophilic pleural effusion, which was documented as the cause of their pleural thickening. The other 4 patients had no pertinent past history such as causative drugs or previous pneumonic illnesses to explain their pleural thickening. These 4 patients' occupations were construction work, kitchen fitting, electrical wholesale and labourer (involved in cleaning pipes lagged with asbestos).

Lung function parameters with their means and standard deviations (SD) are shown in Table 2-3. The mean percentage predicted forced vital capacity (FVC%) was lower in patients with DPT and no CPA obliteration compared to control group; 83.5% vs 98.9%, respectively ($p=0.045$). The predicted FVC% were significantly lower in the unilateral and bilateral groups when compared to the control group; 79.5% ($p<0.001$) and 66.7% ($p<0.001$) respectively (Figure 4).

Similar differences were observed in the percentage predicted values for total lung capacity (TLC), between the control group 91.1%, and those with diffuse pleural thickening without CPA obliteration 77.2% ($p < 0.01$) and the bilateral DPT group 66.7% ($p<0.001$) respectively. However, this relationship was lost between the control group and the unilateral DPT group.

A scatter plot of the individual FVC and TLC percentages predicted are shown in Figures 2-3 and 2-4.

The mean FVC and TLC percentages and trends are shown in Figures 2-5 and 2-6.

Parameter	Control group n = 21	DPT but no CPA involvement n = 10	Unilateral DPT n = 15	Bilateral DPT n = 12
FEV1 (L) (mean ± SD)	2.5 ± 0.6	**2.1 ± 0.5***	**1.9 ± 0.5 ***	**1.7 ± 0.4***
FEV1 % predicted (mean ± SD)	86.9 ± 19.3	**72.0 ± 15.7***	**65.7 ± 17.7***	**57.7 ± 10.0***
FVC (L) (mean ± SD)	3.8 ± 0.7	**3.0 ± 0.9***	**3.0 ± 0.6***	**2.5 ± 0.8***
FVC % predicted (mean ± SD)	98.9 ± 15.2	**83.5 ± 25.9***	**79.5 ± 13.0***	**66.7 ± 15.6***
FEV1/FVC ratio (mean ± SD)	68.1 ± 13.1	69.9 ± 13.9	63.9 ± 14.0	64.9 ± 11.8
TLC (L) (mean ± SD)	6.2 ± 0.8	**5.0 ± 0.9***	**5.4 ± 1.3***	**4.4 ± 0.6***
TLC % predicted (mean ± SD)	91.1 ± 9.4	**77.2 ± 16.4***	82.4 ± 20.8	**66.6 ± 8.6***
TLCo % predicted (mean ± SD)	80.1 ± 23.3	**62.8 ± 15.2***	69.5 ± 17.5	**61.4 ± 11.9***
KCO % predicted (mean ± SD)	92.9 ± 24.9	96 ± 17.2	102.5 ± 22.4	104.8 ± 22.0
BMI (mean ± SD)	28.2 ± 3.6	31 ± 4.4	28.9 ± 2.8	28.5 ± 4.4

Table 2-3: Lung function parameters by DPT group

*Mean and standard deviations shown for each parameter. FEV1- forced expiratory volume in 1 second; FVC - forced vital capacity; TLC – total lung capacity; TLCo – gas transfer; KCO–diffusion coefficient. * statistically significant when compared to the control group.*

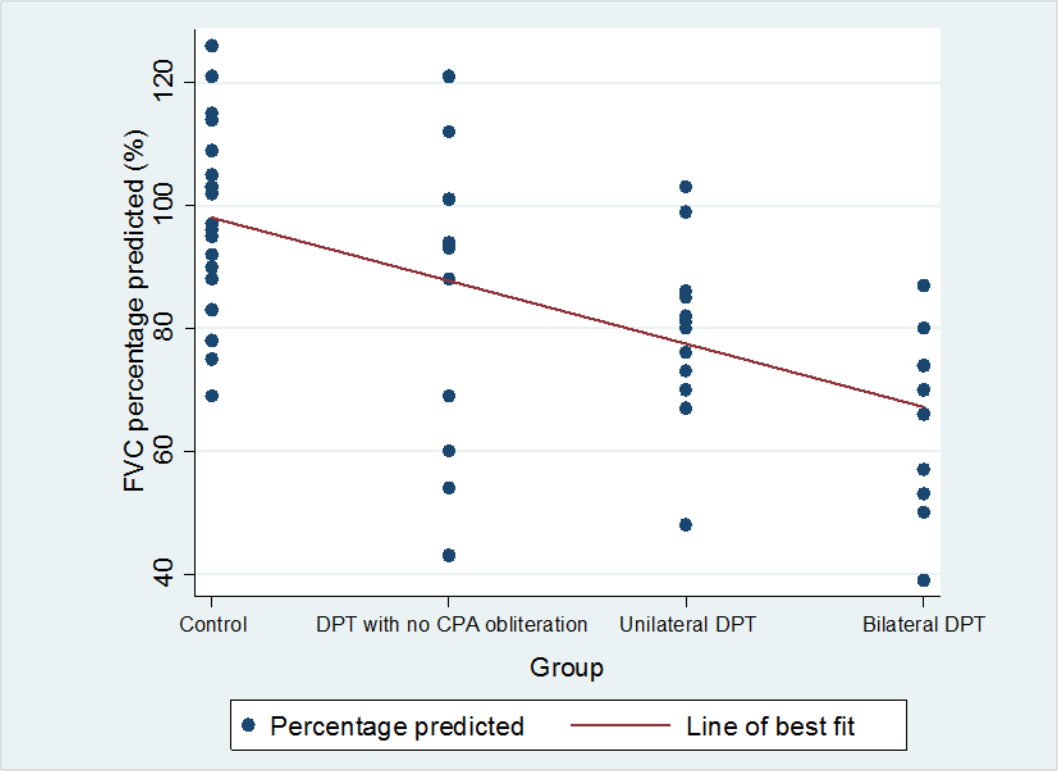

Figure 2-3: Trend of percentage predicted FVC, by group. *Individual FVCs as a percentage of their best predicted value is plotted for patients in each group. The line of best fit shows a downward trend from control group to bilateral DPT. (FVC – forced vital capacity; DPT – diffuse pleural thickening; CPA – costo-phrenic angle)*

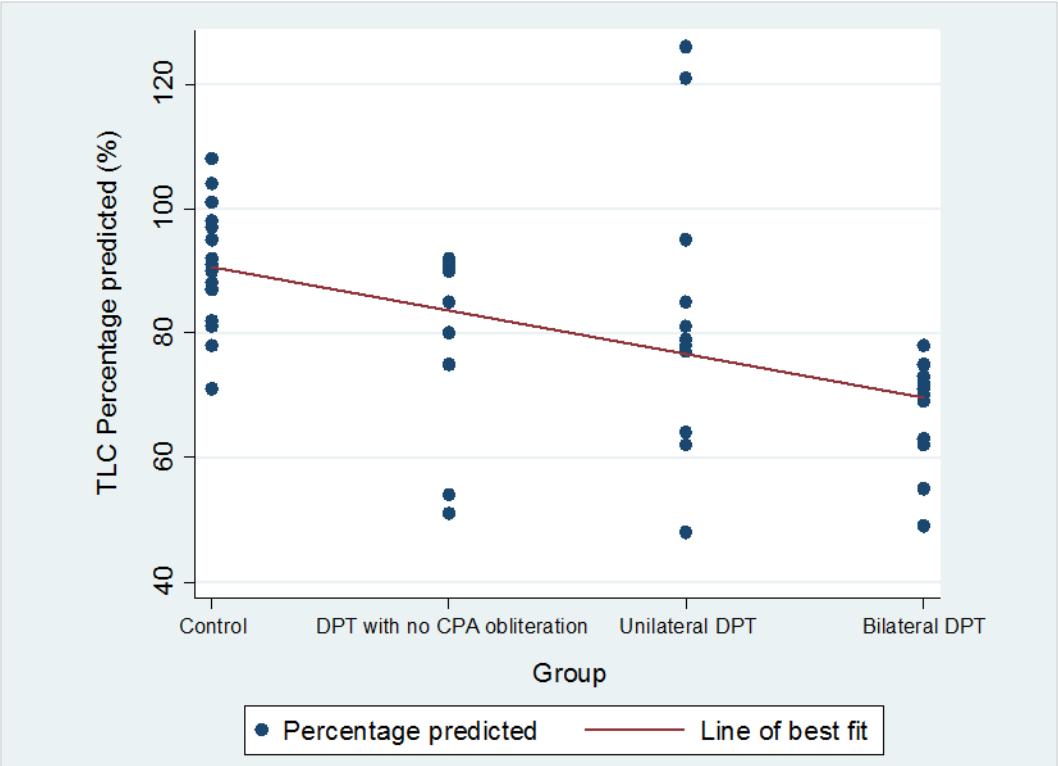

Figure 2-4: Trend of percentage predicted TLC, by group. *Individual TLC as a percentage of predicted value for individual patients in each group. Line of best fit shows a downward trend from control group to bilateral DPT group. (TLC – total lung capacity; DPT – diffuse pleural thickening; CPA – costo-phrenic angle)*

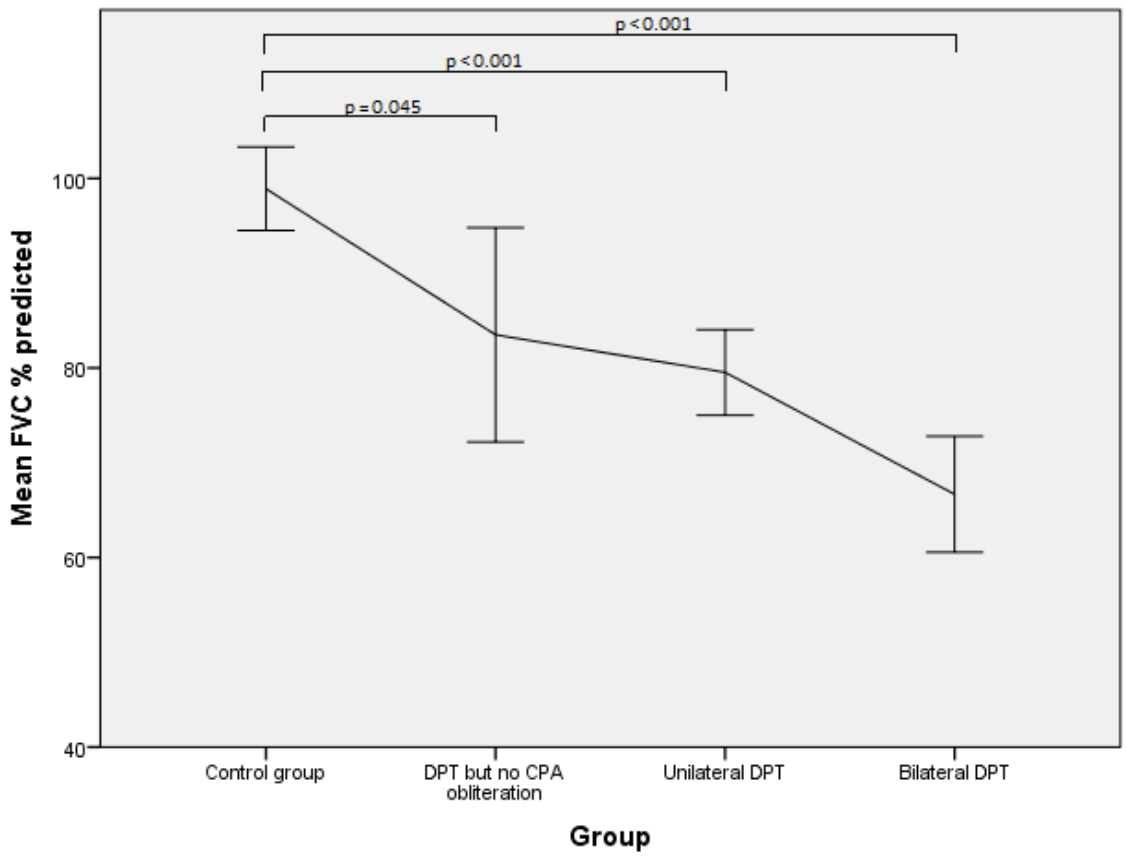

Figure 2-5: Mean FVC percentage predicted with confidence intervals (denoted by upper lower horizontal lines) and trend of FVC percentage predicted. p values show level of significance when FVC for each group is compared with the control group. *(FVC- forced vital capacity; DPT – diffuse pleural thickening; CPA – costo-phrenic angle)*

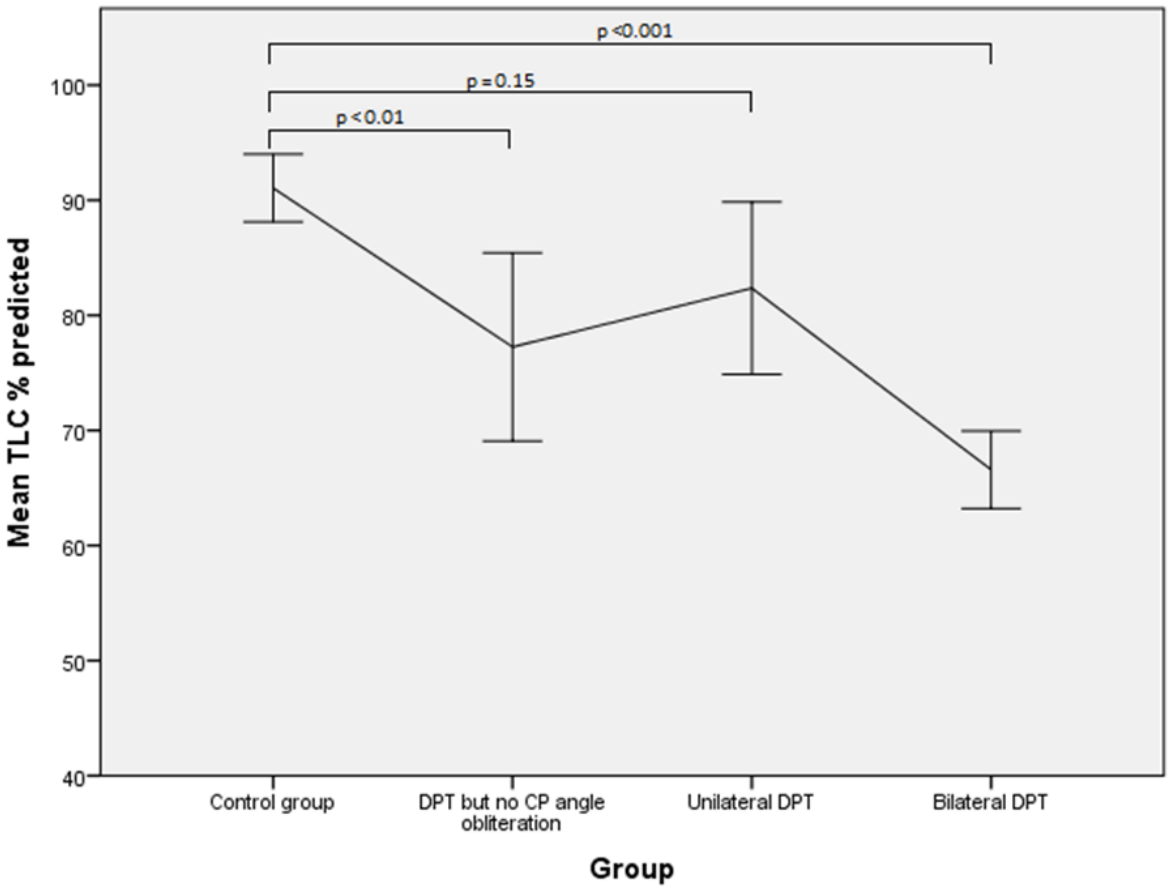

Figure 2-6: Mean TLC percentage predicted with confidence intervals (denoted by upper lower horizontal lines) and trend of TLC percentage predicted. p values show level of significance for TLC against for each group compared to the control group. *(TLC- total lung capacity; DPT – diffuse pleural thickening; CPA – costo-phrenic angle)*

2.4 Discussion

To date, this study is the first to examine the physiological impact of varied distributions of DPT using CT criteria, particularly including a large number of patients in whom there is diffuse pleural thickening without chest radiographic obliteration of the costophrenic angle. Our data highlights the incremental deficit in lung function between bilateral, unilateral and DPT without radiographic obliteration of the CPA, compared to a control group with asbestos-related pleural plaques only. We demonstrate a 33.2% reduction in the FVC percentage predicted between the control group and those in the bilateral DPT group.

A number of previous studies have investigated the respiratory impairment caused by DPT [34, 41, 132-137]. However, the reliance on a chest radiograph to make the initial diagnosis has prevented analysis of individuals in whom there is DPT without obliteration of the CPA. For instance, Singh et al. predicate their hypothesis that diffuse pleural thickening impacts upon lung function by involvement of the diaphragm based on a study of only seven patients all of whom had CPA obliteration [136]. More recently, a study by Ameille et al, designed to evaluate the impact of size-based criteria for DPT vs involvement of the costophrenic recess (n=287) identified the poor correlation of size criteria on chest radiography with lung function [131]. However, assessment of true extent using chest radiography alone is very problematic and only allows for accurate cranio-caudal extent of laterally sited pleural thickening. In this study, the authors acknowledge the superiority of CT over chest radiographs but aimed to provide data applicable to the current guidelines, which favour radiographs [11]. The relative merits of CT based size criteria over chest radiograph measurements is supported in a study by Fujimoto et al, [134] in which they found that size criteria using CT were predictive of respiratory deficit. Finally, a CT based study by Copley et al. [41], demonstrated that pulmonary function deficits are proportional to extent of diffuse pleural thickening (defined in their cohort only as pleural thickening with a tapering margin) 5 out of 50 patients had DPT without obliteration of the CPA. A further shortcoming of the chest radiograph to diagnose DPT is the inability to discriminate between other pathologies that may mimic DPT. In our study, we excluded 16 patients with pleural

effusions; six of these patients had small effusions that could have easily been interpreted as pleural thickening on the chest radiograph in the absence of contemporaneous CT imaging.

It is unclear how the arbitrary size based CT definition proposed by Lynch et al. was reached [39], however it remains widely cited in leading reviews and texts [138]. In our study, we have refined the definition in order to exclude patients with confluent pleural plaques in two ways: first, the morphology of pleural thickening was assessed. Tapered margins are a feature of diffuse pleural thickening compared to the shouldered margins of pleural plaques [41]. Second, the presence of folded lung or pleuroparenchymal bands was assessed confirming that there was fibrosis of the visceral pleura rather than thickening of the parietal pleura alone [139].

The mechanism of breathlessness in DPT remains controversial. The reduction in the lung function vital capacity has been attributed to the reduced expansion of the lower thoracic rib cage and reduced axial height of the rib cage. [136] If the extent of the pleural thickening is significant, this in itself can cause restriction in the movement of the chest wall, a 'lung en cuirasse' effect, despite the lack of extension over the diaphragm [140]. In our study, we clearly demonstrate a significant difference in the predicted FVC and the TLC in those with DPT but no CPA obliteration (Figures 2-5 and 2-6) when compared to a matched control group who have evidence of previous asbestos exposure – pleural plaques, but no other underlying pleuro-parenchymal pathology. The trend of the lung function demonstrates that patients with DPT, but no CPA obliteration sits between the control group and the unilateral DPT group.

The current definitions of DPT based on chest radiography have been a pragmatic choice reflecting world-wide availability of a low-radiation, cost-effective technique. They also reflect the high level of inter-observer agreement in defining obliteration of the CPA as opposed to size criteria on chest radiography [40, 131]. However, CT is now acknowledged as the gold standard for imaging the pleura. The Industrial Injuries Disablement Benefit scheme currently uses the ILO's classification of pneumoconiosis criteria when assessing patients' eligibility for compensation, which requires

'obliteration of the CP angle' [37]. This study highlights that there is a group of patients with DPT who do not meet the current definitions laid out by the ILO yet have significant respiratory impairment.

Our study is not without limitations; firstly, although this is the largest published series of patients with diffuse pleural thickening on CT imaging, the numbers of subjects in each subgroup remains small. Secondly, although every effort was made to match the groups for age, body mass index and cumulative smoking history we were unable to adjust for the extent of emphysema present on CT imaging. We did however manage to exclude any patients with a co-existing pleural effusion or underlying pulmonary fibrosis.

Particular care must be taken during the initial assessment of patients with new diffuse pleural thickening. Six patients (6/64, 9%) in our study who were referred as benign pleural thickening were discovered to have pleural malignancy after initial investigations (5 mesotheliomas and 1 metastatic breast cancer). Our standard operative procedure for DPT follow up is a minimum of two years with interval CT imaging at 6, 12 and 24 months. This allows us to assess any progression and exclude a developing underlying pleural malignancy. Some patients may require a confirmatory pleural biopsy to exclude underlying malignancy, particularly in the presence of concerning symptoms such as chest wall pain or weight loss at presentation, or in the presence of equivocal CT findings.

The next chapter explores if an alternative imaging modality such as MRI could be helpful in directing future management and avoiding pleural biopsy in some of these patients.

Footnote

This diffuse pleural thickening chapter is published in the British Journal of Radiology following peer review. My involvement in this study was to screen and identify patients, measuring the DPT on CTs scans and evaluating chest radiographs with Dr Edey. I conducted all the necessary analyses and authored the final manuscript which was reviewed by all co-authors of the final paper.

Chapter acknowledgements

I would like to thank the radiologists, Dr Anthony Edey and Dr Mike Darby for their assistance with the diffuse pleural thickening assessments on CT. I would also like to thank Jason Viner for his help in retrieving pulmonary function tests.

CHAPTER 3 ROLE OF MRI IN CHARACTERISING EQUIVOCAL PLEURAL THICKENING ON CT.

3.1 Introduction

Advanced pleural malignancy is well characterised on thoracic computed tomography (CT), but in early disease it may be difficult to detect and differentiate from benign pleural thickening. Usually patients under go biopsy or serial CT imaging for confirmation of malignancy. Magnetic resonance imaging (MRI) has been suggested as a problem-solving tool to aid imaging diagnosis of pleural malignancy and benefits from easy accessibility and low costs relative to PET-CT [141]. Leung et al. described the classical features of malignancy on CT as circumferential pleural thickening (PT), PT measuring more than 1 cm, nodular pleural thickening, and PT extending to the mediastinum [59]. However, in the early stages of pleural malignancy, these features can be subtle and similar to the benign PT that accompany diffuse pleural thickening [67].

Different MRI techniques have been examined in studies assessing pleural thickening suspicious for pleural malignancy [65, 69, 142]. These techniques include diffusion weighted imaging (DWI) and derived apparent diffusion coefficient (ADC) values [142, 143], simple visual assessment of the pleura to identify multiple foci of restricted diffusion appearing as high signal areas on b=1000 images on DWI images - also known 'pleural pointillism' [72], and dynamic contrast enhanced (DCE) techniques investigating contrast uptake and wash out patterns to provide information regarding tissue vasculature and likelihood of malignancy [69, 144]. None of these MRI techniques are currently in routine use in the diagnostic pathway of MPM. Often in early pleural malignancy, the radiological features described by Leung et al. may be subtle if present, warranting further investigation, frequently with pleural biopsy. The potential added value of MRI lies in cases where CT features are not diagnostic. Previous studies investigating the role of MRI in pleural disease have investigated patients with established pleural malignancy, where the diagnosis is fairly evident on CT [66, 69].

This pilot study aims to investigate the role of MRI using the techniques mentioned above to differentiate between benign and malignant pleural thickening in those with equivocal CT features

where there is a suspicion of pleural pathology, but not overtly malignant on the basis of the CT alone. In addition, the study explores different MRI assessment techniques to determine which technique would be most accurate at discriminating benign from malignant pleural thickening.

3.2 Methods:

We designed and conducted a prospective single centre pilot study of pleural MRI in patients with pleural thickening on CT. Patients were recruited from the North Bristol NHS Trust pleural clinic between April 2016 and December 2017. All patients were discussed at a dedicated pleural multi-disciplinary meeting.

Patients in whom there was diagnostic uncertainty of pleural malignancy and had one of the following features on CT were recruited to the 'indeterminate' arm of the study:

1. Smooth pleural thickening increasing over time but < 10mm in thickness
2. Subtle pleural thickening extending over the mediastinum but no other suspicious features
3. Pleural thickening < 10mm with minimal nodularity
4. CT suspicious for pleural malignancy but previous biopsies were benign

A control population comprised of both benign diffuse pleural thickening patients and malignant pleural disease patients. The benign control group either had biopsy proven benign pleural disease or had been under follow-up with no radiological progression of their pleural thickening for > 18 months. The malignant control group had biopsy proven mesothelioma.

All recruited patients had a DWI and DCE MRI scan at the same sitting, with both scans taking just over 10 minutes (see below for image acquisition details). Following the MRI scan, they continued with usual care as per clinician discretion to establish a final diagnosis, with either biopsies or interval follow-up scans.

3.2.1 MRI image acquisition

All images were acquired on a Philips Ingenia 3 Tesla MRI scanner (Philips Healthcare, Best, The Netherlands) using the manufacturer's anterior torso coil and built-in posterior coil, giving a total of

32 channels. DW-MRI was performed using a single shot spin-echo echo-planar sequence with parameters as described below in Table 3-1. To minimise motion artefact, a navigator pulse was used. This uses the position of the right hemi-diaphragm as the physiologic trigger so that image acquisition is synchronised to the respiratory cycle. DCE-MRI was performed with a T1-weighted three-dimensional echo gradient sequence with parameters described in Table 3-1. Mean examination duration for DCE-MRI scans were 3 minutes and 8 seconds. Gadolinium contrast (Gadovist) was injected (0.1mmol/kg body weight) after 5 non-contrast images, using a power injector. Scans were obtained during continuous shallow breathing.

	DWI	DCE
Sequence	Spin-echo, echo planar	Gradient echo 3D
Orientation	Axial	Axial
Scan duration (min:s)	6:18	3:08
Repetition time (ms)	7000	4.5
Echo Time (ms)	71	2.3
Flip angle	90°	10°
Field of view (mm)	390 (RL) x 312 (AP) x 220 (FH)	390 (RL) x 312 (AP) x 220 (FH)
Acquistion matrix	80 x 78	280 x 195
Reconstructed pixel spacing (mm)	1.01 x 1.01	0.61 x 0.61
Parallel imaging:	Sense: factor 2	Sense: factor 2
b values	0,50,100,750,1000	
Dynamic frame time (s)	n/a	9.2
Bandwidth (kHz/pixel)	3.345	0.687

Table 3-1:DWI and DCE image acquisition parameters. *(DWI – diffusion weighted imaging; DCE – dynamic contrast enhanced; RL – right:left; AP – Anterior:posterior; FH – foot:head)*

3.2.2 MRI analysis

The MRI scans were clinically reported by a thoracic radiologist with an interest in pleural MRI (with 13 years of experience in thoracic radiology). Data acquisition for research purposes was performed by the same radiologist, as described below.

3.2.3 MRI data acquisition

The DWI and DCE MRI scans were retrieved from the picture archive and communication system (PACS) to a 3D Synapse (FUJIFILM Medical Systems, USA) work station. Within 3D Synapse, the respiratory gated DWI images were analysed first. Areas of restricted diffusion were selected on these DWI images with b-0 and b-1000 datasets viewed simultaneously (b values are an operator selected parameter that defines the strength of the gradient and duration. No optimum value but b-0 to b-1000 are often used for assessing the pleura and other anatomical regions such as the brain). Using the free hand drawing tool, up to 3 regions of interest (ROI) were drawn around areas of restriction on the pleura, for each patient. If no regions of restricted diffusion were detected, the most significant area of pleural thickening appreciable on the MRI, was chosen and a ROI was drawn in this area. ADC values were automatically generated for the ROIs by built-in software (Fuji Synapse 3D) and saved separately as a comma separated value (CSV) file.

Using 3D Synapse the dynamic imaging scans were analysed next. The location of ROIs was matched anatomically with those drawn on the DWI data. The ROI drawn on the DWI scan was identified and a corresponding ROI was drawn on the dynamic contrast enhanced (DCE) scan using the free hand drawing tool. Fuji Synapse 3Ds built in software allows raw data to be generated for contrast enhancement over time for each region of interest. This data is then exported to Microsoft Excel (Microsoft Corporation, USA) for further analysis. Three ROIs and contrast uptake graphs are shown in Figures 3-1a and 3-1b.

A separate ROI was drawn in the aorta of all scans for standardising purposes.

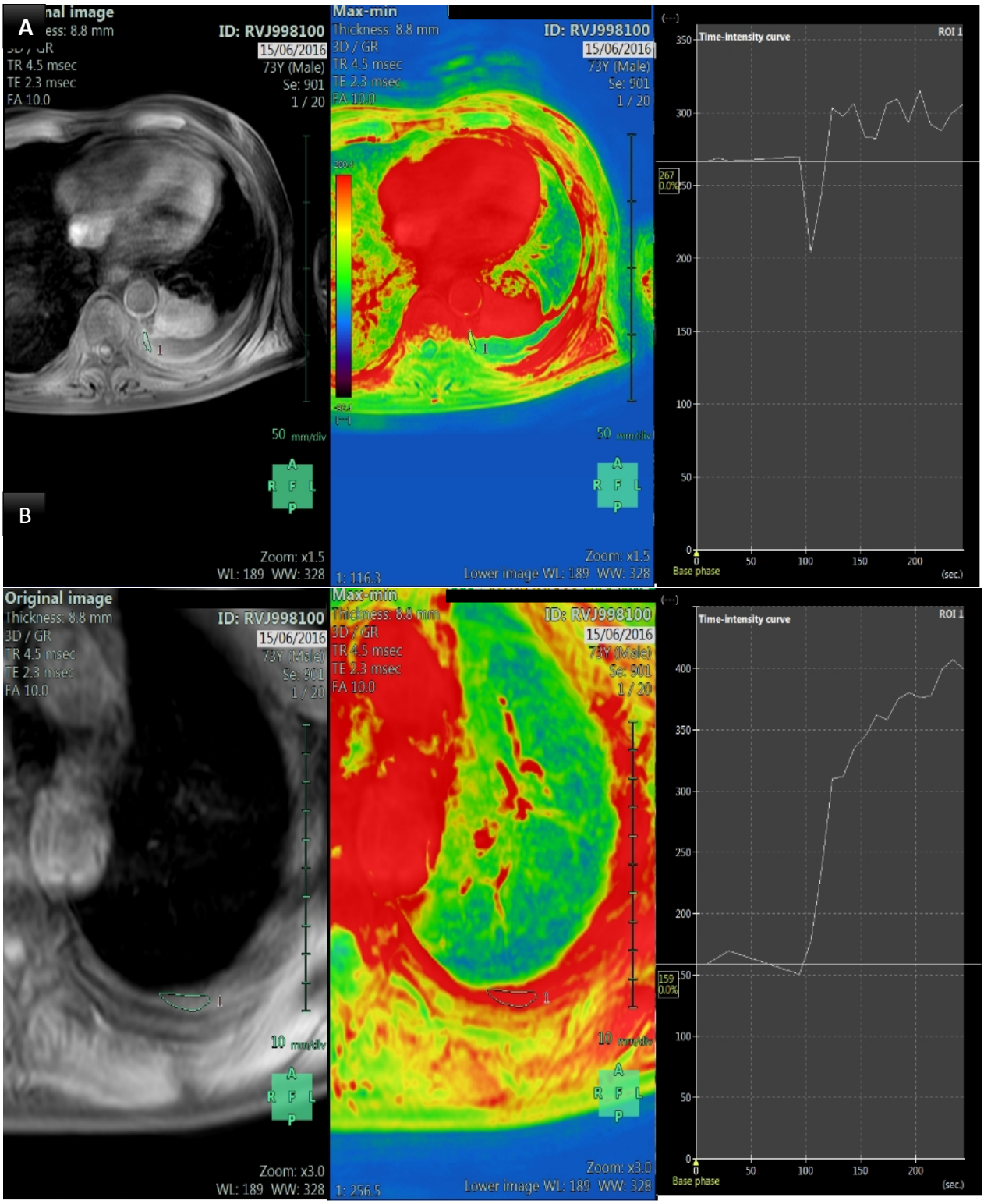

Figure 3-1a: Axial contrast enhanced fat-suppressed T1 weighted MRI images for patient M2. *Images A and B showing regions of interest drawn around the pleura with the corresponding time intensity curves.*

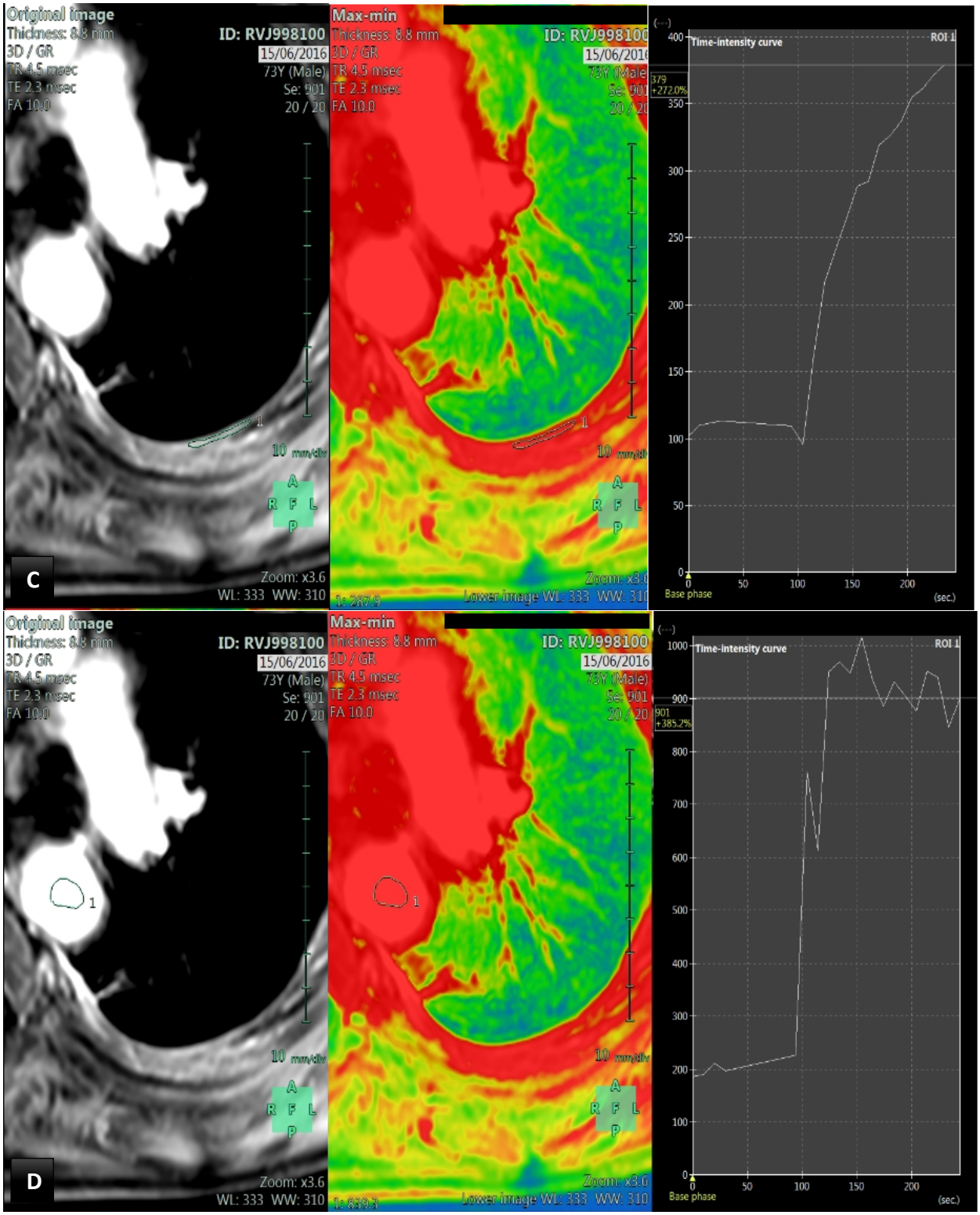

Figure 3-1b: Axial contrast enhanced fat-suppressed T1 weighted MRI images for patient M2. *Image C showing the 3rd region of interest drawn around the pleura with the corresponding time intensity curve. Image D shows the region of interest drawn in the aorta which is then used for standardising the raw data (discussed below).*

3.2.4 MRI data analysis:

3.2.4.1 ADC data acquired from DWI scans

For each ROI a minimum, maximum and a mean ADC value was generated using commercially available Fuji Synapse 3D (FUJIFILM Medical Systems, USA) software. The average of the mean ADCs for the 3 ROIs was used for analytical purposes from here on.

3.2.4.2 Pleural pointillism on DWI scans

This novel technique introduced by Coolen et al. in 2015 involves visually examining the pleura to identify areas of restricted diffusion based on review of b-0 and b-1000 images. Restricted diffusion appears as focal hyperintensities on b-1000 [72]. Pleural pointillism is present when at least 2 of these hyperintense areas are present on b-1000 images. We adopted the same technique for visual assessment of signal intensity in our patients (Figure 3-2).

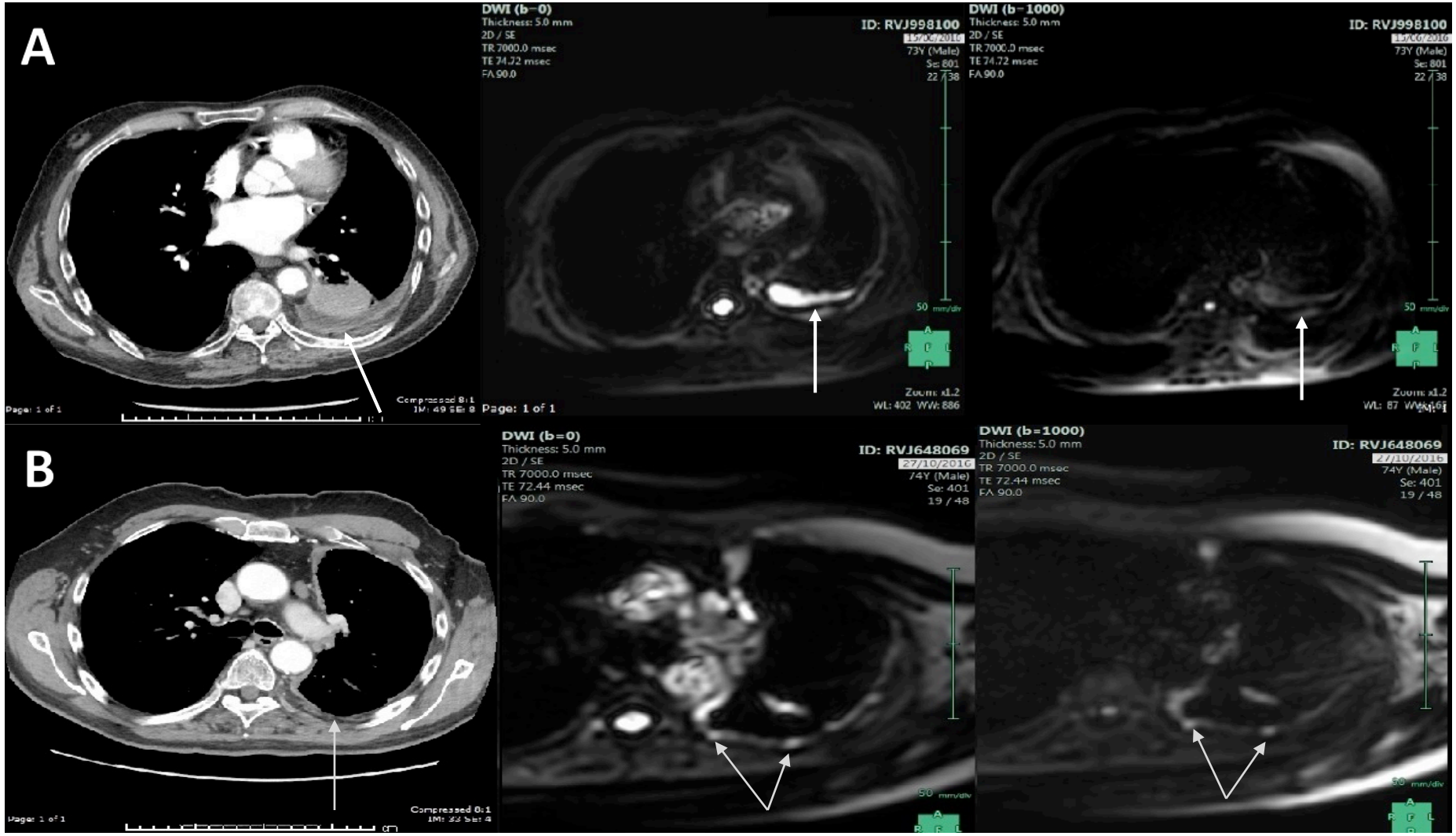

Figure 3-2: Axial CT image and corresponding DWI T2 weighted axial images on b=0 and b=1000 sequences showing pleural pointillism. Figure A shows benign pleural thickening on the right (arrow), with a small pleural effusion and folded lung above the effusion. On the corresponding b=1000 and b=0 images the pleura is seen as a dark band with no areas of hyper-intensity. Note fluid is bright on T2 weighted images hence the pleural effusion and spinal canal seen clearly on the middle image but less clearly on the b=1000 image. Figure B shows axial CT image of nodular pleural thickening which is seen as hyperintense areas on b=0 and b=1000 images.

3.2.5 DCE data preparation for analysis

An example of the exported raw data for contrast enhancement for 3 ROIs and aorta is shown in Table 3-2. The baseline was calculated by averaging the points prior to one frame length before enhancement of the curve (Figures 3-1a and 3-1b). The aorta curve was used to identify the start of enhancement due to the contrast agent. The example below shows the raw data generated from patient M2, the 3 ROIs and aorta (Table 3-2). The corresponding contrast enhancement curves over time are shown in Figure 3-3.

For analysis, 2 parameters were examined from the DCE data; the area under the curve at 60 seconds and maximum signal intensity gradient. Calculated as shown below.

Time point (seconds)	ROI1	ROI2	ROI3	Aorta
10	266.5	158.9	101.8	185.6
20	266.3	159.3	109.7	189.6
30	269.1	164.2	111.5	211
40	266.3	169.7	112.9	196.4
50	269.8	150.6	109.3	226.9
60	204.1	177.2	95.7	762.3
70	244.4	236.1	162.1	613.7
80	303.2	310.1	216.7	950.3
90	297.3	312	239.8	968.9
100	305.8	335	263.4	947.2
110	283.1	345.2	288.5	1016.1
120	282.3	361.6	291.8	935.8
130	306.2	358.2	318.9	886
140	309.4	375	326.1	932.2
150	292.8	380.4	336.4	905.8
160	315.3	376.2	355.1	877
170	292.2	377.5	361.3	951.6
180	287.5	399.3	371.7	939.5
190	299.9	407	379.3	845.1
200	305.7	399	378.9	900.5

Table 3-2:DCE data for patient ID M2. *ROI: region of interest.*

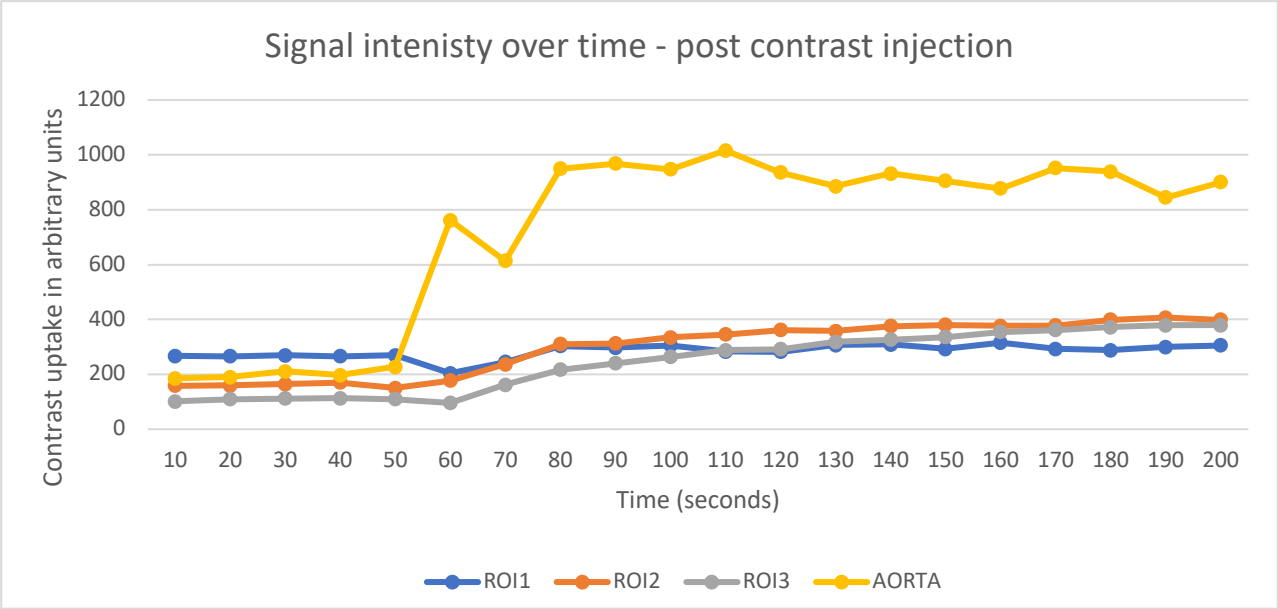

Figure 3-3*: Contrast enhancement graph for patient M2 corresponding to the values in the table above. Aorta curve shown in yellow starts to enhance at 50 seconds

The aorta curve, shown in yellow, starts rising when contrast is injected at 50 seconds. Therefore, this was chosen as the baseline. The baseline adjusted values for ROI1 are shown below, as per the method described earlier:

Average for the baseline for ROI1 = (266.5 + 266.3 + 269.1 + 266.3)/4 = 267

Adjusted against aorta for time point 5 = (269.8 – 267)/267 = 0.01

The adjusted data allows comparison of different sized ROI by giving a normalised fractional increase in signal. All time points are corrected using the same formula. Table 3-3 shows the baseline adjusted data and Figure 3-4 the adjusted enhancement curves for direct comparison.

Time (in seconds)	ROI1 (au)	ROI2 (au)	ROI3 (au)
0	0.010298	-0.07622	0.002982
10	-0.23572	0.08695	-0.12182
20	-0.08482	0.448244	0.487497
30	0.135368	0.902162	0.988529
40	0.113275	0.913817	1.200505
50	0.145104	1.0549	1.417068
60	0.060101	1.117467	1.647396
70	0.057105	1.218065	1.677678
80	0.146602	1.197209	1.926359
90	0.158585	1.300261	1.992429
100	0.096424	1.333384	2.086947
110	0.180678	1.307622	2.258546
120	0.094177	1.315596	2.315439
130	0.076577	1.449318	2.410874
140	0.123011	1.49655	2.480615
150	0.144729	1.447477	2.476944

Table 3-3: Baseline adjusted values. (ROI- region of interest; au – arbitrary units)

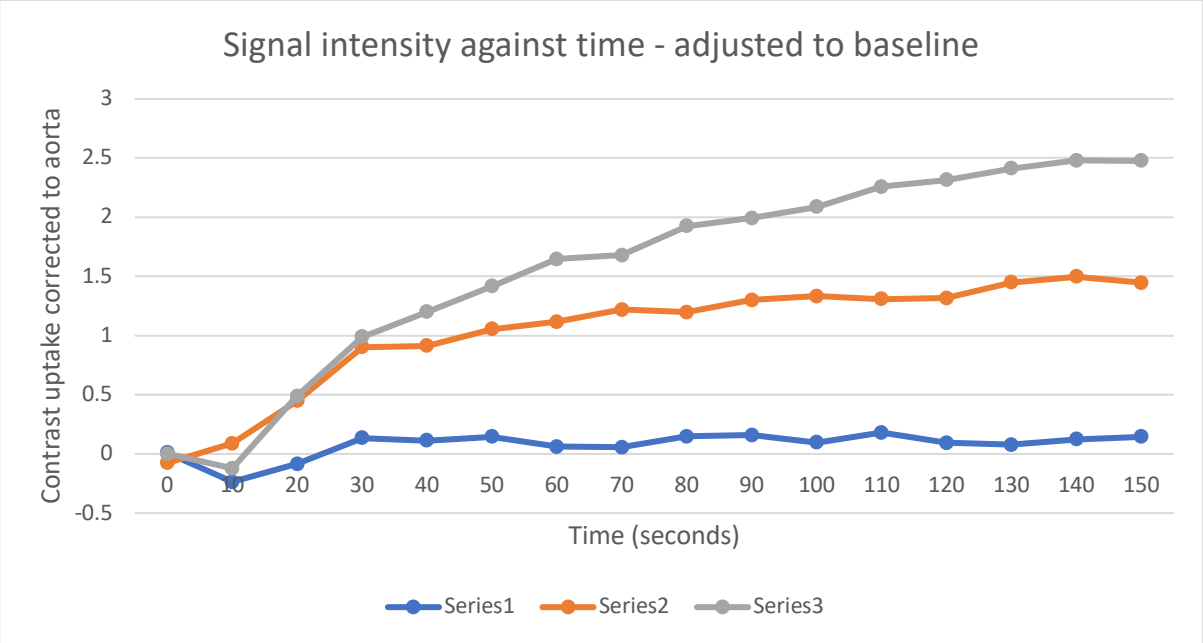

Figure 3-4: Contrast uptake corrected to the Aorta for the 3 regions of interest

The corrected values were then used to calculate the area under the curve (AUC) for the first 60 seconds of enhancement. The trapezium rule (Figure 3-5) was used to calculate the AUC at each time point, as discussed below.

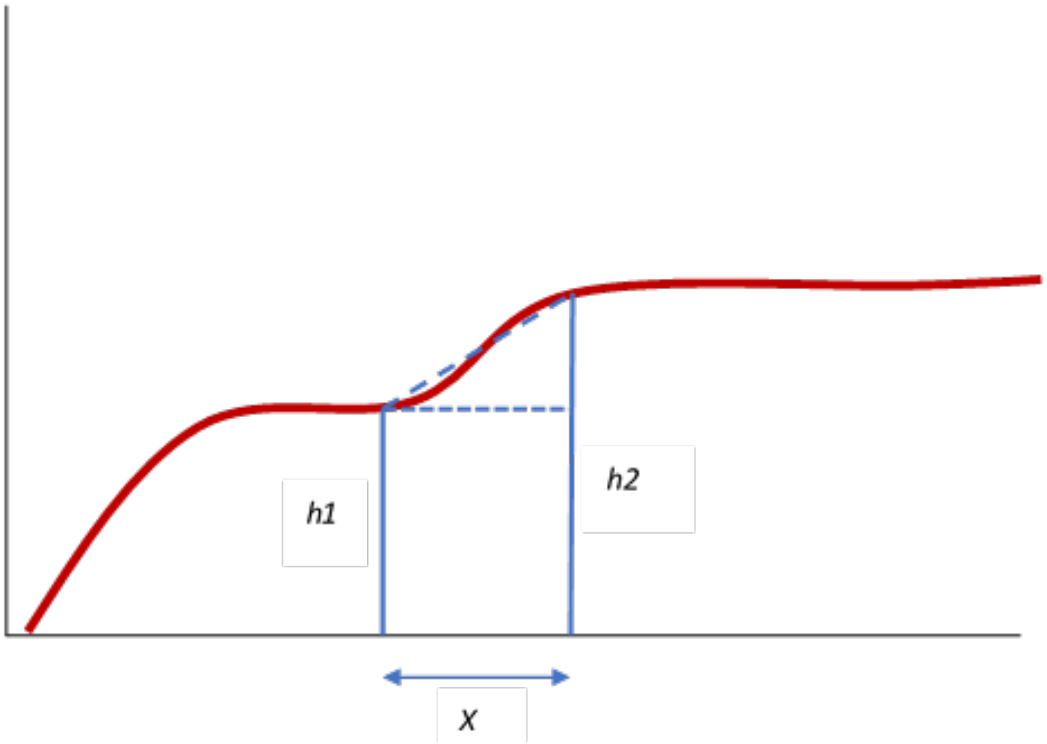

Figure 3-5: Calculation of the area under the curve using Trapezium rule

First, the area of the square (area beneath the dotted blue line) is calculated: *h1 * X*

Second, the area of the triangle is calculated*: (h2-h1)*X / 2*

Total AUC for duration *X = (h1*X) + (h2-h1)*X/2 = h1X + ½ h1X – ½ h2X =* ***½ h1X + ½ h2X***

When using the Trapezium rule we assume the curve to be a straight line for the duration X (as shown by the diagonal blue dotted line on the example).

Calculation of maximum signal intensity gradient (MSIG):

To calculate the gradient of the curve at each time point: *(h2-h1)/X*

The signal gradient is calculated for each 10 second time period for the 3 curves. The largest value corresponds to the steepest gradient of the signal intensity curve. The largest value of the 3 curves is used for subsequent analysis exploring MSIG.

Figure 3-6 shows 3 baseline adjusted graphs for a benign control, malignant control and an indeterminate group patient who was subsequently confirmed as a malignant case.

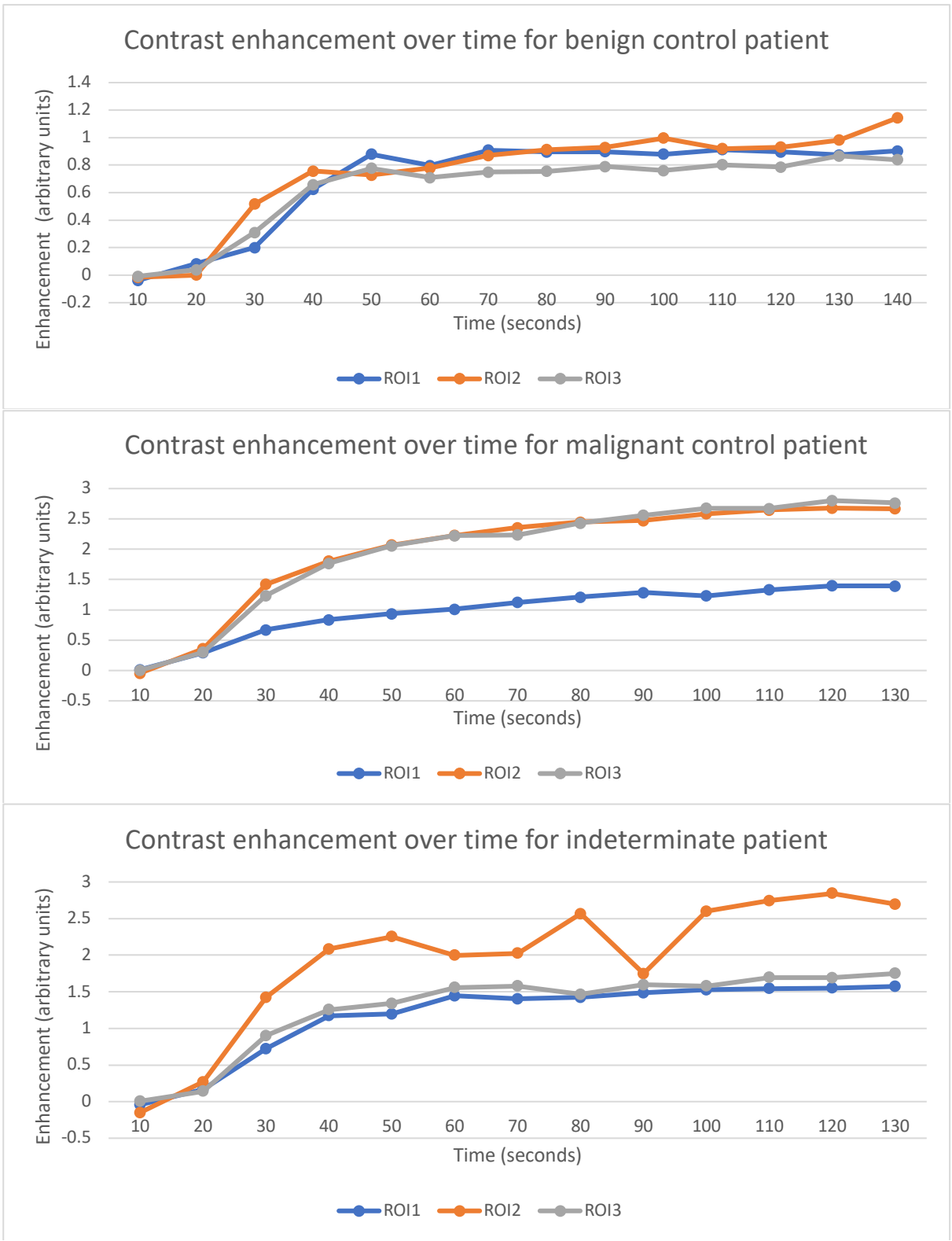

Figure 3-6: Contrast enhancement against time for benign and malignant control group patients and indeterminate group patient

3.2.6 Statistical analysis:

All statistical analyses were carried out in Stata version 15 (StataCorp, Texas, USA). As the ADC and DCE data was not normally distributed the median values with interquartile ranges (IQR) are reported. Mann-Whitney U test was used to compare the means of non-parametric data. A p value less than 0.05 was considered significant, unless otherwise specified in the text. The optimal cut-off for diagnostic tests was calculated using the Liu method [145] on Stata. Intra-observer variability was tested using Bland Altman method for continuous variables.

3.3 Results

Twenty-seven patients were recruited to the study. Two were excluded; 1 patient had the scan while the MRI protocol was being finalised, therefore the data from his scan was not used for analysis and 1 patient declined the study after recruitment. Patient demographics, asbestos exposure history and diagnoses at recruitment for the remaining 25 patients are listed in Table 3-4.

Demographics	**n = 25**
Age (mean ± SD) in years	72 ± 8
Male	23 (92%)
Asbestos Exposure history **Definite exposure** **Probable exposure** **No exposure** **Not documented**	 16 (64%) 3 (12%) 5 (20%) 1 (4 %)
Diagnosis at recruitment **Benign (control group)** **Malignant (control group)** **Indeterminate (intervention group)**	 13 (52%) 3 (12%) 9 (36%)

Table 3-4: Baseline characteristics. (SD – standard deviation)

Mean age at recruitment was 72 (±8) years. A majority of the patients were male (92%). Nine patients were recruited into the indeterminate group if they met the criteria mentioned previously. Of these 9 patients, 3 were subsequently confirmed as malignant while 6 were benign. Sixteen patients were recruited to the control arm; 13 of these with confirmed benign pleural disease and 3 with confirmed

malignant pleural mesothelioma (Figure 3-7). Two thirds of patients admitted to a history of previous definite asbestos exposure.

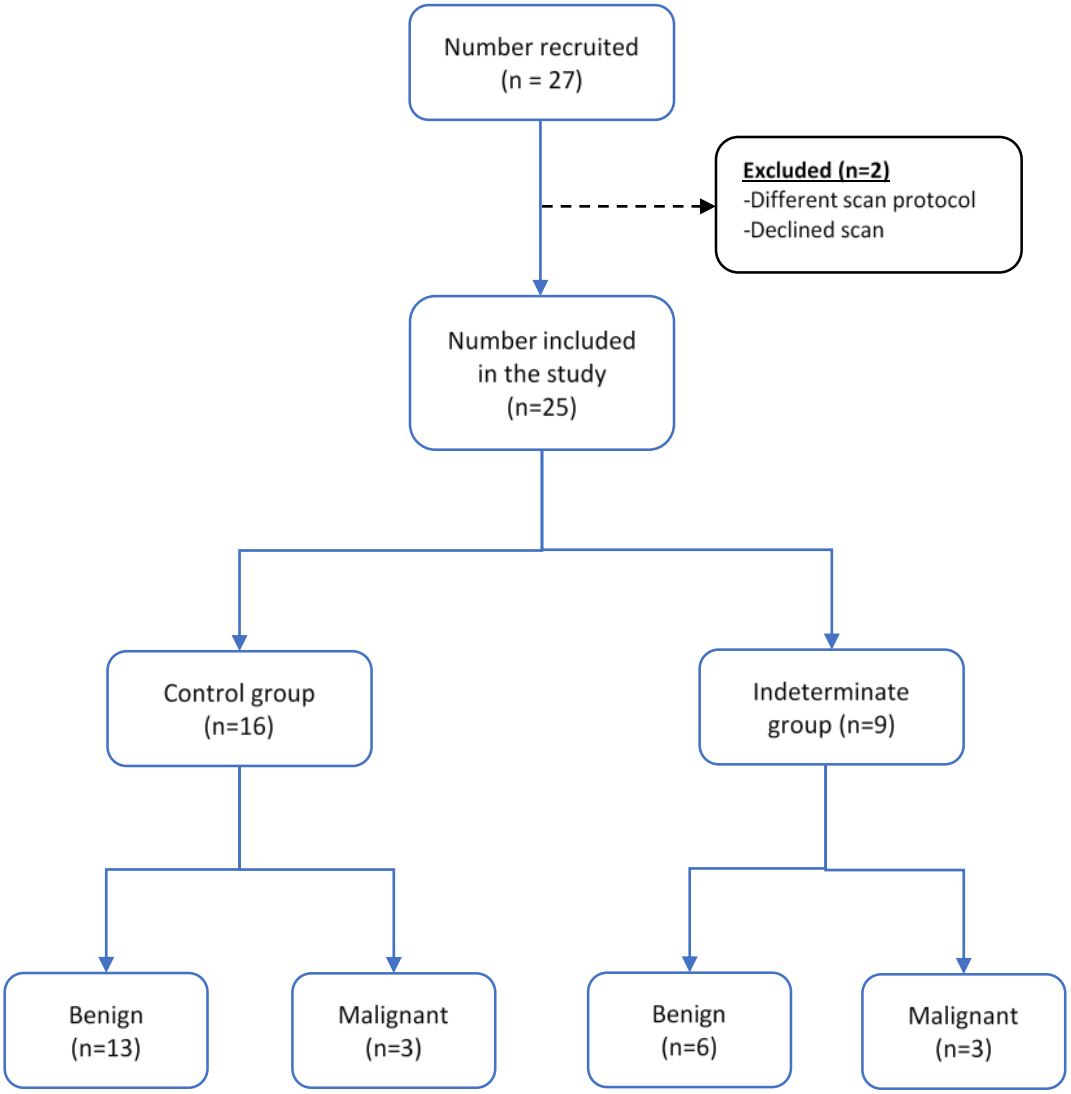

Figure 3-7: Flow chart of patients in each group

Following their MRI scan 7 patients had pleural biopsies (6 CT guided biopsies and 1 ultrasound guided biopsy) 3 of these were confirmed as malignant. Of the 2 patients who did not have biopsies 1 patient had 20 months of follow-up with interval scans with no development of a pleural malignancy. The other patient had 12 months of interval CT scan follow-up and died of an unrelated malignancy. A PET-CT scan at the time did not indicate the pleura to be involved.

Table 3-5 shows the individual data including CT features for classifying patients as ‘indeterminate’. Individual MRI parameters, presence or absence of pleural pointillism sign, and the patients’ final diagnoses are also shown in Table 3-5.

Patient ID	Morphological features	DWI data			DCE data		Pleural pointillism sign	Final diagnosis
	CT	Mean ADC (mm^2/sec)	Min ADC (mm^2/sec)	Max ADC (mm^2/sec)	AUC-60 (au)	MSIG (au)	Present or absent	Benign/malignant
2	Progressive pleural thickening with benign biopsies	1.32	0.99	1.65	7.99	0.06	Absent	Benign pleural disease
3	PT < 10mm with subtle nodularity	1.15	0.18	2.51	18.62	0.03	Absent	Benign pleural disease
4	High CT and clinical suspicion but benign biopsies	1.48	0.94	2.12	109.93	0.11	Present	Epithelioid mesothelioma
7	PT with subtle nodularity and benign biopsies	1.48	1.12	1.78	13.38	0.04	Absent	Benign pleural disease
9	PT < 10 mm with subtle nodularity	1.51	0.92	2.00	28.18	0.04	Present	Epithelioid Mesothelioma
13	PT < 10mm with subtle pleural nodularity	1.26	0.79	1.66	76.95	0.09	Present	Benign pleural disease
18	Progressive pleural thickening but < 10mm	2.10	1.55	2.76	85.77	0.14	Present	Epithelioid Mesothelioma
19	PT < 10mm with subtle pleural nodularity	2.05	1.4	2.72	77.64	0.11	Absent	Benign pleural disease
23	PT < 10mm with subtle pleural nodularity	1.81	1.09	2.48	25.39	0.17	Absent	Benign pleural disease

Table 3-5: Individual data for the patients in the indeterminate group. *(PT – Pleural thickening; DWI – diffusion weighted imaging; DCE – dynamic contrast enhanced; ADC- apparent diffusion coefficient; AUC60 - area under curve at 60 seconds; MSIG - maximum signal intensity gradient; au – arbitrary units)*

3.3.1 DWI and DCE data analysis:

For each scan, a minimum, maximum and a mean ADC was reported for each ROI giving a total of 9 ADC values per scan. The average of the 3 was used for analysis purposes. For DCE data, the mean area under the curve at 60 seconds (AUC60) and the maximum signal intensity gradient (MSIG) were used for analyses.

Using our control population, we derived an optimum cut-off for both DWI and DCE data to discriminate between benign and malignant pleural thickening. Diagnostic performance indices such as sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and overall area under the receiver operating curve (AUROC) were also derived and are shown in the Table 3-6 below. These derived cut-offs were then used in the indeterminate cohort to examine their performance.

	Mean ADC (mm^2/sec)	Min ADC (mm^2/sec)	Max ADC (mm^2/sec)	AUC at 60s	MSIG
Benign controls (n=13)	1.66 (1.5 – 1.86)	1.11 (0.95 – 1.24)	2.25 (2.03 – 2.5)	7.25 (4.26 – 26.85)	0.04 (0.02-0.05)
Malignant controls (n=3)	1.68 (1.65 – 1.80)	1.24 (1.17 – 1.26)	2.32 (2.10 – 2.52)	44.04 (16.75 – 73.61)	0.08 (0.06-0.11)
Optimal cut-off	**1.68**	**1.17**	**2.23**	**23.01**	**0.06**
Sensitivity	67%	100%	67%	67%	100%
Specificity	54%	46%	38%	77%	77%
PPV	25%	30%	20%	40%	50%
NPV	87%	100%	83%	91%	100%
AUROC	60%	80%	53%	72%	88%
Cut-off values tested in the indeterminate group (n=9)					
Sensitivity	33%	33%	33%	67%	67%
Specificity	67%	83%	50%	67%	33%
PPV	33%	50%	25%	50%	33%
NPV	67%	71%	60%	80%	67%
AUROC	50%	58%	42%	67%	50%

Table 3-6: Derivation of cut-offs for diffusion weighted and dynamic contrast values. *Using the control population an optimal cut-off was derived for the control group. Diagnostic performance at this cut-off is shown first followed by the diagnostic performance when using these cut-offs in the indeterminate group. (ADC-apparent diffusion coefficient; AUC - area under curve; MSIG - maximum signal intensity gradient; PPV – positive predictive value; NPV – negative predictive value; AUROC – area under receiver operating curve)*

3.3.2 Pleural pointillism:

For our indeterminate group, MRI visual assessment using pleural pointillism alone had a sensitivity of 100%, specificity of 83%, PPV 75% and NPV of 100% with AUROC 91% (Table 3-7).

	Pointillism present	Pointillism absent	Total
Malignant	3	0	3
Benign	1	5	6
Total	4	5	9

Table 3-7: Diagnostic performance of pleural pointillism in the indeterminate cohort

When considering all benign and malignant diagnoses in our cohort of patients (control and indeterminate group together), there was no difference in the median ADC values. The benign group had a median ADC value of 1.62 mm^2/sec (IQR 1.46 – 1.83 mm^2/sec) while the malignant group had a median ADC of 1.66 mm^2/sec (IQR 1.51 – 1.80 mm^2/sec) p=0.51 (Table 3-8).

	Benign (n=19)	Malignant (n=6)	p value
Median mean ADC (mm^2/sec) (IQR)	1.62 (1.46 – 1.83)	1.66 (1.51 – 1.80)	p = 0.51
Median min ADC (mm^2/sec) (IQR)	1.08 (0.87 – 1.24)	1.21 (0.94 – 1.26)	p = 0.44
Median max ADC (mm^2/sec) (IQR)	2.25 (1.87 – 2.51)	2.22 (2.10 – 2.52)	p = 0.77
AUC60 (IQR)	9.64 (4.91 – 30.30)	58.82 (23.01 - 77.4)	**p = 0.008***
MSIG (IQR)	0.05 (0.02 – 0.09)	0.09 (0.06 – 0.11)	p = 0.07

Table 3-8: Diffusion weighted, and dynamic contrast enhanced data separated by disease cohort. *(ADC - apparent diffusion coefficient; AUC - area under curve; MSIG - maximum signal intensity gradient)*

Figure 3-8 is a Box-and-Whisker plot of the mean ADC values for the benign and malignant groups. The central line of the box denotes the median ADC for each group. As shown by the outliers, the median ADC values for the benign group was widely spread.

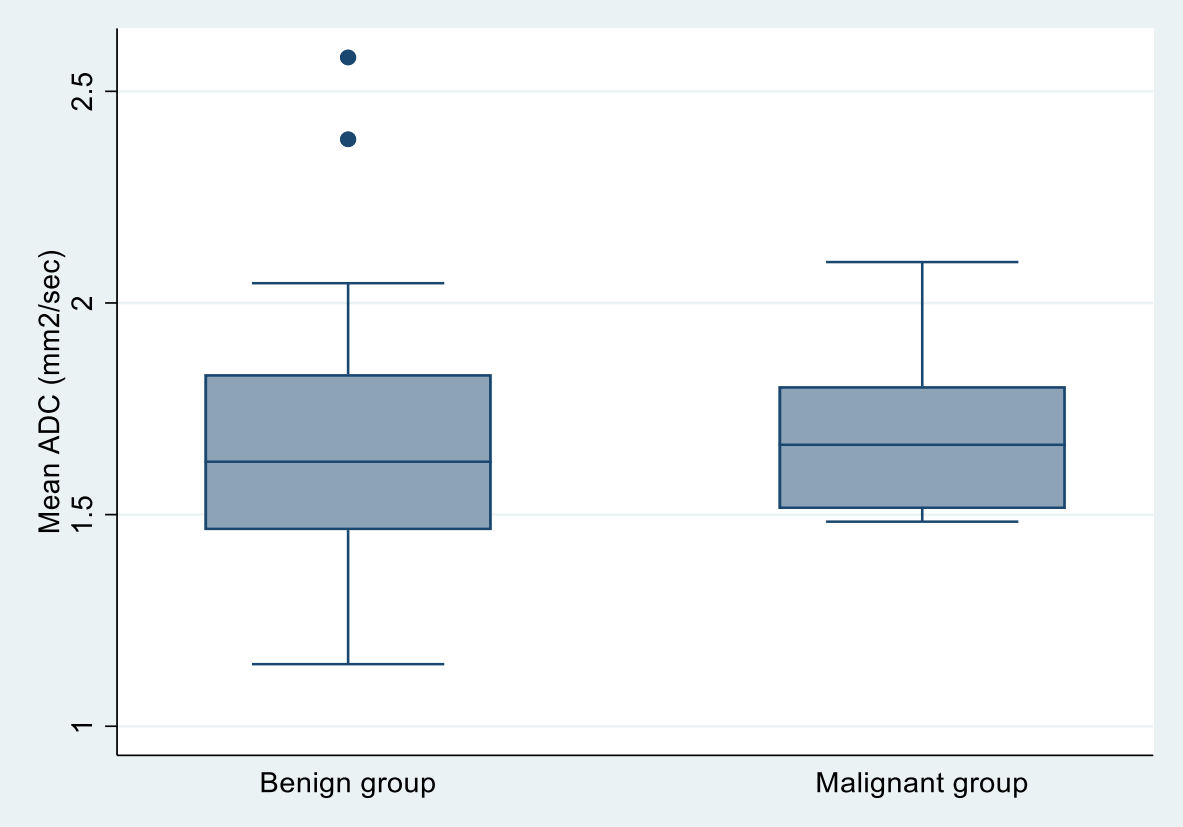

Figure 3-8: Box-and-whisker distribution of the median values for the mead ADCs in the benign and malignant groups. The horizontal line in the centre of the box depicts the median, the upper and lower margins of the box shows the 75th and 25th quartiles. The horizontal lines of the whiskers show the range, highest to lowest values. Note 2 outliers shown separately for the benign group as 2 solid circles.

Coolen et al., have previously published data proposing an ADC value of 1.52 mm^2/sec to be an optimum cut-off for discriminating benign from malignant pleural thickening [70]. Applying this cut-off in our cohort we obtained a sensitivity of 83%, specificity of 37%, PPV of 29%, NPV 87.5% and accuracy of 48% for our study population.

3.3.3 Intra-observer variability

To check for intra-observer variability the same method described above was used to draw ROIs on 3 patients, 1 each from the benign control, malignant control and indeterminate groups. The overall correlation between ROIs was moderate at r = 0.65. Interestingly, the correlation between the benign and malignant patients was r =0.974 and r=0.855, respectively. For the patient in the indeterminate group this dropped to r=0.122.

3.4 Discussion

This is the first MRI pilot study to incorporate functional MRI assessment methods in equivocal pleural thickening on CT. Our results suggest that visual assessment using pleural pointillism is the most robust method for assessing early malignant pleural disease on MRI. Our study is pragmatic and was specifically aimed at identifying those with equivocal signs on CT rather than established malignant pleural thickening, as this is the group that usually presents a clinical conundrum. Patients may go on to have unnecessary biopsies or multiple interval CT scans until a definitive diagnosis is made.

MRI as an imaging modality has certain advantages over CT and PET scans. It is free of ionising radiation, relatively easily accessible and well tolerated. MRI is also significantly superior at soft tissue delineation and spatial resolution [65, 146]. For these reasons MRI has been under investigation for over 2 decades by researchers with an interest in pleural disease [147]. Various quantitative and non-quantitative methods have been investigated as discussed earlier.

Using the DWI data in our control population, we determined an optimal cut-off for the mean ADC at 1.68 mm^2/sec for differentiating benign from malignant pleural thickening in our cohort. This was then validated in our indeterminate group. With a sensitivity of 33%, specificity of 67%, PPV of 25% and NPV of 87.5% this derived cut-off value did not perform well in the indeterminate group. Exploring the minimum and maximum ADC values using derived cut-offs in the indeterminate cohort, which again did not prove to be useful. Similar results were observed in a study by Inan et al with 34 patients where the overlap in the ADC values between benign and malignant groups prevented them from identifying a discriminative cut-off [142].

Studies investigating DWI techniques have reported mean ADC values ranging from 0.99 to 2.0, as potential cut-offs for discriminating benign from malignant pleural thickening [66, 70] however, these absolute numbers are unlikely to be transferable between studies due to inter scanner and software variability. As discussed in the introduction chapter, the ADC is derived from the signal loss associated with thermal motion of water molecules with the switching of the magnetic field [148]. The diffusivity of water molecules is dependent on the cellularity of the tissue and the integrity of the cell membrane.

MPM is a heterogenous malignancy, therefore the cut-off values may vary from study to study depending on the cohort under investigation. In the study by Gill et al [66] they found a mean ADC value of 1.31 mm^2/sec for their epithelioid patients and 0.99 mm^2/sec for the sarcomatoid patients. In the current cohort the median ADC value for all epithelioid patients (n=5) was 1.64 mm^2/sec while the only sarcomatoid patient had an ADC of 1.80 mm^2/sec. Our sample size was too small to confirm or refute findings from previous literature reporting lower ADC values for sarcomatoid and biphasic sub-types (1 sarcomatoid and no biphasic patients) in the current study. Coolen et al., reported a mean ADC value of 1.52 mm^2/sec to be an optimum cut-off for discriminating malignant pleural thickening, with a 71% sensitivity and 100% specificity [70]. Using this cut-off, we were able to demonstrate a better sensitivity than they reported at 83%, but worse specificity at 37% and an overall accuracy of only 48%.

Our DCE data performed better than DWI data in keeping with previously published literature [70, 144]. Using the derived cut-off 23.01au, patients with malignancy in the indeterminate group were identified with a 67% sensitivity and specificity, 50% PPV and 80% NPV. Furthermore, when exploring the full cohort separated by disease status, benign or malignant, there was a statistically significant difference in the mean AUC60 between the 2 groups; 9.64au versus 58.82au (p=0.008), respectively. The higher AUC60 and MSIG are in keeping with what would be expected in malignant tissue; increased neovascularisation leading to rapid contrast uptake in tumour tissue. Other studies using DCE-MRI report similar results for early contrast enhancement. Tsim et al., recently published a study with 58 patients undergoing DCE-MRI looking at early contrast enhancement, signal intensity peaking at or before 4.5 minutes as a marker of malignancy [144]. They reported an 83% sensitivity and specificity using this method alone. When combined with MRI morphology the sensitivity increased to 92% while the specificity dropped slightly to 78%. In the Tsim et al. study 15 ROIs were specified on each scan, increasing the accuracy of the data obtained. In the current study only 3 ROIs were specified on each scan and this may have been a limitation leading to false negative results. The higher the

number of ROIs the more accurate the data derived is likely to be. However, this is a very laborious task and unlikely to be practical in routine clinical use.

The Tsim et al. study used scan duration of 4.5 minutes as the cut-off following their pilot investigations and confirmation that malignant patients would reach a peak signal intensity post-gadolinium contrast injection, at or before this time point. Mean total scan duration in their study was 8 minutes while the mean scan duration for our patients was 3 minutes and 8 seconds. Tsim et al also showed correlation between micro vessel density in histopathological samples and MSIG in their study, in keeping with the hypothesis that the increased vasculature leads to early contrast enhancement [144]. Similar to our data there was significant variation in the contrast enhancement curves between ROIs (Table 1-2 difference between ROI1 and ROI3).

The most striking results in our study is the simple yet accurate visual assessment of the pleura using pleural pointillism on MRI. In our indeterminate cohort pleural pointillism had a sensitivity of 100%, specificity of 83%, positive and negative predictive values of 75% and 100%, respectively. However, larger prospective studies are required for validation before this technique can be recommended in the routine diagnostic pathway of patients with suspicious pleural thickening and equivocal CT findings.

Practicality, patient tolerability, efficiency and cost are some of the key factors to consider when adopting an imaging modality for routine clinical use. Although a number of small studies have shown encouraging results with the use of functional DCE-MRI, adopting this in routine clinical practise may not be very feasible. Drawing regions of interest around subtle areas of pleural thickening is time consuming with potential to inter and intra observer variability [143]. Justification of a contrast enhanced scan is difficult, when the non-contrast pleural pointillism on DWI is superior to DCE-MRI, as shown here. However, with advancing technology/software, improved algorithms/magnetic fields and artificial intelligence there maybe scope for improvement of this technique in the future. Furthermore, when comparing to other imaging modalities such as PET-CT which has been extensively investigated for diagnosing pleural malignancy [76], DWI-MRI certainly has several advantages. A DWI-

MRI scan takes just over 3 minutes, as opposed to a PET scan that can take up to 20 minutes (after a 60-minute sedentary wait following the injection of the 18-FDG). With PET scans patients should fast for at least 6 hours prior to the scan and those with diabetes should have good glycaemic control. Furthermore, with low volume tumour (early pleural malignancy) and tumours with low proliferative indices such as early epithelioid mesothelioma, the false negative rate tends to be higher with PET-CT [76].

Our study has a number of limitations. Our study population was small, particularly the number in the indeterminate cohort. However, this is a reflection of the incidence of such cases. They are rare, but when present, diagnosing these patients can be challenging. Our control population of confirmed benign (n=13) and malignant (n=3) which served as the discovery cohort was also small. A larger discovery population is likely to give more accurate cut-off data, particularly considering the heterogeneity of MPM. By opening more sites, we could have increased our population, but this would have weakened the strength of the study due to using different scanners. Competing diagnostic studies such as the TARGET trial (next chapter) meant a very small number of patients were preferentially entered to TARGET, which is unfortunately another weakness of the study as not all consecutive patients were entered.

One of the weaknesses of the study is the number of ROIs drawn on the scans. For practicality and eventually ease of clinical utility, only 3 ROI were drawn where restricted diffusion was evident on the DWI MRI scan. Due to the heterogeneity of the disease these areas of restricted diffusion were quite subtle and subject to significant intra-observer variability that could have led to false negative results. If the number ROI were higher as in the Tsim et al. study, maybe a stronger signal would have emerged. However, as mentioned earlier drawing ROIs and transposing these to the DCE scans is not practical in routine clinical application. Hence a more acceptable compromise of 3 ROIs was used in this study. Furthermore, the ROIs were drawn in conjunction by a radiologist and me, a non-radiologist. Perhaps the accuracy of ROI would have been higher had 2 thoracic radiologists with experience in MRI,

performed this task. Ideally, all ROI should have been drawn twice independently by the investigators and assessed for inter and intra observer variability to strengthen the findings.

Another limitation of our study is the duration of the DCE scan. The DCE scan was only 3 minutes in duration, this was mainly for patient tolerability. However, this meant we did not obtain any wash-out data for the dynamic studies which may have given additional information that could be useful.

Despite these limitations, we have demonstrated that whilst there may be a slight advantage to using functional MRI for assessment of early suspicious pleural thickening, overall, visual assessment using pleural pointillism may be superior, without the need for a time-consuming process to quantify DCE data. Pleural pointillism using DWI MRI may have a role in supporting a diagnosis of pleural malignancy, but on its own it cannot be used as confirmatory investigations without further validation in larger prospective trials. The role of MRI in pleural disease may lie with assessment of soft tissue invasion (such as through diaphragm and chest wall) [149] if this is likely to have an impact on management, for example upstaging T stage of MPM which would preclude a patient from having surgery [150]. However, in its current state functional MRI does not have an additional role in the diagnostic pathway of patients with equivocal pleural thickening on CT.

Chapter acknowledgements:

I would like to thank Dr Edey for his help with the radiology assessments and the hours spent drawing regions of interest around tumours with me. I would also like to thank Dr Claire Doody, medical physicist who helped with finalising the scan protocols and acquiring data from the MRI scans.

CHAPTER 4 A RANDOMISED CONTROLLED TRIAL TO COMPARE THE DIAGNOSTIC YIELD OF POSITRON EMISSION TOMOGRAPHY COMPUTED TOMOGRAPHY (PET-CT) TARGETED PLEURAL BIOPSY VERSUS CT-GUIDED PLEURAL BIOPSY IN SUSPECTED PLEURAL MALIGNANCY. (TARGET TRIAL)

4.1 Introduction

Patients with high a clinico-radiological suspicion of pleural malignancy usually proceed to a pleural biopsy, either radiologically or thoracoscopically. In a proportion of patients these biopsies maybe non-diagnostic, requiring a further biopsy to confirm underlying malignancy. The yield of repeated CT guided biopsies is low; local audit data from our trust indicated that only 3 out of 15 (20%) repeat pleural biopsies for suspected pleural malignancy (all later confirmed to be cancer) were positive. This compares to a yield of 4 of 6 patients (66%) in the arm that underwent PET-CT with the pleural biopsy targeting the area of highest metabolic activity on the scan. This highlights a potential role for PET-CT in this group of patients, which warrants further investigation.

The TARGET trial was designed to investigate this role of PET-CT in pleural malignancy, for patients with a non-diagnostic first biopsy. The study idea was conceived by Professor Nick Maskell and was later refined by me in the process of applying for funding. Several funding options were explored for the proposed multicentre randomised controlled trial (RCT). Two grant applications were submitted to the British Lung Foundation (BLF) and the National Institute for Health Research (NIHR) research for patient benefit (RfPB) funding streams, simultaneously. A condensed version of the NIHR grant application can be found in Appendix I.

Much work was undertaken to ensure appropriate support was in place to design and conduct this trial. Potential recruitment centres from across the UK were contacted at an early stage to ensure their willingness to participate in the trial. In addition, the local research & innovation (R&I) department, research design service (RDS) and the clinical trials & evaluation unit (CTEU) in Bristol

were involved from the early design stages of the trial. The Bristol CTEU provided statistical, database and administrative support for the duration of the trial. The proposed study plan was presented to a mesothelioma focus group for their input from a patient and public perspective. This proved very useful and their feedback had an impact on the final design of the final study.

Both grant applications were successful but BLF later withdrew their grant (of £50,000) as NIHR was able to support the trial fully with £344,811.

The trial protocol and other trial related documents necessary for the running of the trial, such as the patient information leaflet (PIL), consent forms, and case record forms (CRF) were developed by me. The clinical input necessary for setting-up and running the trial was provided by me, in the role of the trial coordinator. In addition, the necessary regulatory approvals such as research ethics committee (REC) review and administration of radioactive substances advisory committee (ARSAC) certification were all applied for by myself and subsequently presented along with Professor Maskell, to the South West Research Ethics Committee (REC) in Exeter. Following a few minor amendments REC approval was granted on the 15th of July 2015. A CTEU appointed trial manager took charge of the day-to-day administrative duties of the trial from here on.

4.2 Methods

4.2.1 Study objective

The TARGET trial is a UK based multicentre parallel group randomised controlled trial, aiming to evaluate whether a PET-CT targeted CT guided biopsy is superior to a standard CT guided biopsy in patients with suspected pleural malignancy who have undergone one non-diagnostic biopsy.

4.2.2 Study outcomes

The primary outcome of the study is 'pleural malignancy correctly identified on the second biopsy'.

The primary aim of the study was to determine the sensitivity and specificity of the PET-CT targeted second biopsy as a part of the trial. Patients were followed up for 12 months or to the end of the trial - if recruited in the last 6 months of the recruitment period. Patients with a non-diagnostic biopsy in the trial may have had further biopsies during this time via other means, which may confirm the diagnosis. Some patients may be given a clinico-radiological diagnosis of pleural malignancy due to characteristic progressive features on subsequent radiology. All suspected cancer diagnoses were discussed in a mesothelioma or lung cancer multi-disciplinary team (MDT) meeting and members were in consensus of the diagnosis, prior to classifying the disease as malignancy.

The secondary outcomes of the study were as below:

- Total number of invasive procedures (video-assisted thoracic surgery –VATS- or radiology guided biopsies) undertaken following randomisation to confirm the diagnosis
- Time from randomisation to cancer diagnosis (those not diagnosed with cancer to be censored at last follow-up)
- Time from randomisation to death (survivors to be censored at last follow-up)
- Total number of hospital attendances following randomisation to confirm the diagnosis
- Procedure related adverse events
- Uptake of chemotherapy following a positive diagnosis, in the 12 months following recruitment.

- Serum mesothelin levels measured at baseline, 6 and 12-month follow-up visits for those followed up for 12 months
- PET scan parameters - Total Glycolytic Volume (TGV), maximum and mean standard uptake value (SUV) (PET-CT group only)
- Estimated costs associated with health-related resource use from randomisation to diagnosis

At the conclusion of the study, biopsies and radiology results are to be reviewed by an independent adjudication committee. The committee will be blinded to the results.

4.2.3 Participant identification

Patients were identified via the local lung cancer and mesothelioma MDTs. Patients suspected of pleural malignancy usually have a biopsy and are discussed at the MDT meeting. Therefore, patients with a non-diagnostic biopsy would be identified and screened through the MDT.

4.2.4 Pre-screening, screening and recruitment

Eligible potential patients were given a patient information leaflet. Provided they were happy to participate in the trial, they were asked to consent to the trial and recruited. Following a baseline assessment gathering data on their demographics, investigations to date, they were randomised either to the standard arm of the trial, or a PET-CT scan followed by a CT guided biopsy to an area identified on the PET-CT scan.

4.2.5 Eligibility criteria

4.2.5.1 Inclusion criteria:

Patients were eligible if they met all the criteria below:

- Pleural thickening on CT suspicious for pleural malignancy
- Have had any form of pleural biopsy in the last 12 months (either by thoracoscopy or under radiological guidance) which was non-diagnostic for cancer

- Lung Cancer/mesothelioma MDT decision to perform a further CT-guided biopsy to pursue a diagnosis

4.2.5.2 Exclusion criteria

Patients did not enter the study if they met any of the criteria below:

- Unsuitable for a CT guided biopsy – inability to co-operate, lie still for the duration of the biopsy, uncorrectable coagulopathy, inability to tolerate a pneumothorax, severe underlying lung disease (patients with an FEV1 < 35% assessed using simple spirometry)
- Unable to give written informed consent
- Pregnancy or lactation
- Age <18 years
- Pleural thickening not amenable to a radiologically guided biopsy
- Talc pleurodesis in the previous 6 months

4.2.6 Randomisation and blinding procedures:

Patients were allocated on a 1:1 basis to either the intervention (PET-CT prior to CT guided biopsy) or comparator (CT guided biopsy only) arm. The allocation was blocked using varying block sizes and stratified according to enrolling centre. Only authorised personnel were given access to randomise patients and access was password protected.

Concealed randomisation ensured selection bias excluded. The sequence of random allocations were generated by computer and was concealed from all clinical and research personnel until a participant was recruited.

Due to the nature of the investigations performed, neither participants nor investigators were blinded to allocation.

4.2.7 Research Procedures:

All patients had a baseline assessment at the time of their recruitment to the trial. Patients also had blood tests which included a full blood count, urea and electrolytes a clotting screen and a trial specific

blood test Mesothelin at their baseline assessment. All Mesothelin blood tests were analysed at the lead centre NBT. Other centres were asked to send their samples securely to the NBT laboratory.

Patients had simple spirometry at their baseline assessment to ensure they were able to tolerate a pneumothorax in the unlikely event this was a complication of the CT guided biopsy. Patients were randomised at the end of their baseline assessment.

Those randomised to the PET-CT arm had the PET-CT scan followed by a CT guided biopsy, within a 2-week period from randomisation. Patients in the standard arm went straight to a CT guided biopsy (Figure 4-1).

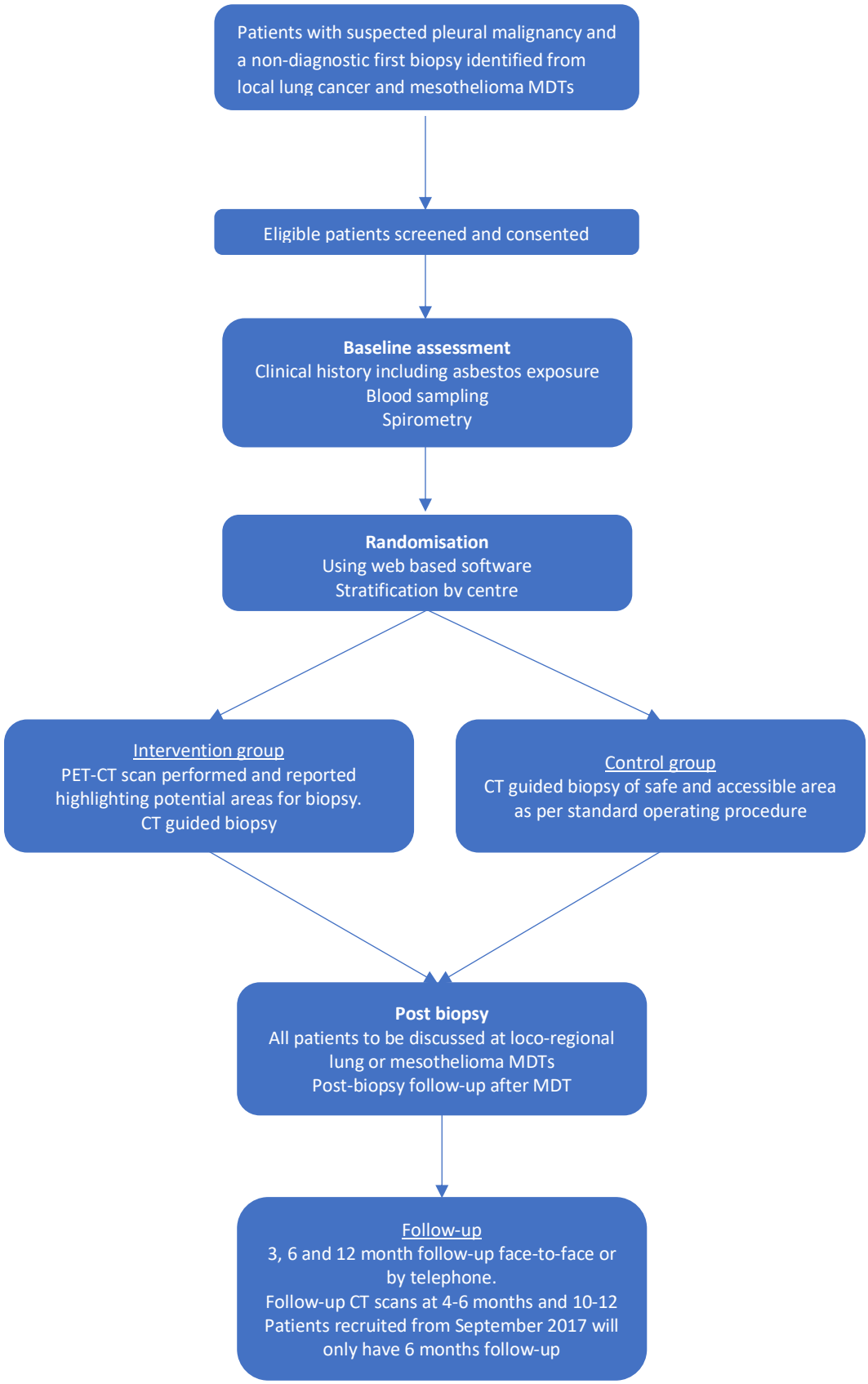

Figure 4-1: Trial flow chart

The Mesothelin blood test was a trial specific blood test, and was performed at baseline, 6 and 12-month follow-up visits on patients who were able to attend these follow-up visits.

Patients also had a CT scan between 4-6 months and another at 10-12 months. This is of particular importance for those with a non-diagnostic biopsy as a part of the trial, as the expectation was an underlying malignancy that was yet to be diagnosed would become apparent during this follow-up period.

4.2.8 Follow-up visits

The first follow-up visit was scheduled for when participants attended clinic for their biopsy results. This was usually 1-2 weeks after their biopsy. Adverse events relating to the PET-CT scan and CT guided biopsy were captured at this visit. In addition, information pertaining to their final diagnosis and any further interventions undergone were also captured.

Three further follow-up visits occurred at 3, 6 and 12 months from randomisation (Figure 4-1). Patients recruited in the last 6 months of recruitment only had 6 months of follow-up. At each follow-up visit, information regarding any further intervention patients had undergone, adverse events they had experienced, and treatment received were captured.

4.3 Statistical analysis

An intention to treat analysis is planned which will include all randomised participants unless consent to use data is withdrawn. Study protocol adherence and reasons for deviation will be described. Recruitment and participant flow will be described using a Consolidated Standards of Reporting Trials (CONSORT) flow diagram. If the number of withdrawals differ by group, the sensitivity of the findings to this attrition bias will be explored.

The number of positive biopsies will be compared, as a proportion of the participants recruited and as a proportion of those with a confirmed diagnosis of mesothelioma on second biopsy using logistic regression. Other binary outcomes will be analysed similarly. Time-to-event outcomes (e.g. time to a confirmed diagnosis of mesothelioma) will be compared using survival methods.

Differences between groups will be quantified and reported with 95% confidence intervals. If the data is sufficient to allow parameter estimation, we will adjust for centre as a random effect.

The ability of the serum mesothelin levels to predict a positive diagnosis (sensitivity, specificity, positive and negative predictive values, area under curve) will be assessed for the study cohort as a whole. Similar analyses of the value of the PET parameters to predict a positive diagnosis will be restricted to participants in the PET-CT group.

4.4 Safety reporting

Standard definitions and clinical judgement was used when reporting any adverse events (AE) relating to the trial. As the only research intervention in this trial was a PET-CT scan, significant events were not expected. A list of expected adverse events relating to the PET-CT scan and CT guided biopsy were listed in the protocol.

4.5 Study overview

The trial was funded by NIHR Research for patient benefit funding stream (Ref: PB-PG-0214-33095). The necessary ethical and regulatory approvals were obtained from the South West – Exeter research ethics committee in May 2015 (15/SW/0156). The trial was registered with the International Standard Randomised Controlled Trials Number (ISRCTN ref: 14024829) registry and adopted by the NIHR clinical research network portfolio, as an NIHR funded study. The lead centre for the trial was North Bristol NHS Trust (NBT) and the study was sponsored by the research and innovation department at NBT.

The first trial steering committee (TSC) meeting was held on the 13th of July 2015. The meeting was chaired by an independent member of the TSC. The background of the trial, design of the trial and trial related procedures were presented to the TSC by me, as the trial coordinator.

4.6 Set-up and recruitment

4.6.1 Lead centre set-up

The site-initiation visit for the lead centre North Bristol Trust (NBT) took place on the 04th of September 2015 shortly after which the trial opened to recruitment at NBT.

4.6.2 Set-up and opening of other sites

Due to unforeseen circumstances, significant delays incurred prior to opening the other sites as listed below:

- withdrawal of some of the centres who originally agreed to participate in the trial
- identifying and assessing capability of new centres to participate in the trial
- changes to the clinical diagnostic pathway leading to some centres being unable to deliver the trial protocol
- changes to the Health Research Authority (HRA) system
- ARSAC certification delays for each centre

In the 18-month period from initial agreement to participating in the trial (February 2014), to approaching the sites again for setting up the trial (Aug 2015), there were changes in circumstances at some of the centres restricting them from participating in the trial. These circumstances varied from changes in personnel who were able to deliver the trial, ie reduced capacity or change of PI who initially agreed to the trial, to changes in the diagnostic pathway. For example, at the concept of this study most centres would repeat a non-diagnostic biopsy, using CT guidance, but with better access to surgical pathways and establishment of mesothelioma MDTs more patients were being referred for surgical biopsies. Therefore, some centres felt they would be unable to deliver the study protocol of a second CT guided biopsy.

Remedial measures were taken to identify other suitable sites:

- The trial was published in the BTS monthly newsletter informing the wider respiratory community about the trial and inviting centres interested to contact the lead centre
- Informing the clinical research networks (CRNs) in the UK about the study as a way of engaging national centres

- Poster presentation of the trial at 2 international conferences for more exposure - the British thoracic oncology group meeting in Dublin 2016 which was attended by thoracic and oncology physicians, and the iMig meeting in May 2016, attended by a large network of mesothelioma specialists.
- Publicising the trial in the Mesothelioma UK newsletter – this newsletter reaches most clinicians and specialist nurses managing mesothelioma
- The trial was presented at the National pleural research meetings from 2015 to 2017 to raise awareness and recruit centres that maybe interested.

Following these measures, we were successful in opening a total of 10 sites to recruitment (Table 4-1).

4.6.3 Recruitment

Recruitment was slower than expected. Due to the niche population under consideration in this study, low patient numbers were expected, however the recruitment rate was much lower than anticipated. Therefore, the following remedial measures were taken in an attempt to boost recruitment.

- Increasing the number of recruiting centres from 6 to 10
- Relaxing the eligibility criteria so more patients would be eligible for the trial, without compromising the trial results
- Extending the recruitment period by 12 months.

Table 4-1 shows recruitment centres with respective PIs and recruitment to date.

Figure 4-2 is a recruitment graph with predicted recruitment against adjusted recruitment target (taking into consideration the extension), and actual recruitment.

Figure 4-3 is a CONSORT diagram of recruitment and data completeness rates to end of June 2018.

Site	Name of principal investigator	Date opened to recruitment	Number of patients screened	Number of patients randomised
North Bristol NHS Trust	Prof Nick Maskell (CI)	04/09/2015	**32**	**27**
Gloucestershire Hospitals NHS Foundation Trust	Dr Henry Steer	27/04/2016	**2**	**2**
Glasgow Royal Infirmary	Dr Kevin Blyth	04/05/2016	**10**	**5**
Oxford University Hospitals	Dr Najib Rahman	18/05/2016	**9**	**8**
University Hospitals of North Midlands	Dr Shahul Khan	16/08/2016	**3**	**3**
Norfolk and Norwich University Hospitals NHSFT	Dr Eleanor Mishra	21/02/2017	**3**	**0**
Sheffield Teaching Hospitals NHSFT	Dr Leon Lewis	17/03/2017	**9**	**4**
Portsmouth Hospitals NHS Trust	Dr Hitasha Rupani	03/04/2017	**0**	**0**
Aneurin Bevan University Health Board	Dr Alina Ionescu	19/07/2017	**3**	**3**
Blackpool Teaching Hospitals NHSFT	Dr Tarek Saba	15/11/2017	**0**	**0**
TOTAL			**71**	**52**

Table 4-1: TARGET recruitment sites and numbers recruited to date

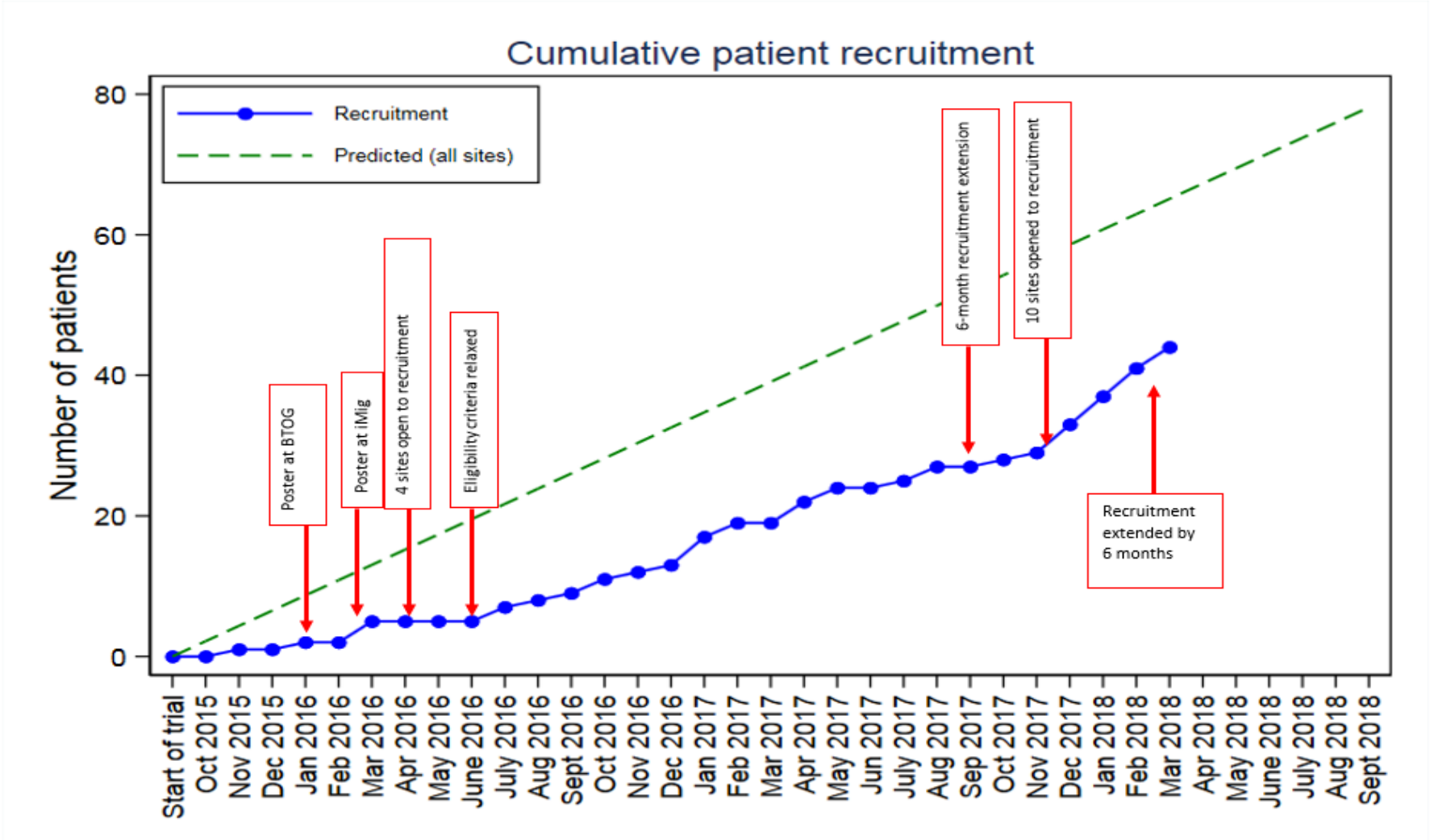

Figure 4-2: Recruitment graph against predicted recruitment. (With key action taken to boost recruit shown in red arrows)

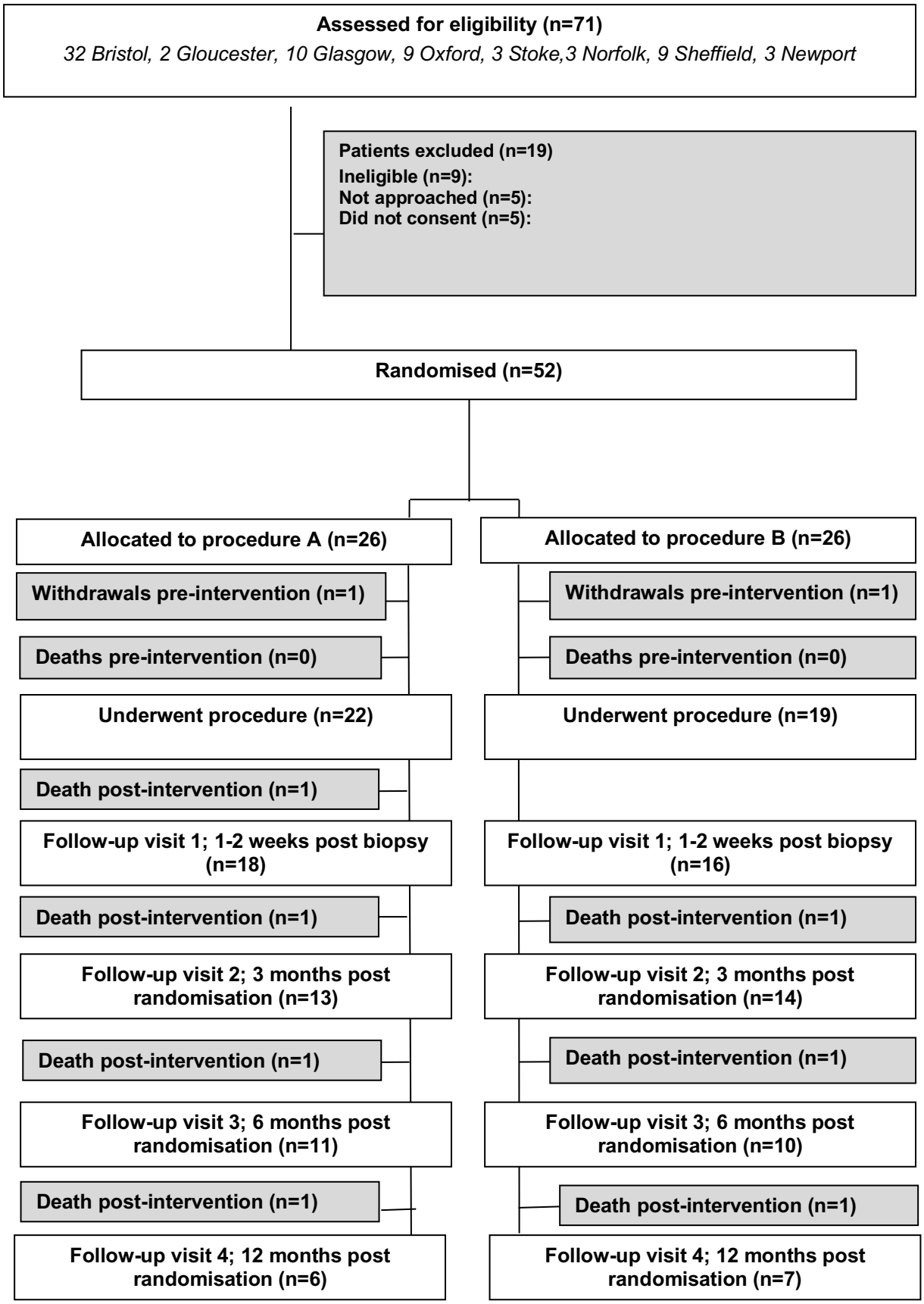

Figure 4-3:CONSORT diagram for TARGET trial

4.6.4 Trial Status

The 10 centres continued to recruit patients until September 2018. A 6-month follow-up period will then conclude trial related patient activity in March 2019. Following data cleaning and validation the database will be locked, and the data will be analysed according to the pre-published statistical analysis plan.

4.6.5 Ethics Amendments

Since original REC approval the trial protocol and some of the PILs underwent several amendments. Two of the amendments were major amendments relating to changes to eligibility criteria in an attempt to boost recruitment as stated below:

- Exclusion criteria changed from 'pleural thickening not amenable to 'Tru-cut biopsy' to 'pleural thickening not amenable to radiologically guided biopsy'. As Tru-cut refers to a specific type of biopsy method, this was replaced with 'radiologically' to encompass standard biopsy methods.
- Inclusion criterion 'any form of pleural biopsy in the previous 6 months' was changed to 'any form of pleural biopsy in the previous 12 months' to include more patients who would otherwise be ineligible.
- Exclusion criterion 'prior Talc pleurodesis' was changed to 'Talc pleurodesis in the previous 6 months' due to emergence of new evidence suggesting no increased Fluorodeoxyglucose (FDG) uptake on PET 6 months post talc pleurodesis [119].

Several minor amendments relating to addition of new sites and extensions to trial recruitment period were also approved by the REC.

4.7 Discussion

Pleural malignancy, particularly MPM can be a challenging disease to diagnose. The heterogeneity of the disease and the tumour itself, the difficulty of establishing the diagnosis on pathology and its slow indolent presentation are some reasons why MPM can be a diagnostic challenge. A lack of diagnosis can disadvantage the patient by delays in oncological treatment or stopping them from entry into clinical trials.

The role of PET-CT in MPM is still not firmly established although the literature does suggest a potential purpose in staging the disease when patients are considered for surgery, a PET-CT scan can be useful to exclude distant metastases [149]. As PET-CT scans can reliably highlight areas of increased metabolic activity this would be a useful method of targeting biopsies to confirm the diagnosis of pleural malignancy.

If the trial confirms superiority of PET-CT targeted biopsies, this could potentially minimise the number of invasive investigations patients are currently subjected to. In addition to the obvious patient benefits it is also likely to have a health economic benefit.

The Target Trial enabled me to learn about the processes involved in designing and conducting a multicentre clinical trial. I was able to design a study, including all the trial related processes from a preconceived hypothesis by Professor Maskell. I learnt the importance of a fool-proof trial design at the point of applying for funding. Coming from a non-academic background of clinical work, this was new to me. Having had no experience of applying for funding, setting up or conducting a clinical trial, I embarked on applying for funding which involved exploring various funding opportunities, attending grant applications work-shops and identifying the necessary personnel to help me with the development of a grant application. I learnt the importance of collaborating with the research design service, local research and development teams including financial officers who helped me in costing the application appropriately. Having not been aware of the importance of patients and public involvement in setting up clinical trials, I was soon organising a focus group of patients and relatives, with mesothelioma and presenting my study design to them. Having never applied for any funding for

a clinical trial, yet being successful in obtaining both grants applied for, is proof what a steep learning curve this has been.

Following on from the successes of obtaining funding and ethical approval, we were then faced with a series of obstacles when attempting to set up other sites. One of the biggest obstacles here, was the change in the diagnostic pathway of patients with suspected pleural malignancy. At the inception of the study idea, most patients had repeated percutaneously guided biopsies to confirm pleural malignancy. With the adoption of regional mesothelioma multi-disciplinary team meetings and the presence of thoracic surgeons in a large number of these, most patients are now being offered surgery as the next choice of biopsy. Hence, a number of centres who originally agreed to participate could not take part in the trial due to a change in their diagnostic pathway. Therefore, new sites needed to be identified. Urgent action was taken to publicise the trial via newsletters that would reach the specific target audience such as the British Thoracic Society newsletter, Mesothelioma UK newsletter and also raising awareness of the trial with the local clinical research networks (CRNs). The annual pleural research update meeting was a good platform to introduce the trial and attract researchers who were already taking part in national pleural trials. The trial was also presented in poster form at 2 international conferences where clinicians managing mesothelioma would congregate; the British Thoracic Oncology Group meeting and the International mesothelioma interest group meeting. Despite reaching a large audience, I soon realised only a small number of centres could take part due to variances in their diagnostic pathways. By this point I had learnt how an initial feasibility assessment could have saved significant time and resource down the line and act as a method of excluding some centres quite early on. Therefore, we started feasibility assessments for all interested sites at a very early stage. Since the advent of the HRA, feasibility and capabilities assessments have now become routine for all new sites under consideration for joining a clinical trial.

Another challenge was that the PET-CT scan, which is the crucial investigation under scrutiny here, was not funded as a research cost. PET-CT scans are expensive and considering this as an excess treatment cost meant the sites having to pay for the scans themselves. The financial implications of

this was cited as a reason by several sites not taking part. This is an important lesson I will bear in mind when designing and costing future research trials.

Having successfully enrolled 10 sites for recruitment we then faced the challenge of slow recruitment or non-recruitment from some sites. We expected the target population to be niche and recruitment was expected to be slow, however recruitment numbers were much lower than we had anticipated. As mentioned previously several, remedial measures were taken after discussion in the TSC meetings. Careful thought was needed to ensure the quality of the trial was not compromised when remedial measures were taken; for example, relaxing the recruitment criteria such as length of time since previous biopsy. Even with the measures undertaken, it was clear we were not going to reach our target within the previously specified time frame of 2 years of recruitment. A time extension was therefore sought and granted by the funder. Simultaneous measures to boost recruitment included regular contact with investigators to ensure the trial remained on the forefront of the investigators' minds, ensuring no potential patients were missed. Regular contact included quarterly Target Trial newsletters, frequent reminder emails and over the phone discussion with PIs at centres that were particularly slow to recruit.

From applying for funding to setting up and conducting the trial, this trial has taught me some valuable lessons which will undoubtedly help me in the future when designing and conducting clinical trials. TARGET is attempting to answer an important clinical question regarding the role of PET-CT in this cohort of difficult to diagnose pleural malignancies. The role of PET-CT in suspected pleural malignancy is poorly studied with mixed results. In addition to directly assessing the pleural characteristics, other benefits will also be explored, such as the overall value of PET-CT in identifying distant metastases which may have an impact on the management of patients and identifying alternative sites for biopsy. Analysis of FDG uptake indices on PET-CT such as TGV and SUV may provide further information to negate the suspicion of pleural malignancy in certain cases, thereby preventing patients from undergoing unnecessary investigation. Furthermore, identification of distant metastases would

provide prognostic information and suitability for certain treatment options. These are some of the secondary benefits of PET-CT scans we would explore as a part of the trial.

Finally, the biomarker serum mesothelin in this cohort may be diagnostically useful in some of the patients in conjunction with the biopsy ± cytology result. Change in Mesothelin levels over the follow up period may also be of benefit to the treating physician with their further management of the patient as discussed in the next chapter.

Footnote

The TARGET protocol is published in the BMJ Open Respiratory Research electronic journal.

Chapter acknowledgements

I would like to thank Jon Pollock from the RDS who's help was invaluable in preparation of the NIHR grant application. Mrs Ann Craig who until quite recently sat on the TSC as a patient representative. Patients and carers of the mesothelioma focus group who dedicated their time to reviewing the trial and provide very constructive feedback.

CHAPTER 5 ZOLEDRONIC ACID IN THE MANAGEMENT OF MALIGNANT PLEURAL MESOTHELIOMA: A FEASIBILITY STUDY

5.1 Background

The poor survival associated with a diagnosis of MPM, even with active treatment was discussed earlier in the introduction chapter. Phase I to III clinical trials investigating chemotherapy, immunotherapy and surgery are actively recruiting patients in a bid to discover treatment options with better outcomes in MPM [98, 151]. The projected peak of mesothelioma is between 2015 and 2025 [50], and although the Western world is likely to see a decline in the incidence after the next decade, this may not be the case in other parts of the world.

Drug repurposing is a very relevant research area in MPM [152]. 'Drug repurposing' refers to finding new indications for approved drugs and is ideal for a disease like MPM, which is rarer than other thoracic malignancies, therefore recruitment to phase I to III trials may prove challenging, in a disease where there is an urgent need for better treatments [153]. By the time the current phase I treatments are approved, the Western world that can afford these treatments may be seeing a decline in the incidence. Although the disease will continue to rise in developing countries, the novel treatments more than likely would be beyond their reach due to cost implications. Several well-known drugs for non-malignant indications have been studied for their potential anti-cancer activity in MPM; Valproate, thalidomide and zoledronic acid to name a few [152].

Zoledronic acid in Mesothelioma

Bisphosphonates are synthetic analogues of naturally occurring pyrophosphate [113]. Bisphosphonates are commonly used in the treatment of osteoporosis and other bone disorders such as Paget's disease, due to their action on inhibiting osteoclast mediated bone resorption [154]. Nitrogen containing bisphosphonates (n-bisphosphonates) have been shown to inhibit various epithelial cancer cells in vitro, by inhibiting the mevalonate pathway [155]. Potential anti-tumour activity of bisphosphonates includes reduced tumour angiogenesis, reduced tumour cell proliferation,

migration, invasion and adhesion, increased tumour cell apoptosis and increased cytotoxicity of gamma-delta T cells, which subsequently lead to reduced tumour vascularization[112, 156].

Studies using n-bisphosphonates, particularly Zoledronic acid (ZA) have shown a survival benefit in patients with breast cancer [157, 158]. *In vivo* studies on mice inoculated with mesothelioma cells, treated with bisphosphonates have shown a significant survival advantage [114], supporting the direct anti-cancer properties of bisphosphonates in MPM. Similar results were seen in other *in vivo* studies of murine models inoculated with small cell and non-small cell lung cancer, both showing a reduction in tumour burden and increased survival in mice treated with n-bisphosphonates [159, 160]

Zoledronic acid (ZA) is a potent nitrogen containing bisphosphonate which has bone independent anti-tumour activity [155]. In addition, when combined with certain chemotherapy agents such as Paclitaxel, Etoposide, Cisplatin and Irinotecan in lung cancers, it has an even greater synergistic effect in induction of apoptosis *in vit*ro [159].

The role of ZA in MPM is unclear. Anecdotal accounts of ZA given for MPM have been described in the literature without any clinical trial evidence to substantiate its effect [161]. A study by Jamil et al[115] recently investigated the role of single agent ZA in a small cohort of patients with advanced MPM, who had either completed chemotherapy or were too frail to receive chemotherapy. They demonstrated some benefit with ZA, where there was a 37.5% rate of clinical benefit (progression free survival + stable disease). Clive et al from our research group conducted a proof of principle RCT investigating the role of ZA in malignant pleural effusions (MPE) [162]. Although, they were unable to demonstrate any clinical or radiological significance in their MPE cohort treated with ZA, 2 patients with MPM who received ZA showed a reduction in tumour bulk on radiology [162].

When used in isolation in advanced MPM there was no improvement in overall survival [115]. There are no human studies investigating the synergistic effect between ZA and chemotherapy. A double blind multi-centre RCT would be best placed to investigate the hypothesis that treatment with the n-

bisphosphonate ZA, in addition to the standard chemotherapy (pemetrexed and cisplatin) confers a survival benefit to patients with MPM compared to chemotherapy alone. We designed a feasibility study to capture the data needed to inform a definitive phase III trial. In the Zol-A trial we aimed to randomise 50 patients to receive either ZA or placebo alongside chemotherapy, over a 12-month period across 3 centres.

A qualitative sub-study was designed to help understand patient experience and acceptability of trial related procedures and explore reasons behind their decisions to accept/decline chemotherapy or participating in a trial.

5.2 Obtaining funding for the trial

With the above study design in mind, I applied for funding via a mesothelioma themed NIHR Research for Patient Benefit (RfPB) funding stream in January 2015 (condensed version of the grant application can be found in Appendix III). During the grant application development, I worked closely with the Research Design Service (RDS) and the local Research and Innovation (R&I) teams. As before with the TARGET trial, I met with a mesothelioma focus group, comprising mesothelioma patients and carers, to discuss the study and ask for their opinion on the study design and trial related procedures. This meeting was very helpful in that they identified a weakness which led to a change in the study design. They made a pragmatic argument that, if patients did not want to receive chemotherapy, but were keen to consider the trial treatment this was not an option in our study design. Audit data from North Bristol has since been published showing up to 40% of patients who are fit and eligible for chemotherapy may decline chemotherapy [163]. This was an important group of patients that we had not taken into consideration prior to the focus group meeting. As a result, we altered the study design to include an open-label ZA arm for those who were eligible but declined chemotherapy but were keen to consider open-label ZA.

I was successful in obtaining the NIHR RfPB grant for the value of £ 287, 465.

5.3 Ethics approval and opening of sites

In the capacity of the trial manager I developed the trial related documents such as the trial protocol, patient information leaflet (PIL), consent forms and case record forms (CRFs). The trial was then presented to the East of England, Cambridge East Research Ethics Committee (REC). Following minor amendments, we were granted a favourable opinion on the 04/05/2016 (ref: 16/EE/0105). As a clinical trial of an investigational medical product (CTIMP), regulatory authority for administering ZA in mesothelioma was sought through the medicines and healthcare products regulatory agency (MHRA). As a multicentre RCT sponsored by the National Health Service (NHS), the study was registered with the Health Research Authority (HRA) prior to setting up other sites. The first trial steering committee meeting was held on the 10/05/2016.

As we stipulated a definitive recruitment period of 12 months to achieve our feasibility outcomes, we were keen to open the 3 recruiting sites, North Bristol NHS Trust (NBT), Bristol Royal Infirmary (BRI) and Royal United Hospital (RUH) Bath, simultaneously. With the involvement of different departments such as pharmacy, oncology and radiology there were delays and hurdles, prior to finally opening the trial across the 3 sites on 04/10/2016.

The trial was registered with ISRCTN, trial registration number 45536692 and was sponsored by North Bristol NHS Trust (R&I ref: 3638). The trial was also registered with the European Union Drug Regulations Authorities clinical trials (EudraCT ref: 2015-004433-26) database.

5.4 Methods:

5.4.1 Feasibility outcomes:

Our primary feasibility outcome was to randomise 50 patients over a 12-month period, however the trial was extended by 1 month (discussed later in this chapter) therefore extending the recruitment period to 13 months. In addition, we had a number of secondary feasibility outcomes largely exploring the acceptability of trial related procedures as stated below:

- Acceptability of recruitment procedures, consent and randomisation, and data collection methods.
- Acceptability of ZA in MPM patients, and the optimal timing and location for ZA administration.
- Qualitative assessment (QA) in a subgroup of up to 10 patients (from both the randomised and non-randomised groups) to evaluate patients experience in the randomisation and recruitment process. Qualitative analysis of the group of patients who declined participation in the trial.
- Quantification of drop-out and data completeness rates
- Estimates of outcome event rates e.g. survival times, measures of mean response and outcome variance (continuous variables such as quality of life) and confidence intervals around estimates of proportions, categorical variables such as recruitment rates) to use for calculating full trial size and number of sites.

5.4.2 Participant identification

Potential participants were identified by the principal investigators (PI) across the three sites, primarily from the local lung cancer/mesothelioma multi-disciplinary team (MDT) meetings. In addition, the regional mesothelioma MDT meeting is held at the lead centre NBT and is led by the chief investigator for the study, Professor Nick Maskell. All new cases of MPM from across the region (including the three hospitals taking part in the trial) are discussed at this meeting. Patients who met the eligibility criteria for Zol-A were identified as potential participants, and the relevant PIs were informed.

5.4.3 Pre-screening, screening and recruitment

All patients with a new diagnosis of mesothelioma between 04/10/2016 and 04/11/2017 were pre-screened for the trial. Eligible patients were approached for a discussion about the trial and if interested were invited to take part in the trial, after reading the PIL. The trial was discussed again

when they returned to consult the specialist mesothelioma nurses, prior to seeing the oncology specialists. If patients were agreeable to taking part in the trial, they were invited to consent at this point.

5.4.4 Eligibility criteria

Inclusion criteria

If the participants met all the following criteria, they were eligible for the study:

- Histo-cytologically confirmed diagnosis of MPM
- World Health Organisation (WHO) performance status (PS) 0-1
- Eligible for first line chemotherapy
- Ability to give informed consent

Exclusion criteria

If the participants met any of the criteria below, they were not eligible for the study:

- Not fit for chemotherapy due to PS or other comorbidities,
- Previous chemotherapy for MPM
- IV bisphosphonate therapy in the preceding 3 months
- Significant renal disease defined as an eGFR < 30ml/min within the preceding 4 weeks
- Current hypocalcaemia receiving treatment or evidence of hypocalcaemia within the preceding 6 weeks
- Age < 18 years
- Severe untreated dental caries
- Concomitant participation in another drug trial for MPM
- Allergy to 18-fluorodeoxyglucose used for PET scans
- Women of child bearing potential (defined as fertile or following menarche and until becoming post-menopausal unless permanently sterilised).

5.4.5 Randomisation and blinding procedures:

Patients were allocated on a 1:1 basis to either the intervention (ZA) or placebo. Allocation was blocked using varying block sizes and stratified according to histological subtype (epithelioid or cytological versus non-epithelioid) using web-based software provided by Sealed Envelope Ltd.

Participants and investigators were blinded to the treatment received. The ZA or placebo was provided in identically matched 100ml 0.9% saline bags by pharmacy. The infusion bag contained the participant trial ID and randomisation kit number. The allocation of treatment pertaining to the relevant randomisation kit number remained within the pharmacies preparing the IMP/placebo.

5.5 Trial interventions

5.5.1 Baseline assessment

A baseline assessment at the point of recruitment captured demographic data, medical history, current treatment, and investigations undergone for the diagnosis of MPM. Baseline blood tests performed at this visit included a full blood count (FBC), electrolyte levels (including adjusted Calcium level, Magnesium and Phosphate), liver function tests and C reactive protein (CRP) level. A research specific blood test, serum Mesothelin was also checked at the baseline assessment. Those receiving ZA/placebo alongside chemotherapy were then randomised.

All participants were started on calcium supplementation if they were not already on them, to prevent hypocalcaemia whilst undergoing treatment.

5.5.2 Scans

The initial CT scan at presentation, which was used for establishing the diagnosis was used as the baseline scan. A baseline PET-CT scan was performed following recruitment and prior to receiving their first cycle of ZA/placebo. A further CT and PET-CT scan was performed after 3 cycles of treatment (Figure 5-1). A final CT scan was performed at 6 months from enrolment. CT scans were performed as standard chest and abdomen scans at patient's local hospital. All PET-CT scans for patients taking part

in the trial were performed at the Cobalt Imaging Centre in Cheltenham using a standardised imaging protocol.

5.5.3 Follow-up assessments

All participants had follow-up appointments 2-3 weeks after their previous cycle of treatment. For patients in the randomised arm this often coincided with their pre-chemotherapy oncology assessment visits. Data on any adverse effects and new symptoms were captured at these follow-up appointments. If the blood tests identified any electrolyte disturbances, additional supplementation was prescribed as necessary.

A final follow-up appointment took place at 6 months from enrolment (Figure 5-1).

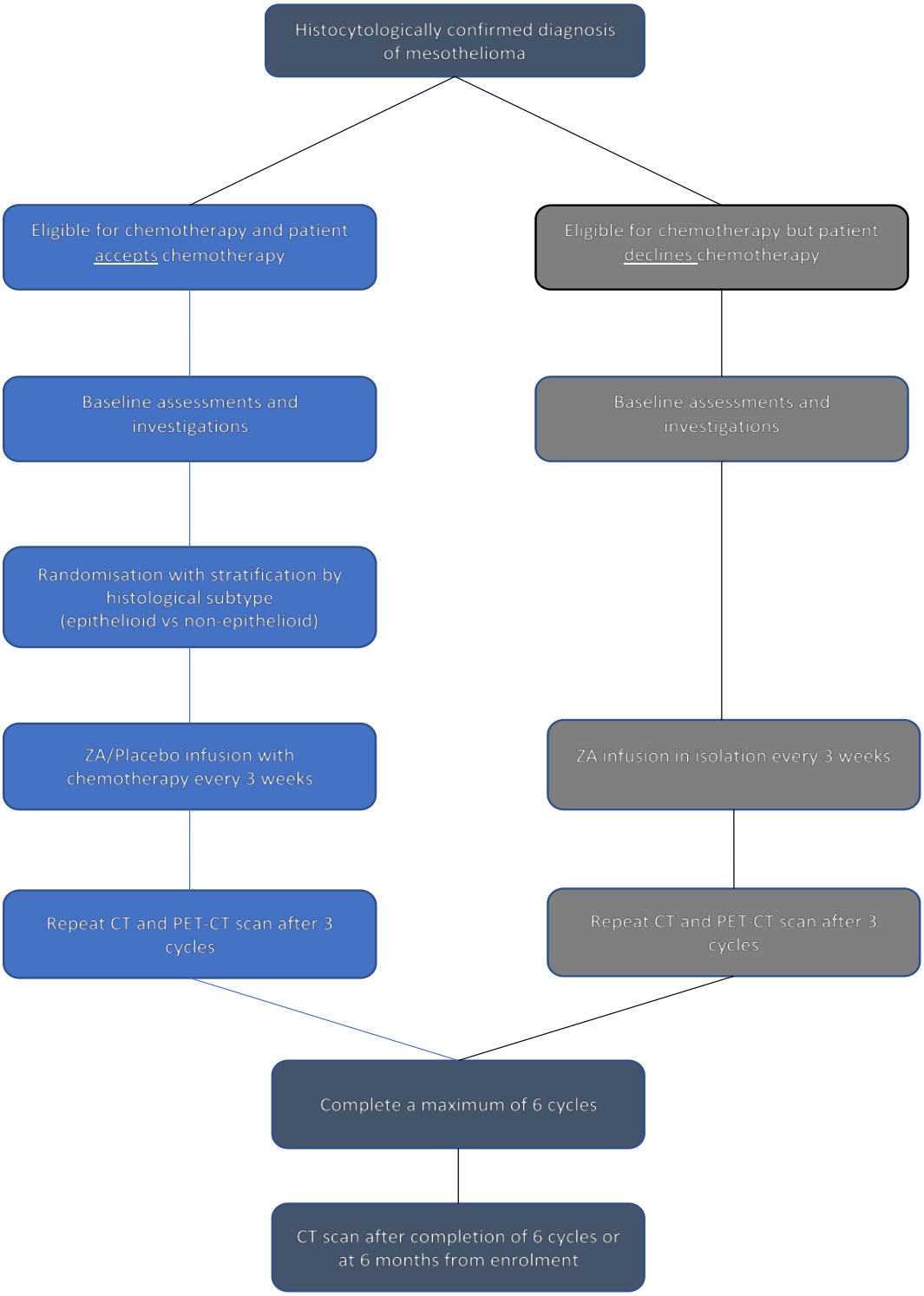

Figure 5-1: Trial flowchart for patients receiving treatment in the Zol-A trial (Randomised patients in the blue arm non-randomised patients in the grey arm)

5.5.4 Mesothelin tests

Patients had a serum mesothelin at baseline and every follow-up visit. A final mesothelin test was performed at the 6-month follow-up visit. All mesothelin samples were analysed using the commercial Mesomark™ ELISA (Fujirebio) according to the manufacturer's recommended methods for sample processing (sample processing is discussed in detail in Chapter 6 section 6.2.2).

5.5.5 IMP/placebo schedule and administration

Patients in both the randomised and non-randomised arms received up to a maximum of 6 cycles of ZA/placebo, at 3 weekly intervals. (Figure 5-1)

Zoledronic acid was given in 100ml of 0.9% saline over a 15-minute period. The exact dose of the ZA was dependent on their most recent renal function (Table 5-1) which was within the preceding 7 days.

Renal Function	Dose of ZA (mg)
eGFR ⩾ 60 ml/min	4.0
eGFR 50-59 ml/min	3.5
eGFR 40-49 ml/min	3.3
eGFR 30-39 ml/min	3.0
eGFR < 30ml/min	0.0

Table 5-1: Dose of Zoledronic acid according to renal function. (eGFR: estimated glomerular filtration rate; ZA – zoledronic acid; mg – milligrams; ml – millilitres; min - minute)

5.5.6 Semi structured interviews

As a feasibility study potentially paving the way to a larger phase III trial we were keen to explore patients' decisions behind their chosen treatment option, acceptability of trial related procedures and their overall experience of participating in the Zol-A trial. Therefore, we designed a qualitative component to this study centred around semi-structured interviews for up to 10 patients.

A purposive maximum variation sample of 7 patients took part in the semi-structured interviews. Patients who had varied experiences in the study were purposely sought and approached in order to obtain a wealth of different information from patients' perspective. The decisions on who to approach were decided upon informal feedback received from both NBT and RUH patients.

Patients were approached at their end-of-treatment follow-up appointment. If they were interested, a PIL relating specifically to the qualitative sub-study was given. This was followed up with a telephone call to ascertain interest in participating. If interested, an appointment was scheduled for the interview either at their home or at the Clinical Research Centre (CRC) at NBT. All patients were interviewed by Mrs Anna Morley (registered nurse), who has experience and an interest in qualitative research.

The interviews were structured across a number of themes relating to the diagnosis of MPM, and patients' participation in the trial. The full list of themes is available in Appendix IV.

A total of 7 interviews took place between 26th of April 2017 and 24th of May 2018. Six of the interviewed patients were randomised whilst one patient was in the open label arm. Three interviews took place at patient's own homes and 4 interviews took place in the CRC at NBT. The interviews ranged from 27 minutes to 42 minutes. All interviews were recorded and subsequently transcribed verbatim by me and checked by the interviewer (AM). The transcriptions were analysed by me using a semantic and deductive thematic analysis.

5.6 Radiology reporting

5.6.1 CT scan reporting

CT scans at baseline, post 3-cycles of treatment and at 6 months from randomisation were analysed in conjunction by 2 radiologists with expertise in thoracic radiology and pleural disease. The baseline scan was reported as per the International Association for the Study of Lung Cancer (IASLC) mesothelioma staging project's 8th edition of the Tumour Node Metastases (TNM) classification system for MPM [19]. All scans were also reported using the recently updated mRECIST version 1.1 guidelines to assess tumour response after 3 cycles of treatment and at 6 months [164]. As per the most recent literature, the minimum measurable tumour thickness was taken as 7mm, with pleural thickening measuring < 7mm classified as non-measurable disease [164, 165]. To calculate an mRECIST score the pleural tumour (if >7mm) is measured on 2 regions at 3 separate anatomical levels on the CT scan. The sum of the 6 measurements gives a final mRECIST score for each tumour. In addition, if

lymph nodes measuring > 15mm on short axis were present, these were also included in the final sum. (A detailed diagrammatic explanation of mRECIST calculation is shown in the Chapter 6 section 6.2.1) Using mRECIST tumour response is classified into 4 categories. Complete response (CR) denotes disappearance of all pleural and non-pleural disease. A partial response (PR) is when the summed measurement is reduced by at least 30% from the baseline scan. A sustained response on 2 scans 4 weeks apart is required for confirmation of above. Progressive disease (PD) is when the summed measurement is increased by > 20% or if any new pleural or extra-pleural lesions are detected. To classify as stable disease (SD), the summed measurements should not meet criteria as PR or PD, as described above [164].

5.6.2 PET-CT scan reporting

Two nuclear medicine experts independently reported the PET-CT scan SUV parameters for the baseline and post cycle-3 PET-CT scans. For reporting purposes, three anatomical levels were defined on the thorax as upper – above the arch of the aorta; middle – between the arch of the aorta and the carina; lower - below the level of the carina. A 15mm spherical region of interest (ROI) was drawn in the most intense area of FDG activity within each of these anatomical regions. SUV values were generated for each ROI using commercially available software, Syngo.via (Molecular Imaging, Siemens Healthcare). The average of the readings from the 2 reporters were used for subsequent analyses.

Using previously published data on tumour response grading for PET-CT criteria [121], a complete metabolic response (CMR) is defined as a complete resolution of FDG SUV uptake in tumour volume so that it is indistinguishable from surrounding tissue. A partial metabolic response (PMR) is when there is a > 25% reduction in FDG SUV after 1 treatment cycle, whereas progressive metabolic disease (PMD) is when there is an increase of > 25% FDG SUV uptake compared to baseline scan. Stable metabolic disease (SMD) is when the increase in the FDG SUV uptake is < 25% or the reduction of uptake is <15% [121].

5.7 Analysis plan

The statistical analysis is as per the feasibility objectives detailed above. The information obtained from this study would hopefully allow us to calculate numbers needed to treat in a full phase III trial. Assuming a 40% response rate for chemotherapy alone, the difference in the number of patients with a disease response between the IMP group and the placebo group will be used to calculate the sample size for the full trial. The quantifiable feasibility outcome such as screening and recruitment information, treatment up take, drop-out rates and radiology findings are reported here with. Inter-observer agreement for PET-CT reporting was tested using the kappa coefficient. The Mann-Whitney-U test was used for comparison of non-parametric data. Simple linear regression was used to examine the relationship between normally distributed variables. The full statistical analysis plan designed with the help of Bristol Clinical Trials and Evaluation Unit (CTEU) can be found in Appendix V.

A framework analysis which consisted of several stages of familiarization of the data (including transcribing the recorded interviews), theme recognition, coding and finally interpretation of the data was used for the qualitative analysis. Interpretation of the data was planned along deductive theme however due to unexpected emergence of certain themes, a combination of deductive and semantic analyses were used.

5.8 Safety reporting

Standard definitions and clinical judgement was used when reporting any adverse events (AE) relating to the trial. Given the nature of the disease and the chemotherapy treatment patients were receiving certain adverse reactions were to be expected as detailed below.

Expected adverse events relating to zoledronic acid

- Poor appetite
- Sore eyes
- Redness and soreness around drip site
- Electrolyte disturbances (hypocalcaemia/hypomagnesaemia/hypophosphataemia)

Expected adverse events relating to chemotherapy

- Flu-like symptoms
- Tiredness/lethargy
- Nausea and vomiting
- Gastrointestinal upset (diarrhoea)
- Skin reaction
- Peripheral neuropathy
- Pancytopaenia
- Neutropaenic sepsis
- Low Folate levels

Expected SAEs relating to ZA and chemotherapy

- Electrolyte disturbances requiring hospital admission for replacement of electrolytes
- Neutropaenic sepsis requiring hospital admission

5.9 Data collection:

All patients who were approached about the trial and given a PIL, were captured on a screening log. Consent, baseline information and blood results were recorded on the specific worksheets and subsequently entered onto an electronic database, locally at the relevant sites. At each pre-chemotherapy visit the patients had an assessment covering any AEs/SAEs secondary to treatment and had repeat blood tests. The results of these and a quality of life measure was documented in the specific worksheets and subsequently entered on to the database. All CT and PET-CT scans were imported to the local centre for assessment.

The trial team involved with conducting the trial at the lead centre and the statistician will have access to the final trial dataset once the database has been locked down.

5.10 Trial management:

A trial steering committee (TSC) comprising of the key members of the trial and a patient representative met before the start of the trial and twice since at regular intervals. An independent data monitoring committee met at the start of the trial. After 10 patients were randomised they reviewed the safety data remotely and confirmed their agreement to continuing on with the trial.

5.11 Results:

The results reported in this chapter were prior to unblinding the patients as we were unable to lock the database due to ongoing data cleaning and awaiting finalising of the statistical analysis plan. Despite not unblinding we have been able to report on most of the feasibility outcomes as discussed below.

5.11.1 Screening and recruitment

Between October 2016 and November 2017, 47 patients were screened across the 3 sites and 22 patients were recruited. Fifteen of the recruited patients were randomised while 7 were enrolled into the open label arm of the study. Figure 5-2 is a CONSORT diagram with recruitment and follow-up data for the Zol-A trial. Table 5-3 summarises the screening data by centre.

Of the 15 potential participants who did not meet the eligibility criteria 13 patients failed based on their WHO performance status. Two patients were ineligible due to only having a clinico radiological diagnosis of MPM rather than a histo-cytological diagnosis.

Of the other 10 patients who did not take part in the trial, 3 patients declined chemotherapy and hence failed inclusion into the randomised arm of the trial. Three patients declined to participate in the trial without giving a reason, while 1 patient declined the trial on the basis the trial schedule was too demanding for them. Two patients fell just outside the trial treatment period and 1 patient delayed their chemotherapy and hence fell outside the trial recruitment period.

Patient demographics and baseline characteristics for the recruited patients are shown in Table 5-4, separated into randomised and non-randomised groups.

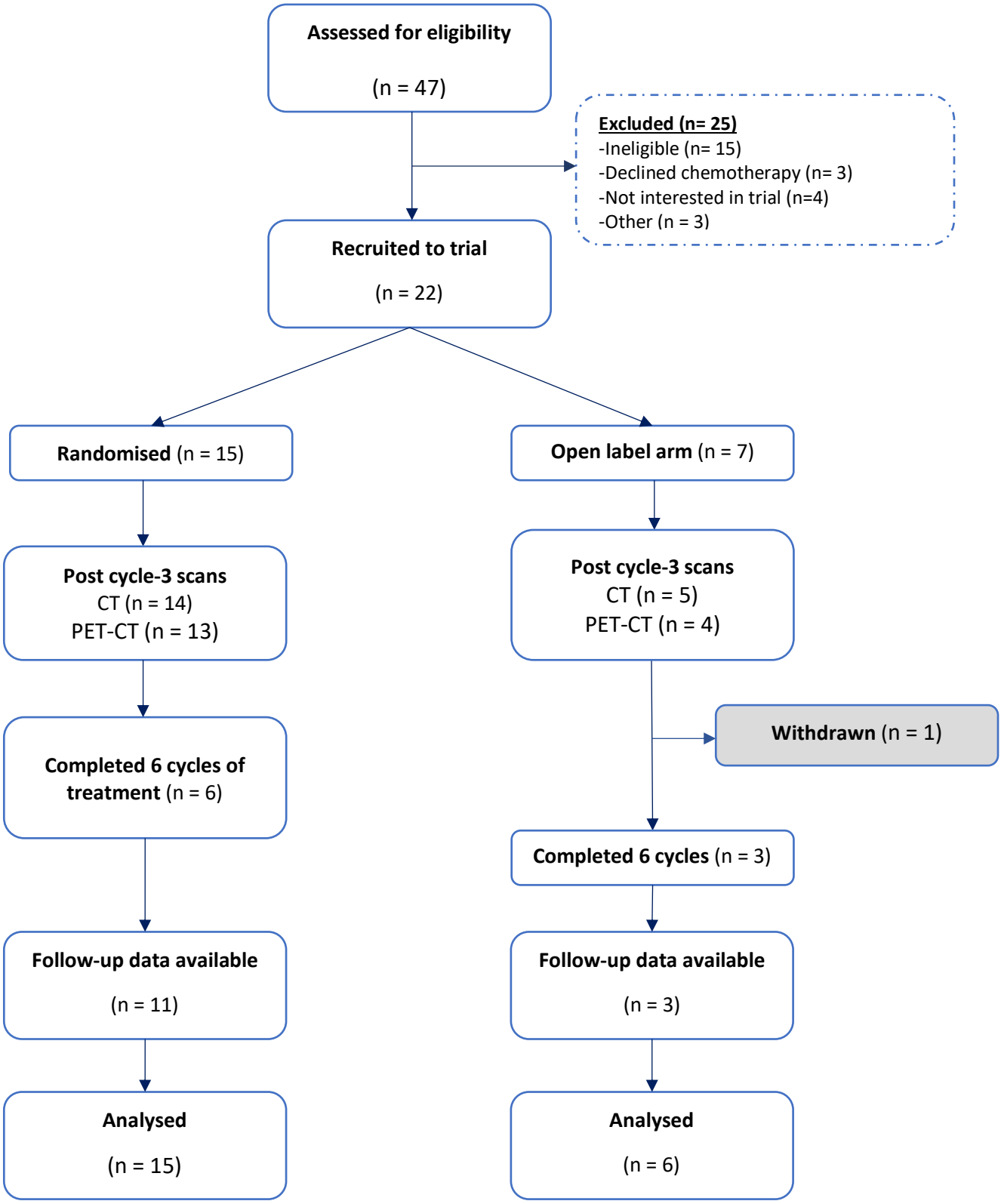

Figure 5-2:CONSORT diagram of patient recruitment in the Zol-A trial

Site	Screened	EXCLUDED FROM STUDY				Recruited (% of screened patients)	Randomised (% of screened patients)
		Ineligible	Declined chemotherapy and trial	Not interested in trial (had chemotherapy)	Other		
NBT	31	9	1	2	3	16 (52%)	9 (29%)
RUH	13	6	2	2	0	3 (23%)	3 (23%)
UHB	3	0	0	0	0	3 (100%)	3 (100%)

Table 5-3: Screening, recruitment and randomisation by centre

Patient characteristics	Randomised (n=15)	Open label (n=7)
Age (years ± SD)	72.2 (±5.6)	82.3 (±5.2)
Sex (M:F)	14:1	6:1
BMI	25.1 (± 2.8)	22.8 (±2.4)
WHO performance status	0.6 (±0.5)	1 (±0)
Laterality Right Left	 7 8	 5 2
Histological sub-type Epithelioid Sarcomatoid Biphasic Mesothelioma NOS	 11 (73%) 2 (13%) 1 (7%) 1 (7%)	 5 (72%) 1 (14%) 1 (14%) 0
Radiology		
CT staging 0 IA IB II IIIA IIIB IV	 1 (7%) 4 (29%) 2 (14%) 3 (22%) 2 (14%) 0 2 (14%)	 0 5 (71%) 0 0 0 2 (29%) 0
PET-CT parameters		
Upper SUV max (median; IQR) Mid SUV max (median; IQR) Lower SUV max (median; IQR)	6.1 (4.3 – 12.3) 6.1 (4.6 – 10.3) 6.0 (5.2 – 9.7)	6.6 (5.8 – 11) 10.9 (6.7 – 11.5) 10.2 (5.8 – 11.2)
Blood markers		
-Hb (g/dL) (median; IQR) -NLR -Calcium -CRP	140 (127 – 148) 4.64 (3.57 – 7.78) 2.4 (2.32 – 2.46) 38 (9 – 80)	130 (121 – 132) 6.26 (2.97 – 10.41) 2.46 (2.41 – 2.48) 33 (9.2 – 49)
Mesothelin level	5.7 (1.9 – 8.3)	4.7 (2.6 – 6.3)
Baseline EQ5D VAS score	80 (75 – 89)	70 (40 – 75)
Number of treatment cycles received (median; IQR)	5 (4 – 6)	5.5 (3 - 6)

Table 5-4: Baseline characteristics, blood results and radiology findings. *(BMI – body mass index; SD – standard deviation; SUV – standard uptake value; IQR – interquartile range; NLR – neutrophil lymphocyte ratio; CRP – C reactive protein; EQ5D – EuroQol five-dimension; VAS – visual analogue scale)*

The mean age of the randomised participants was 72.2 (±5.6) years. A majority, 20/22 (91%) of the patients were male. Epithelioid was the predominant histological subtype with 72% and 73% in the randomised versus open label groups respectively. Overall, when taking into consideration age, stage of disease, WHO performance status and PET parameters, the patients in the open label cohort were older with more advanced disease. However, as the purpose of this group was not for direct comparison with the randomised group, inter group comparisons were not performed.

5.11.2 Treatment uptake and adherence

In the randomised cohort 7/15 (47%) patients received the total complement of 6 or 4 cycles of chemotherapy + ZA/placebo treatment. Four (27%) patients stopped treatment after 5 cycles of treatment; 3 due to drug side effects and 1 due to general deterioration in health condition. One (7%) patients stopped after 4 cycles of treatment due to side effects of chemotherapy. Of the remaining 3 patients 1 stopped after 3 cycles due to progression of disease; 1 after 2 cycles due to general deterioration in overall health and 1 after 1 cycle due to treatment side effects (severe toxicity from chemotherapy). Trial treatment/placebo was given or stopped in conjunction with chemotherapy, no patients stopped one treatment or the other separately.

Of the 7 patients in the open label group 3 (43%) patients completed 6 cycles of open label ZA. Of the other 4 patients, 1 patient stopped after 5 cycles of treatment, by choice; 1 patient stopped after 3 cycles due to progression of disease; 1 stopped after 2 cycles due to general deterioration in health and 1 patients was withdrawn by the principal investigator after 2 cycles of treatment due to significant deterioration in health.

5.11.3 Drop out and data completeness rates

Of the 22 patients 3 patients were lost to follow-up and one patient withdrew. Three patients died prior to their 6-month follow-up appointment.

Although a number of patients (53%) did not complete the full 4 or 6 cycles of treatment they were not classed as ‘drop outs’ from the trial. Their treatment was stopped early, either by patient or

investigative team due to reasons mentioned above. These patients continued to participate in the trial and went on to have the necessary trial related scans and follow-up.

5.11.4 Withdrawals

One patient in the open label arm was withdrawn from the trial as per the principal investigators decision. Patient's general health deteriorated significantly making attendance to hospital too cumbersome. Therefore, a decision was made to withdraw this patient.

No patients withdrew from the randomised arm.

5.11.5 Protocol deviations

Two protocol deviations were reported for 2 patients in the randomised arm of the trial. One patient was given open label ZA from stock by a staff nurse at the chemotherapy suite in error, unaware that the patient was in the Zol-A trial. This was despite a specific trial prescription being available for Zol-A/placebo as per randomisation. The second deviation was a failure to record clinical observations post administration of the Zol-A/placebo infusion.

5.11.6 Outcome events

We did not feel the trial follow-up of 6 months was sufficiently long to collect survival data on overall survival or progression free survival. Therefore, survival was not included in our feasibility outcomes. Quantifiable outcome data on radiology such as mRECIST scores and changes in PET parameters were collected instead. The individual CT and PET-CT data is shown in Table 5-5 for the open label group and Table 5-6 for the randomised patients. Figure 5-3 shows the pre and post PET images for RUH405. There was good inter-observer agreement for overall response using PET-CT SUV values with a kappa co-efficient of 0.79 ($p<0.001$).

Patient ID	CT - Stage	Baseline mRECIST	Mid-cycle mRECIST	% change	Outcome	End of Treatment mRECIST	% change compared to baseline	Outcome	Baseline PET SUVmax sum	Mid-cycle PET SUVmax sum	% change	Outcome
NBT003	1A	0	0	0	SD	0	0	SD	15.4	15.3	-0.7	SMD
NBT004	1A	0	0	0	SD	35	High	PD	25.8	34.4	33.1	PMD
NBT006	3B	53	74	39.4	PD	136	156.6	PD	35.3	21.7	-38.4	PMR
NBT012	1A	20	CT not done	0	NA	CT not done	NA	NA	38.1	Not done	NA	NA
NBT014	1A	0	CT not done	0	NA	CT not done	NA	NA	15.5	Not done	NA	NA
NBT016	3B	56	103	83.9	PD	CT not done	NA	NA	25.7	33.8	31.3	SMD
NBT029	1A	0	64	High	PD	CT not done	NA	NA	28.2	Not done	NA	NA

Table 5-5: Radiology outcomes for the open label patients. (CT - computed tomography; mRECIST - modified response criteria in solid tumours; SD - stable disease; PR - partial response; PD - progressive disease; CR - complete response; SMD - stable metabolic disease; PMR – partial metabolic response; NA – not available)

Patient ID	CT - Stage	Baseline mRECIST	Mid-cycle mRECIST	% change	Outcome	End of Treatment mRECIST	% change compared to baseline	Outcome on CT	Baseline PET SUVmax sum	Mid-cycle PET SUVmax sum	% change	Outcome on PET
NBT005	1B	60	7	-88.3	PR	0	-100	CR	16.1	4.8	-69.8	PMR
NBT015	2	0	92	High	PD	16	High	SD	7.2	6.3	-12.5	SMD
NBT021	3B	106	111	4.7	PD	67	-36.8	PR	44.2	28.3	-35.9	PMR
NBT022	1A	0	0	0	SD	0	0	SD	17.1	7.4	-56.9	PMR
NBT023	4	72	0	-100	CR	0	-100	CR	19.4	7.5	-61.4	PMR
NBT024	3B	94	95	1.06	SD	90	-4.3	SD	22.1	21.7	-1.8	SMD
NBT025	1A	0	24	High	PD	50	High	PD	20.0	15.3	-23.3	SMD
NBT026	1A	0	51	High	PD	61	High	PD	25.0	12.5	-49.9	PMR
NBT030	1B	13	15	15.4	SD	32	146.1	PD	19.5	13.8	-29.2	PMR
RUH405	2	83	0	-100	CR	0	-100	CR	42.8	10.8	-74.9	PMR
RUH410	4	117	38	-67.5	PR	88	-24.8	PD	20.7	13.9	-32.6	PMR
RUH413	1A	0	76	High	PD	CT not done	NA	NA	34.1	Not done	NA	NA
UHB201	2	21	0	-100	CR	CT not done	NA	NA	9.9	Not done	NA	NA
UHB202	0	0	67	0	PD	119	High	PD	15.1	16.4	8.6	SMD
UHB203	1B	0	CT not done	NA	NA	CT not done	NA	NA	19.0	16.6	-12.6	SMD

Table 5-6: Radiology outcomes for the randomised patients *(CT - computed tomography; mRECIST - modified response criteria in solid tumours; SD - stable disease; PR - partial response; PD - progressive disease; CR - complete response; SMD - stable metabolic disease; PMR – partial metabolic response; NA – not available)*

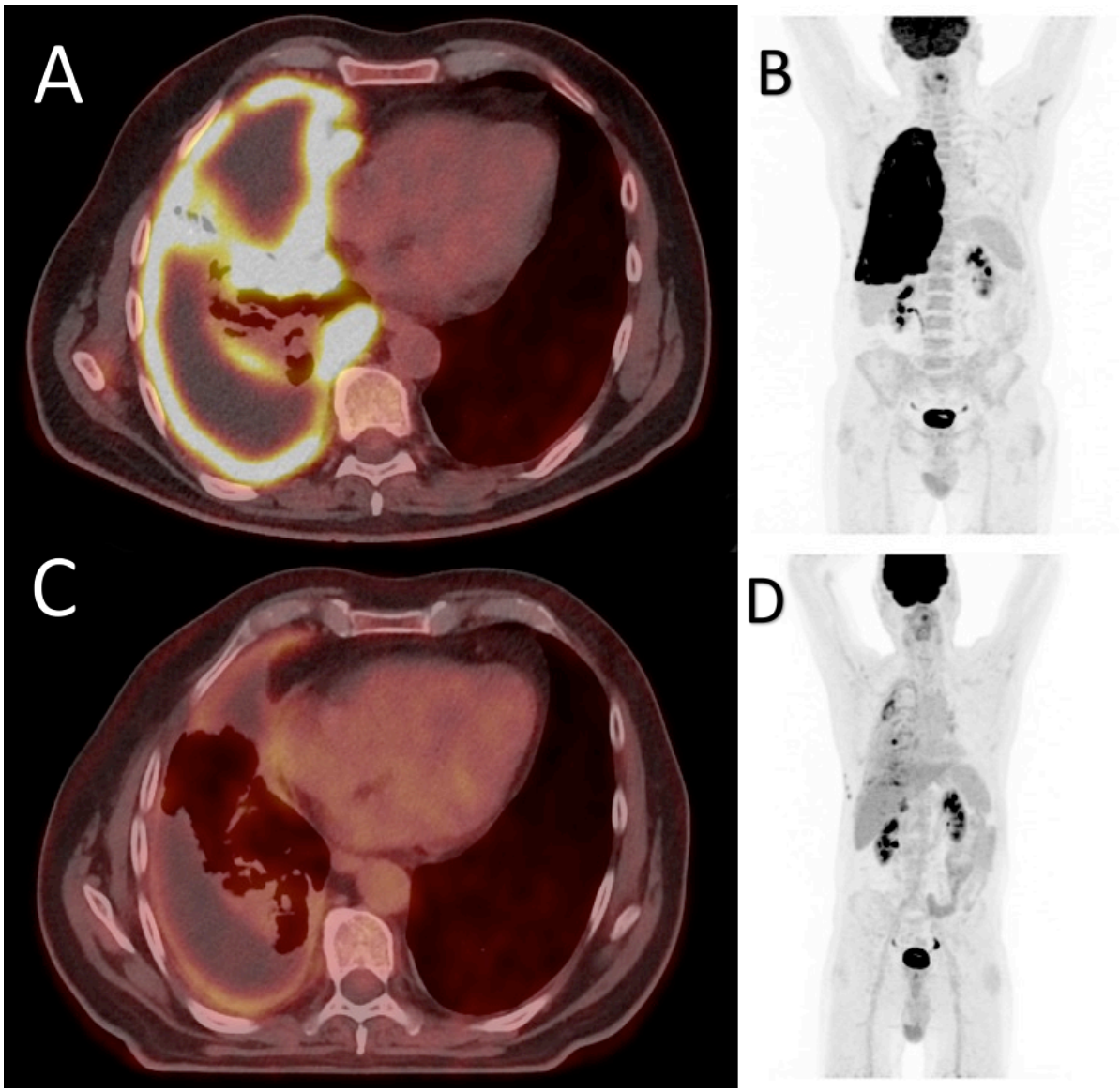

Figure 5-4: Pre and post-3 cycles of treatment PET-CT scans for RUH405 in the randomised arm of the trial. Images A and B showing high uptake on PET scan with the entire pleura displaying avid FDG uptake (bright yellow area on image A and black area on the right chest on image B). Images C and D show a significant reduction in the FDG uptake after 3 cycles of treatment.

5.11.7 Mesothelin

The median baseline mesothelin value across the entire cohort was 5.2 (IQR: 2.1 – 6.7) nmol/L. Excluding the 1 patient with histological subtype Mesothelioma NOS, patients with epithelioid histology had a median baseline mesothelin of 6.05 (IQR: 2.4 – 7.5) nmol/L, while for non-epithelioid patients it was 2.1 (1.3 – 4.3) nmol/L, a significant difference between the 2 groups ($p<0.05$).

Mesothelin levels after 3 cycles of treatment and CT data for the same time point were available for comparison for 14 patients. Percentage change in mesothelin tracked CT response fairly accurately ($p=0.01$). Patients starting with a baseline mesothelin >2.5nmol/L tracked the disease even better ($p=0.008$).

Radiological response	Median percentage change in mesothelin compared to baseline
Complete Response (n=2)	82 % reduction (SD± 13%)
Partial Response (n=2)	63 % reduction (SD± 6%)
Stable Disease (n=5)	23% reduction (SD ± 33%)
Progressive Disease (n=5)	21% Increase (SD ± 88%)

Table 5-7: Mesothelin performance against CT findings

The waterfall plot in Figure 5-5 shows the percentage change in mesothelin for individual patients at 3 cycles post-treatment. The colours of the bars depict the response shown on CT as per mRECIST criteria (see legend).

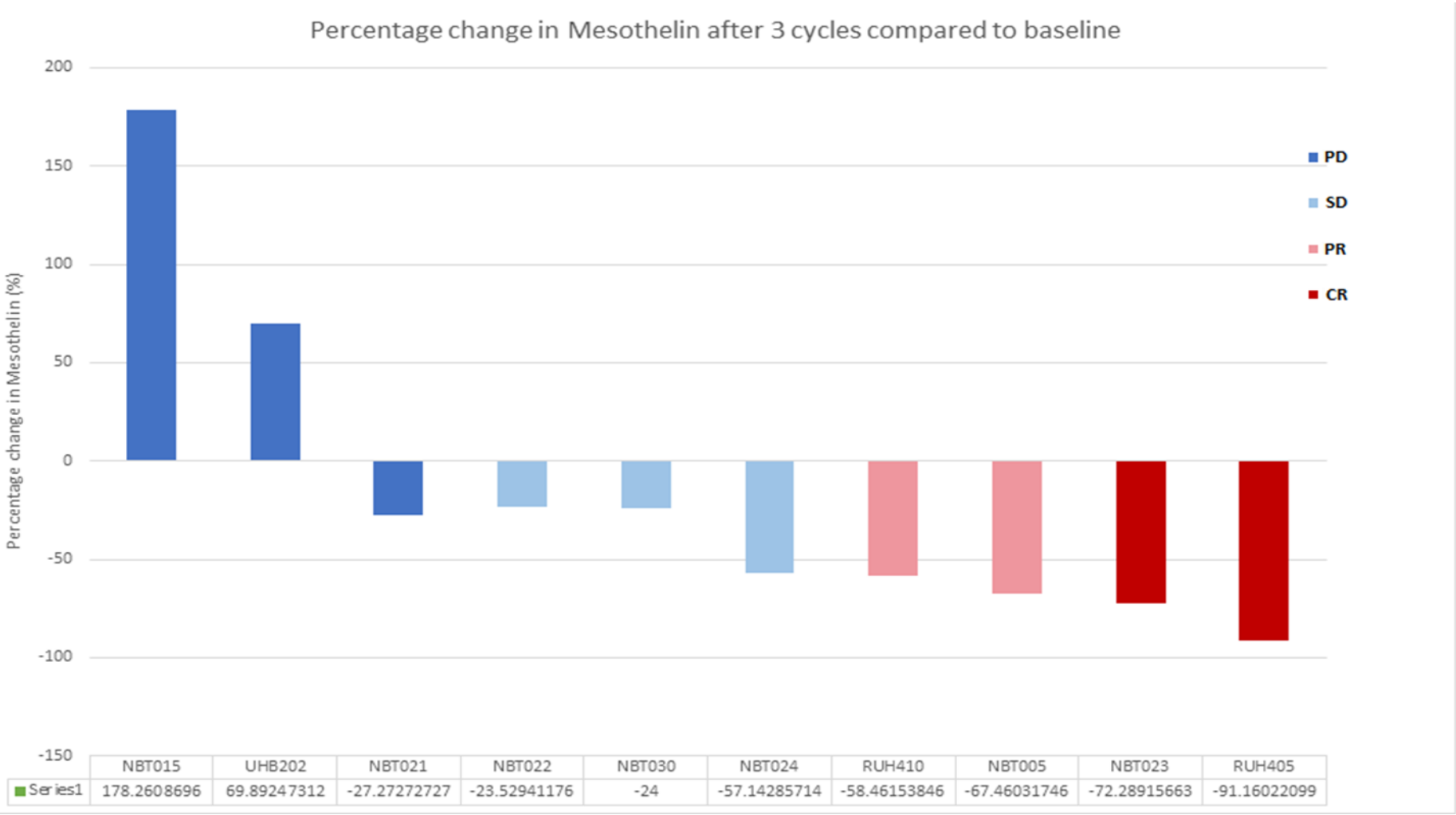

Figure 5-5: Percentage change in mesothelin after 3 cycles of treatment for patients in the randomised group. The colour of the bar shows their response on CT as per mRECIST. Table at the bottom shows the individual percentage changes. (PD – progressive disease; SD – stable disease; PR – partial response; CR – complete response)

5.11.8 Safety data

One hundred and nineteen adverse events (AEs) were reported for the duration of the trial. 31/119 (26%) were expected AEs as stipulated in the protocol. See table 5-8. Eighty-eight other adverse events were reported ranging from constipation to breathlessness.

Averse Event	Frequency (%)
Flu-like symptoms	2 (1.68)
Nausea	7 (5.88)
Poor appetite	3 (2.52)
Vomiting	4 (3.36)
Gastro-intestinal upset (diarrhoea)	1 (0.84)
Tiredness	8 (6.72)
Skin Reaction	4 (3.36)
Electrolyte disturbances	2 (1.68)
Other **Constipation** **Chest infection** **Fatigue** **Anaemia**	88 (73.95) 11 7 4 4

Table 5-8: Adverse events reported and the reported frequency

Sixteen SAEs were reported for 11 of the patients (Table 5-9). Only 1 of these SAEs were considered to be 'possibly related' to the trial drug. Twelve out of the 16 SAEs were due to hospital admission or prolongation mainly due to side effects of chemotherapy or progression of MPM. None of these were thought to be related to the trial drug.

Patient ID	Serious adverse event	Reason	Relatedness
NBT012	Required Hospitalisation / Prolongation	General deterioration	Not related
NBT014	Required Hospitalisation / Prolongation	Lower respiratory tract requiring IV antibiotics	Not related
NBT014	Resulted in Death	Progression of mesothelioma	Not related
NBT015	Required Hospitalisation / Prolongation	Collapse – pulmonary emboli	Unlikely to be related
NBT021	Required Hospitalisation / Prolongation	Diarrhoea and vomiting	Unlikely to be related
NBT022	Required Hospitalisation / Prolongation	Sepsis and derange bloods	Possibly related
NBT029	Resulted in Death	Progression of mesothelioma	Not related
NBT030	Required Hospitalisation / Prolongation	Increased pleural effusion	Unlikely to be related
NBT030	Required Hospitalisation / Prolongation	Pneumonia	Unlikely to be related
RUH410	Required Hospitalisation / Prolongation	Neutropaenic sepsis	Unlikely to be related
RUH410	Required Hospitalisation / Prolongation	Lower respiratory tract infection	Unlikely to be related
RUH413	Required Hospitalisation / Prolongation	Opioid toxicity	Not related
RUH413	Required Hospitalisation / Prolongation	Lower respiratory tract infection	Not related
UHB201	Life-threatening	Neutropaenic sepsis	Not related
UHB203	Required Hospitalisation / Prolongation	Rash to Co-amoxiclav	Not related
UHB203	Life-threatening	Rash to Amoxicillin	Not related

Table 5-9: Serious adverse events, reasons for reporting and relatedness to investigational medicinal product

5.12 Results of the qualitative sub-study

The findings of the qualitative sub-study are reported along deductive themes generated by the questions posed to the patients and any other interesting themes that emerged which were considered useful.

Initial thoughts on the diagnosis of mesothelioma

All patients interviewed expressed either 'shock' or 'surprise' at the diagnosis of mesothelioma. 4/7 patients talked about diagnostic delays and being treated for other conditions such as heart conditions for a number of months before a definitive diagnosis of mesothelioma was made. The opening question asked about the patients understanding of mesothelioma, and most patients answered the question by referring to the diagnostic delay. This suggests an element of anxiety/concern around the diagnostic delay. No direct reference was made to advanced stage of disease with delay in diagnosis, but a perceived late stage of disease could be one of the concerns. Two patients also voiced their feelings that they had never smoked in their life yet, developed a cancer associated with the lung.

"Well I was quite surprised to hear the diagnosis, you know, originally…….. Considering that the GPs didn't have any clue as to what it was……" [NBT015]

"It was big shock. I've never been involved with anything to do with asbestos in my life and therefore something totally out of the blue." [RUH410]

MPM and its relatedness to asbestos

Although most of the patients interviewed had an awareness of asbestos, and related health problems, their diagnosis was as a consequence of previous asbestos exposure was surprising to all of them. 5/7 patients were disappointed that they were exposed during previous occupations, and that health and safety regulations were not in place to protect them. Despite the initial disappointment of

the occupationally related malignancy, patients appeared to have come to terms with the cause and effect, seemingly accepting the diagnosis.

"…..but asbestos has been known to cause this [mesothelioma] since early 1900s……..They have known, the people in charge have known this is a killer! Asbestos is a killer since the early 1900s. I mean turn of the century. And still used it……"[RUH410]

"……I was working on building sites. Back then there was no health and safety as regards to asbestos dust, general thing it was there. You worked around it……" [RUH405]

Thoughts on a terminal disease with a lack of effective treatment

The terminal nature of the diagnosis and the lack of curative treatment rendered them powerless and out of control. Taking part in the trial was seen as one way of taking control back and not letting the disease take over their lives.

"……it was a shock when I first thought about it obviously. Nobody likes being told that they are that close to dying in the end……." [NBT005]

"I mean we try to continue as if this wasn't this mesothelioma wasn't there to a certain extent" [NBT030]

Symptoms and disability secondary to MPM

There were mixed opinions about the symptom burden and associated disability. Some had a clear expectation that they should be more ill than they were, for a malignancy with such a poor prognosis. Whereas others did not want to class themselves as ill, which was almost felt as giving into the disease.

"we've gone from really doing what we want when we want, to… hang on… we can't do that today. I'm not very well, I don't feel like doing it" [NBT030]

One patient discussed the pleural effusion and MPM as 2 separate entities altogether saying:

"The worse thing about is this, was the cancer never made me ill. The fluid, that my body made to fight the cancer, was what was making me ill" [RUH410]

Information regarding mesothelioma

Patients were asked about information received at the time of diagnosis. Patients were satisfied with information regarding diagnosis and available treatments, or lack of it. However, 5/7 admitted they or their spouses wanted to find more information by searching the internet. This could be driven by a sense of hopelessness at the lack of treatment options, wanting to be in control of their disease, wanting to understand more about the disease and what it entails. All patients preferred written information as opposed to verbal information as they thought a leaflet could be taken away to study at a later time, keep it as a resource they could refer back to in the future in case they forgot verbal information.

"…. I generally don't. [Names husband] used the internet and had a look at a little bit" [RUH410]

"When I was first diagnosed [wife named] was on the internet all the time. Reading all about it"[NBT015]

Thoughts about taking part and understanding of the trial

We explored participants' decisions behind taking part in a research trial. Altruism was the main reason for participation in a majority of cases. In 5 out of the 7 cases patients wanted to help find a treatment to cure mesothelioma. In 2 cases patients were driven more by desperation of wanting better treatment and the addition of another drug to the chemotherapy was perceived as a better option than chemotherapy in isolation. A sense of desperation for better treatments was apparent in all 7 interviews. A willingness to try 'anything' if it would help. Two of the participants were very grateful a trial was available for them to consider.

"……we've got to try and find a cure if we could. But if nobody wants to take part in these trials, you're never going to know, are you? They might strike lucky one day!" [NBT005]

"……. if it didn't help me it might help others in the future. So… it's worth you know trying….." [NBT015]

"…..I saw nothing really other than a benefit of going down the trial because I had a 50% chance of getting something that may help me personally of…. Of…. Treating…. Or going some way towards treating this cancer……." [RUH410]

"……all I knew was I'm like a guinea pig in that respect, and whatever comes out it'll benefit somebody else. And at the end of the day I have nothing to lose. So, had to be done. Amazing that……. There's nothing to lose is there?" [NBT003]

Other perceived benefits

All patients felt they would have more contact and support from the research team and health care professionals by taking part in a trial. More contact with trial teams, research nurses and doctors were reassuring for the patients. 1 patient felt taking part in a trial would allow quicker access to treatment. Patients also felt PET scans would offer additional information and was reassured by the extra scans offered as a part of the trial.

"Positive thing is having so many extra people looking after you…. Basically, if you've got any problems, you are on the end of a phone. It doesn't matter which one of you that we spoke to, you were all brilliant." [NBT015]

"…. it was wonderful to have all the support of the research team…."[NBT003]

Receiving chemotherapy and IMP/placebo at the same time

Chemotherapy administration usually takes a number of hours at each sitting. Addition of the trial drug extended this duration. We asked patients about their thoughts on receiving both chemotherapy and the trial drug on the same day. All randomised patients agreed it was better to have all the treatments in 1 sitting rather than have another day trip for the trial drug. They accepted it was a long day, yet one less trip to hospital if all treatments can be delivered in the same sitting.

"So um…. It possibly made it a little longer day but I was having a long day anyway, so I wouldn't have wanted to have to go back and do it at a different day" [RUH410]

Trial related scans

All interviewed patients felt PET-CT scans to be a positive addition. The trial scans were performed at the Cobalt centre in Cheltenham for all participants. Patients did not find travelling to be much of an issue. They were happy to be offered a scan that may potentially provide the medical professionals with additional information than the usual clinical CT scans.

"And then you know as it progressed further, I'm being sent to Cheltenham for the PET-CT scans, which we wouldn't have had if we weren't part of the trial" [NBT015]

Interviewer: The extra PET scans, how did you find those?

"Not a problem at all. It was excellent. They ordered a taxi. It was a day out." [NBT003]

Chemotherapy and trial drug/placebo administration for NBT patients

NBT patients usually have chemotherapy at Bristol Haematology and Oncology Centre (BHOC). Therefore, they received the trial drug alongside chemotherapy at BHOC with each cycle. Significant delays were incurred which was informally fed back to us via patients, but the same issues were highlighted by the patients as shown below.

"The only problem I got or had was…. It [trial drug] not being available when I turned up like. You know. I was given obviously dates and times when I should be there. But sometimes I was sat there for 2 hours coz they never had the drug" [NBT005]

"Not with the actual drug…. But when we went to oncology sometimes the drug wasn't ready or you know, we were there for 8.30 most of the sessions, sometimes we didn't get the drugs till 1 o'clock" [NBT015]

Randomisation

All patients had a degree of understanding about randomisation, including the one patient who was not randomised. However, only 1 person had a clear understanding about the full benefits of randomisation in clinical trials. None of the randomised participants had strong feelings about whether they received ZA or placebo, but all hoped they received the trial drug. One participant was firm in his belief he did not receive the drug as he did not experience any of the side effects mentioned in the patient information leaflet. The lack of side effects was perceived as not receiving the treatment. All participants had a good understanding of the double-blind nature of the trial; that the investigators were also blinded to which treatment they were allocated to.

"Well really….a bit of a lucky dip really. As they explain the placebo, you might be getting it. Never really talked about it sort of ……. No one was in the know as such. No one could tell me I didn't ask" [RUH405]

"Well I'm presuming that… that…. It's absolutely random as to whether you get the zoledronic acid or you get a placebo. So you don't know which one you are getting, so you can't make up symptoms or or or…. Responses." [RUH410]

Other interesting themes

Being in control of the disease

One of the recurring themes throughout all interviews was a need to be in control of their situation. Participants' desperate need to know disease activity with treatment. A majority of patients felt having more scans would be reassuring. They wanted more information from the clinicians with each cycle of treatment. The PET-CT scans were considered a positive aspect of participating in the trial by 2 of the patients.

"…….I need to know like… "is it getting better" is it not like, you know…. Have they stabilised it……?"

"……I would've had a scan every time I had a chemo sessions really……" [NBT005]

Progression of disease in the future

Patients expressed wishes for more information regarding disease progression and the future after completion of chemotherapy. A definite uncertainty about the future and an 'in-limbo' feeling was apparent amongst all patients interviewed. This may be due to participants responding well to treatment which is in contrast to the information given at the time of diagnosis. There was an expectation of a sudden deterioration and patients had almost stopped living their life waiting for the end.

"……the only worry really is… no one seems to know what will happen in the future to me…… Even [names consultant] says 'no one's had 'you' here before'. So we don't know what's going to happen. So that's only problem…. How long I'm going to live….or… or what….." [NBT005]

Information sharing between clinicians and patients

Two of the patients had strong feelings about the lack of information being communicated through to the patients. They believed the clinicians were aware of their prognosis but did not wish to upset them by discussing a poor prognosis. A definite sense of helplessness was evident in these 2 participants.

"…..I'm sure they [doctors] know, or they can estimate, but perhaps they are unwilling to upset you…. Or I don't' know……." [NBT003]

"…….perhaps there isn't nothing else to know about it. You know. Doc… [names consultant] and all those people up here they don't talk at your level anyway do they? So they are like 'ohhh you got it" you got it…." [NBT005]

Although the themes were deductive from the questions posed to the patients some unrelated themes did surface. Hence a semantic analysis was carried out as above to appreciate and recognise these emerging themes.

5.13 Discussion

The Zol-A multicentre feasibility RCT is the first trial to explore recruitment, patient acceptability and tolerability of Zoledronic acid or placebo concurrently with first line chemotherapy, for patients with MPM. It is also the first study to investigate Zoledronic acid in isolation for patients with MPM, who are relatively fit and well, and are eligible for chemotherapy. Whilst we failed to achieve our ambitious primary feasibility outcome of randomising 50 patients over a 13-month period across 3 sites, we obtained useful data regarding the overall feasibility of a full phase III trial.

As mentioned previously the data discussed here is blinded data. Therefore, the final results regarding effect sizes or sample size for a full phase III study could not be reported herein.

We obtained good screening data from 2 of the centres participating in the study (NBT and RUH), the screening data from the third centre only included three randomised patients. All screening logs were available for review. Of the 47 patients screened, 13 were ineligible due to their WHO performance status, and 2 due to a clinico-radiological diagnosis only. Therefore, the primary reason for ineligibility was patients being frail and old, assuming the 2 patients who were unable to have biopsies were probably too frail for further investigation. It is also interesting to note that the open label group, which comprised of patients who were fit, yet declined chemo, were older (mean age 72.2 years for the randomised group versus 82.3 years for the open label group). Older patients maybe disinclined to accept the toxic side effects of chemotherapy yet would be willing to accept non-chemotherapy trials, if they were available [163]. In total 10/32 (31%) patients declined chemotherapy despite being eligible for first line treatment (7 patients in the open label group and 3 patients who declined chemotherapy and the trial). The response rate with first-line chemotherapy in MPM is moderate with only a 3-month additional survival benefit, and only 40% of patients responding to the treatment [109]. The trade-off between 3 months of extended survival for a potentially worse quality of life may deter some patients from taking up 1st line treatment. With more effective treatments this trend may change in the future.

Mesothelioma is a relatively rare thoracic malignancy and clinical trials in MPM struggle to recruit patients. A number of MPM treatment trials have prematurely terminated due to poor recruitment (NCT00597116; NCT00003508). However, patients who are fit, and likely to take-up treatment are keen to participate in first line clinical trials. First line treatment trials are sparse in MPM at present and most other treatment trials stipulate completion of first line chemotherapy as an eligibility criterion. Therefore, patient choices are limited, they are obliged to have a poorly effective treatment in order to be eligible for clinical trials further along. A trial such as Zol-A will be well placed for those interested in a first line clinical trial alongside chemotherapy.

It became apparent that an eligibility criterion specifying 'measurable' disease according to mRECIST criteria (i.e. a tumour thickness>10mm) was inappropriate as some patients have early disease and the tumour thickness would not meet this criterion. Relaxing the eligibility criteria was considered when recruitment in the trial was slow to start with. Measurable disease was removed from our eligibility criteria at this point. The results from the CT scans do not suffer as a consequence of removing this criterion. In fact, 6 of the recruited patients did not have measurable disease at recruitment, as shown in Table 5-6. A full list of amendments since original REC approval is listed in table 5-10 at the end of the discussion section.

Attrition is a common issue with MPM trials due to the rapid deterioration and death of patients. In the current study 3/22 (14%) patients were lost to follow-up and 3/22 (14%) patients died during the relatively short 6-month trial period. Two of the 3 patients who were lost to follow-up were from peripheral hospitals, having to travel further to receive treatment at the regional oncology department. This is an important factor to take into consideration when designing a future trial. Patients may have to travel to different sites, at times different cities, for chemotherapy treatment as chemotherapy administration is only at sites with oncological support. Alternative forms of trial follow-up such as telephone follow-up and locally performed CTs and blood tests need to be incorporated to reduce loss to follow-up. The 3 deaths in the short trial period reflects the

unpredictability and rapidity of progression of mesothelioma, even in cases where patients were fit and well at the start. When designing a full trial these numbers should to be taken into consideration. It is unsurprising that a number of patients (8/15; 53%) stopped treatment early due to the side effects (27%) or lack of effectiveness (13%). Identifying those who are unlikely to respond or those with a poor prognosis is useful for both patients and researchers. The Brims decisions tree analysis model is one method of risk stratifying MPM patients, however at the time of designing this trial, the Brims model had not been published [166]. This model can be incorporated in future mesothelioma studies, so an informative discussion can be held with the patients about their prognosis and positive/negative impact of chemotherapy.

Tumour response in mesothelioma is difficult to measure objectively. MPM does not grow spherically like other solid tumours, instead like a 'rind' following the contours of the pleura. Response to treatment may not be uniform throughout the tumour. The modified RECIST criteria allows an objective assessment of tumour response and to date this is the best available tool for measuring MPM response in clinical trials [116]. Using the most recently published mRECIST version 1.1 criteria for assessing tumour response, we were still unable to measure the tumour in 21/56 (38%) available scans in the study as the minimum tumour measurement did not meet the >7mm cut-off criteria [164]. But until another imaging modality or biomarker is proven better, CT will continue to be used despite its deficiencies. Therefore, it is reassuring to know that the patients had no concerns about the number of CT scans offered as a part of the trial and were happy to undergo regular scans.

In addition to the CT scans we explored PET-CT as a more reliable alternative for tumour response in MPM. At the time of writing this chapter we only had the standard uptake value (SUV) data from PET-CT scans available for comparison, not the TGV data which can probably give a more accurate reflection of global tumour response. Thirteen patients had both a baseline and a mid-cycle PET-CT scan. Of these, 8/13 (62%) had a partial metabolic response (PMR) and 5/13 (38%) had stable metabolic disease (SMD). The mean reduction in the SUVmax for PMR was 51.3% (range 29.2% – 74.9%). In the SMD group, the mean change in the SUVmax was a reduction of 8.3% (range 8.6% to -

23.3%). Without longer term survival or CT data it is difficult to comment on the significance of the large reduction in SUVmax, and the number of responders by PET-CT criteria. None of the patients in the randomised group had disease progression by PET-CT SUV criteria, despite 5 patients showing disease progression on CT by mRECIST criteria. This variability between CT and PET-CT response was seen across all categories of response. For example, 3 out of the 5 patients who had SMD on PET-CT had progressive disease by mRECIST on CT. Similarly, of the 8 patients who had a PMR on PET-CT, 2 patients each had CR, PR, SD and PD by mRECIST criteria. It is therefore difficult to comment on the accuracy of the PET-CT findings without longer term survival data. The significant reduction in the FDG uptake can partly be explained by the tumour stunning effect that has been previously reported with malignant tumours receiving chemotherapy [119]. A pseudo-response effect is seen depending on timing of the PET-CT scan and treatment cycle. We may be in a position to explore the PET-CT characteristics further when the TGV data is available. The investigators were concerned about the PET-CT scans being an extra burden to patients, particularly with the extra travel to Cheltenham. Contrary to the investigators concerns, patients felt the extra-scans were reassuring as they may provide additional information that may not be available on routine standard care (CT scans).

Mesothelin is a less invasive method of monitoring response in MPM and probably the most reliable biomarker investigated in MPM for this purpose [93]. In this study we monitored levels of mesothelin in parallel with each cycle of chemotherapy. Patients who had a baseline serum Mesothelin level > 2.5nmol tracked the disease well (p=0.008). Those who had a partial/complete response showed a significant reduction in their mesothelin compared to the baseline values. As would be expected, those with progressive disease had an increase in the mesothelin levels, compared to baseline. Mesothelin levels are related to tumour volume [96]. Therefore, if patients were classified as responders by mRECIST criteria due to a reduction in tumour volume, the mesothelin level is also expected to reduce. This is confirmed in our study. There were a number of missing data points with Mesothelin. The main issue around this being some patients not returning to hospital to have their pre-chemotherapy bloods. For some patients attending the GP practice was more convenient for the pre-chemotherapy

bloods. Unfortunately, as the Mesothelin test was a research blood test, they were unable to have their mesothelin test performed at GPs. The missed mesothelin tests were not reported as protocol deviations, a pragmatic decision was made at the start to allow the patients to have their blood tests at GPs where needed but every effort was made to repeat the mesothelin at their next visit to hospital. One way of overcoming the hurdle of missing mesothelin levels maybe to perform the mesothelin blood test on the day of their chemotherapy at the time of iv access.

There were no safety concerns regarding administering ZA at the same time as chemotherapy. No immediate complications of extravasation, flushing or low BP was recorded for any of the patients after receiving the infusion. In total 119 AEs and 16 SAEs were reported for the duration of the trial. All expected AEs and SAEs stipulated in the protocol were captured and reported. The commonest reported AE was constipation 11/119 (9%), the IMP was thought to be related in 4 of these cases, in 6 cases the IMP was not thought to be related and data was missing for 1 case. Electrolyte disturbance was reported twice, for the same patient where Magnesium supplementation was prescribed. As shown in Table 1-8 the SAEs were largely expected, and predominantly due to the side effects of chemotherapy. One patient had deranged electrolytes on a background of sepsis and diarrhoea. And a low magnesium on this context was considered 'possibly related' to ZA.

Two protocol deviations were reported, one of which was a significant deviation where a patient received 'stock' held ZA in error. This was the first patient to be randomised in the trial and the first to receive treatment at the chemotherapy suite. Due to unfamiliarity of the trial, and familiarity of a stock drug that is administered to cancer patients regularly, ZA was administered open label on this occasion despite the prescription clearly stating ZA or placebo. Immediate measures were taken to prevent any future recurrences. This highlights the importance of communication and raising awareness of new trials when setting up sites.

The information obtained from the semi-structured interviews suggest that the trial related procedures were broadly acceptable to patients. A repeated complaint was the time delay associated with administering the drug, on the day of their chemotherapy. This issue was primarily with one site

and when identified measures were taken to expedite the prescription completion and delivery process which had a significant impact on lessening the time delays. Despite a long chemotherapy day made even longer by administration of a trial drug, patients preferred to have all treatment in one sitting rather than at two visits. Patients were also happy with the structure of the trial follow-up being embedded with oncology clinical visits.

Overall, the SSIs gave some insight into patients' reasons for taking part in a clinical trial. Altruism was cited as one of the main reasons while the need to take back control and find a cure for MPM was cited as another. Most patients had a good understanding of what randomisation entailed and the need for randomisation in clinical treatment trials.

Uncertainty regarding the future was another recurrent theme that emerged from the SSIs. Whether this was with regards to progression of disease, leaving loved ones behind, or simply not knowing what to expect next, after chemotherapy had finished. These themes are important not only from a trial perspective but from the point of managing such patients in the future. Follow-up and long-term care of MPM is not well described and the information gleaned here suggests that the patients may be suffering as a result. Not just from a clinical trial perspective but as a clinician caring for MPM patients we should aim for a more structured long-term follow-up arrangement for patients with MPM, whether this is with respiratory, oncology or their own GP. There is also a role for exploring whether this follow-up should be in combination with imaging or biomarker tests (discussed in the next chapter).

Other interesting themes unrelated to the questions posed to the patients, also emerged. Patients perceived the clinicians to have more knowledge of their disease process than they were informed. The withholding of information on occasions was thought to protect them from further disappointment. The thirst for information and the urge to be in control could be due to the need for control in a battle they felt they had lost.

In summary, although I am unable to report the full results of the Zol-A study, valuable feasibility data was obtained from the Zol-A study as discussed here. Whilst we were unable to meet the primary

feasibility outcome, important data regarding safety, tolerability and overall acceptability of the trial is available. When patients have been unblinded to treatment, the full results will be published in a peer reviewed clinical journal.

5.14 Trial protocol amendments

The substantial amendment 1 (SA01) was undertaken to allow more independence to the research nurses to allow randomisation. We also made a change to our inclusion criteria. We had previously stipulated that patients should have measurable pleural disease as per modified response evaluation criteria in solid tumours (mRECIST) criteria for them to be eligible for the study. However, a large proportion of our patients do not have measurable disease at presentation. Therefore, the eligibility criteria were amended slightly from mRECIST criteria to 'measurable disease on CT'. This inclusion criterion was removed altogether after the trial has been open for 3 months as it was preventing patients from participating in the trial. SA04 was another important amendment. We noted a number of patients declined chemotherapy and declined participating in the trial altogether. This is a well-known phenomenon in MPM as well as other oncology trials, some terminating prematurely due to poor accrual (NCT00597116; NCT00003508). In an attempt to explore patients' reasons behind their decisions not to take part in a clinical trial we sought REC approval to interview patients who declined the trial. All amendments since the original REC approval in May 2016 are summarised in Table 5-10.

List of amendments	Summary of change
SA01 14/09/2016	**Personnel randomizing to the trial has changed from pharmacy to research members as the randomization software allows randomization whilst protecting the blind.** **Minor change to inclusion criteria- removed 'modified RECIST' from measurable disease section.**
SA02 25/01/2017	**Change to eligibility criteria - remove 'measurable disease on CT (tumour thickness > 5mm)'** **Dr Steve Walker added as sub-investigator** **Radiological data collection is further explained in section 5.13** **Plan of analysis (Section 6.1) details how the radiological information will be used to calculate the sample size for the full study**
SA03 26/04/2017	**Addition of Patient appointment schedule v1.0 22/03/17**

SA04 08/09/2017	**Request to interview patients who decline participation in the trial Clarify number of patients for semi-structured interview (up to data saturation rather than the previously stated 10)**
NSA01 20/09/2017	**Extend recruitment period by 1 month, to November 2017**

Table 5-10: List of Amendments since initial REC approval

Chapter acknowledgements

I would like to thank the following oncologists for their support with recruitment to the trial; Doctors Alfredo Addeo and Charlie Commins for recruitment at NBT and UHB; Drs Ashley Cox and Matt Sephton at RUH; Dr Adam Dangoor at UHB. I would also like to thank the research and pharmacy teams at UHB and RUH for their help with day to day conduct of the trial. Finally, thank you to Mr Rob Thompson, our patient representative who tirelessly attended all except one TSC meeting, despite slowly progressive mesothelioma.

CHAPTER 6 A PROSPECTIVE STUDY OF SERIAL SERUM MESOTHELIN FOR MONITORING MESOTHELIOMA.

6.1 Background

Monitoring disease activity post chemotherapy is important in mesothelioma. The previous chapters have demonstrated potential roles for PET-CT and CT, however these modalities subject patients to high doses of radiation, are time consuming and expensive. Hence a less invasive, easily accessible and cheaper method of monitoring disease progression would be welcomed both by clinicians and patients. Specifically, for those in a remission stage under regular observation prior to second or third line treatments, a simple blood test performed at regular intervals as opposed to 3 or 6 monthly CT scans offers an appealing alternative to identify disease progression.

As a diagnostic biomarker Mesothelin has the most robust evidence compared to a number of other biomarkers investigated in mesothelioma [93]. No studies have assessed the utility of Mesothelin in monitoring of patients not receiving chemotherapy or other forms of active treatment. In this prospective cohort study, we aim to assess the ability of serum Mesothelin to monitor disease in patients who have completed chemotherapy or those receiving best supportive care (BSC).

I designed the study with Professor Maskell. At its inception we presented the study concept to the local oncologists treating mesothelioma at NBT, for their suggestions and support for the study. We planned to recruit all patients with a diagnosis of mesothelioma who were under active monitoring, having completed prior chemotherapy or receiving best supportive care. Ethical approval was granted for an amendment appended to the existing pleural investigation trial (South West REC Ref: 08/H0102/11).

6.2 Methods

From February 2014 to October 2016 consecutive patients were prospectively recruited to the study alongside normal clinical care. Patients were invited to participate if they had a histocytologically confirmed diagnosis of malignant pleural mesothelioma (MPM) and were under active monitoring for the disease, aged over 18 years and had an expected life expectancy greater than 3 months. Patients who were receiving chemotherapy were not included, until they had completed their treatment. All patients gave written informed consent. At baseline, patient demographics, method of diagnosis of MPM and WHO performance status were recorded. Blood samples were taken at baseline and a full staging CT scan was performed. This study had no impact on the treatments received by the patient, but prior treatment information was recorded.

6.2.1 CT imaging and reporting

All patients had a baseline full staging CT scan. Patients who had just completed chemotherapy had CT scans every 3 months. Those on best supportive care had CT scans every 6 months. CT scans were reported by two methods. Firstly, they were all reported in a standard reporting fashion for clinical purposes by a consultant thoracic radiologist who classified the scans into radiological progression, stability or partial response but did not use formal reporting criteria (herein described as the 'clinically reported CT'). Secondly, another independent radiologist and I assessed the same CT scans using the modified RECIST criteria (herein described as the 'mRECIST CT') [118].

Assessment of pleural tumour by mRECIST criteria involves measuring 2 sites of tumour at 3 different anatomical levels, where the tumour thickness is measured perpendicular to the chest wall or mediastinum. The thickness of the tumour measured should be at least 1cm, lesions less than 1cm are considered non-measurable [118]. The resultant 6 pleural thickness measurements are then summed up to give one univariate measure (Figure 6-1).

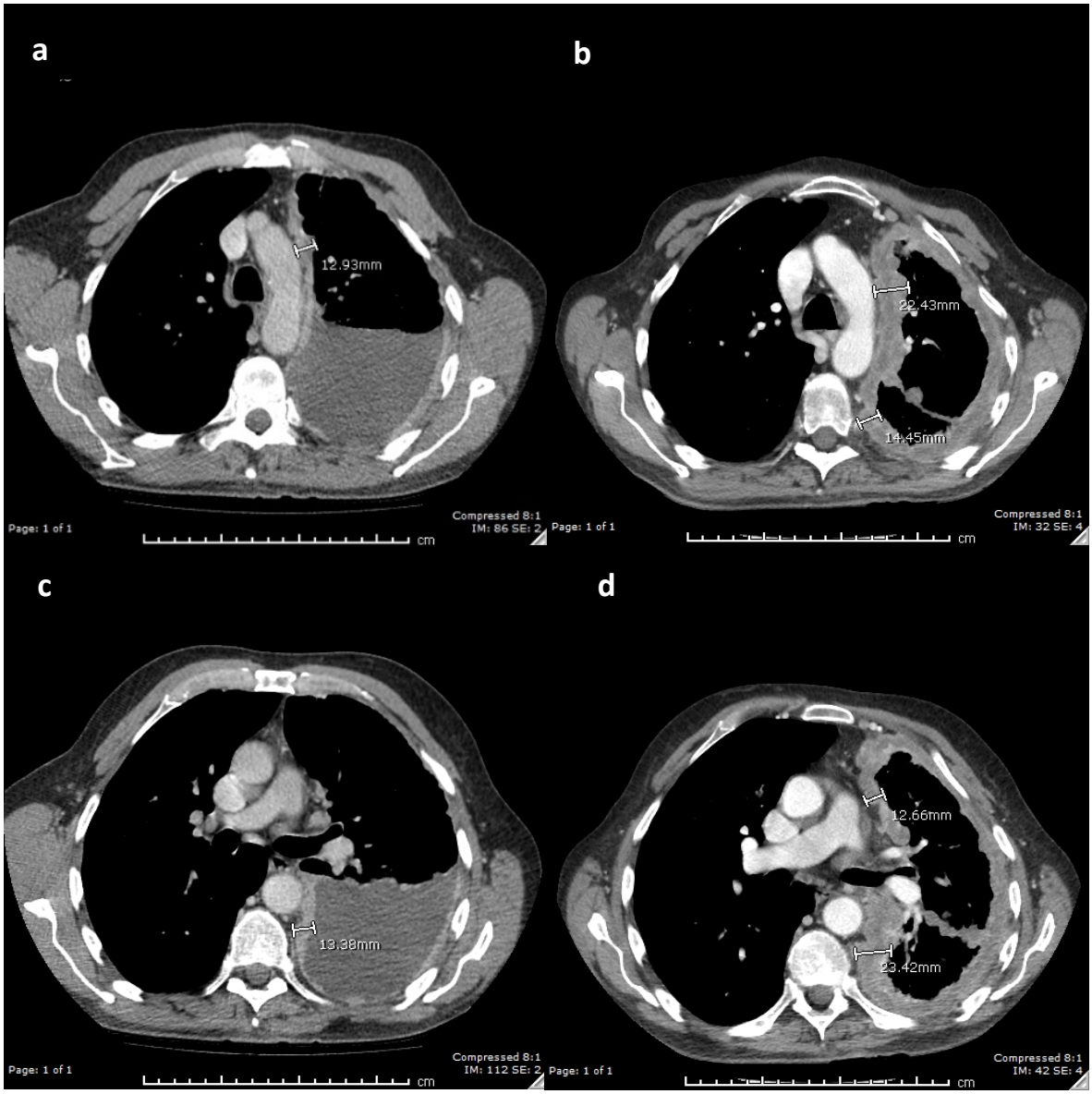

Figure 6-1: Measurement of tumour thickness for calculation of mRECIST. Tumour thickness measured perpendicular to the mediastinum. Measurement of 2 sites at 2 levels shown here. Images *a* and *c* are from the baseline scan. Images *b* and *d* from a subsequent scan 6 months later, showing significant progression of disease.

The mRECIST criteria define a complete response (CR) as the disappearance of all target lesions with no visible tumour elsewhere and partial response (PR) as at least a 30% reduction in cumulative tumour measurement. Progressive disease (PD) is defined as an increase of 20% in cumulative tumour measurement or emergence of new lesions. Stable disease (SD) is defined as not fulfilling the criteria for PR of PD. All reporting physicians were blinded to mesothelin results.

Percentage reduction in tumour is calculated using the formula below [118]:

$$\text{mRECIST score percentage change} = \frac{(\text{mRECIST score on scan 2} - \text{mRECIST score on scan 1})}{\text{mRECIST score on scan 1}} * 100$$

(where scan 2 is a subsequent scan to scan 1 usually 3 or 6 months after scan 1 in our study)

6.2.2 Mesothelin sample processing

Serum samples for Mesothelin testing were collected at respiratory or oncology clinic appointments. They were routinely analysed using the commercial Mesomark™ ELISA (Fujirebio) according to the manufacturer's recommended methods for sample processing, by investigators unware of the patients clinical or radiological characteristics. Mesothelin samples were processed using a manual enzyme linked immunosorbent assay (ELISA). Samples were batch processed with 12-14 samples at a time with calibration samples and controls analysed in parallel. Spun down whole blood was stored at -20 degrees Celsius until they were ready for processing. Two separate monoclonal antibodies are used in routine mesothelin sample testing, one to capture the mesothelin peptide and the second for the detection of it. Diluted patient serum (10µL per sample) is added to antibody coated wells which were then incubated on a 700rpm plate shaker for 60 minutes. This was followed by a rinse-cycle to remove any residue. Next the conjugate monoclonal antibody (100µL) was added and incubated on a 700rpm plate shaker for another hour. Once this was rinsed through a substrate was added which binds to the conjugate antibody allowing detection of serum mesothelin related peptide (SMRP also called Mesothelin) by ELISA. The optical density of ELISA is directly proportional to amount of SMRP present. Using the calibrators and controls the samples can then be interpreted to give a value in nanomolar. All samples were processed in duplicates and the mean of the 2 samples was counted as the final value. For calibrators, controls and samples the coefficient of variation should be ≤ 15%.
Baseline blood tests of liver and renal function were collected but not performed again unless clinically indicated.

6.2.3 Statistical analysis

Mesothelin levels were not normally distributed, therefore results were presented as medians with interquartile ranges (IQR). Comparison of baseline mesothelin levels between groups was performed using the Mann Whitney U test, with a p value less than 0.05 considered statistically significant. The non-parametric Mantel-Cox log-rank test was used to assess survival differences between groups. The Cox proportional hazards model was used to investigate independent and combined multivariable relationships between age, histological sub-types, treatment status and baseline mesothelin. Data imputation was not used for missing values.

The ability of serum mesothelin to predict radiological progression was assessed using timepoint analysis. A mesothelin measurement with a paired CT scan (defined as performed within a maximum of 31 days of each other) was called a 'timepoint'. These timepoints were grouped depending on the time from baseline into 3 monthly intervals. Two timepoints in the same patient allowed for a 'comparison' between the percentage change in mesothelin level and the radiological report (both clinically reported and mRECIST CTs). Patients were grouped into whether their CT scan showed progression of disease or stable disease/partial response. The 'baseline' for treatment naïve patients was when they were enrolled into the study. For patients who had received chemotherapy, the post-chemotherapy mesothelin and CT scan was considered the new point of reference, or baseline.

For the timepoint analysis a change in mesothelin was defined as a relative change from the previous mesothelin level, either 'falling' or 'rising', with separate analyses performed using 10%, 15% and 25% cut-offs. The thresholds were calculated by the following method;

$$\% \text{ change in mesothelin} = \frac{(\text{Later timepoint mesothelin} - \text{Earlier timepoint mesothelin})}{\text{earlier timepoint mesothelin}} * 100$$

The ability of mesothelin to predict radiological progression was assessed using sensitivity, specificity, predictive values and accuracy. Exploratory subgroup analysis was carried out based on baseline mesothelin level and histological type.

To further assess the ability of changes in mesothelin to track disease, independent of radiological assessments, the impact of a rising level on survival was calculated and compared to stable mesothelin levels and other poor prognostic indicators.

6.3 Results

6.3.1 Participants

In total, 41 patients with malignant pleural mesothelioma were recruited to this study and had a mesothelin taken at baseline alongside usual care (see Table 6-1). The majority were males (35 males vs 6 females) and the cohort had a median age of 72 (range 58-83). Twenty-three patients had received prior chemotherapy in the form of Pemetrexed and Cisplatin, with no patients being referred for surgical intervention. Eighteen patients received no chemotherapy either due to choice (n=8), poor performance status (n=6), or intention for delayed chemotherapy by the physician (n=4). Patients in the non-chemotherapy group were older on average (75 vs 69) but had a similar distribution of histological subtypes. Overall, 25 (61%) were epithelioid histological sub-type, while 9 (22%) were sarcomatoid, 4 (10%) biphasic and 3 (7%) were cytological diagnoses only therefore the exact subtype was not known.

6.3.2 Baseline mesothelin

All 41 patients had a serum mesothelin performed at baseline, with a median of 2.3nmol/L (IQR 1.2-6.6) across the entire cohort. Baseline mesothelin was significantly higher in the epithelioid (3.1 nmol/L IQR 1.25-13.6) compared to non-epithelioid (1.3 nmol/L IQR 0.75-2.95) histological subtypes (p=0.04). Survival of the entire cohort was 15 months (IQR 10-23). When analysed as a bivariate variable (dichotomizing the cohort at a mesothelin level of 2nmol/) there was no difference in survival between those with a low (<2 nmol/L) or high (≥2 nmol/L) baseline mesothelin with median survival times of 11 (IQR 7.5 to 20) months and 15 (IQR 10 to 26) months, respectively (p=0.191). Additionally, baseline mesothelin did not impact on survival when analysed as a continuous variable (p=0.193). There was no significant difference on baseline mesothelin or change in mesothelin levels depending on the patients' baseline renal function (eGFR).

	Post-chemotherapy (n=23)	Non-chemotherapy (n=18)
Median age (range)	69 (58-77)	75 (67-83)
Male (%)	22 (96%)	13(72%)
Histology -Epithelioid (n) -Biphasic (n) -Sarcomatoid (n) -Unknown (n)	 14 3 5 1	 11 1 4 2
Baseline Mesothelin -Median (nmol/L) -IQR (nmol/L)	 1.9 (1.2 – 13.4)	 3.4 (1.2 – 6.4)
Survival (months) -Median -IQR	 17 (14 – 26)	 10 (7 – 22)

Table 6-1: Baseline characteristics, mesothelin and survival.

6.3.3 Change in mesothelin and radiological progression

At baseline, all 41 patients had a CT scan within 31 days of a serum mesothelin measurement for comparison to later timepoints. The numbers of patients with data recorded at subsequent timepoints were: 2 at 3 months, 20 at 6 months, 3 at 9 months, 13 at 12 months, and 5 at later timepoints. This allowed for 43 time-point comparisons, with 25 time-points in the post-chemo group and 18 time-points in the non-chemotherapy group. The median time between mesothelin measurement and CT was 13 days (range 0 to 31 days).

When all comparisons were amalgamated across the cohort there was no change in median mesothelin levels in patients who had not progressed on the clinically reported CT (0.0 nmol/l (IQR -0.6 – 0.4)). This compared to a rise of 1.8 nmol/l (IQR 0.7 – 1.8) in patients with radiological progression of disease on the clinically reported CTs ($p < 0.01$).

Mesothelin Cut-off as a percentage change	10% change from previous mesothelin		15% change from previous mesothelin		25% change from previous mesothelin	
CT reporting method	Clinically reported CT (n=43)	mRECIST (n=25)	Clinically reported CT (n = 43)	mRECIST (n=25)	Clinically reported CT (n=43)	mRECIST (n=25)
Sensitivity (%)	95.8 (78.8-99.8)	90.9 (58.7-99.7)	83.3 (62.6-95.3)	72.7 (39.0-93.9)	80.0 (59.3-93.2)	72.7 (39.0-93.9)
Specificity (%)	73.7 (48.8-90.8)	57.1 (28.9-82.3)	84.2 (60.4-96.6)	71.4 (41.9-91.6)	84.2 (60.4-96.6)	71.4 (41.9-91.6)
PPV	82.1 (68.3-90.8)	62.5 (46.9-75.8)	87.0 (69.9-95.0)	66.7 (44.7-83.2)	87.0 (69.9-95.0)	66.7 (44.7-83.2)
NPV	93.3 (66.8-99.0)	88.9 (53.9-98.2)	80 (61.7-90.9)	76.9 (54.6-90.2)	76.2 (58.8-87.8)	76.9 (54.6-90.2)
Accuracy	86.0%		83.7%		81%	

Table 6-2: Ability of Mesothelin to predict radiological progression depending on cut-off used and radiological reporting method. (PPV – positive predictive value; NPV – negative predictive value)

Results from Table 6-2 demonstrated the ability of a rising mesothelin to predict radiological progression on both clinically reported and mRECIST CT in individual patients. Due to non-measurable disease (pleural thickening < 1cm) only 25 of the scans could be reported according to mRECIST criteria. A variety of pre-defined mesothelin cut-offs were tested. Regardless of cut-off used, mesothelin tracked the clinically reported CT results with greater accuracy than mRECIST CT reports. Resultantly, the clinically reported CT has been used for ongoing analyses. Figure 6-2 is a waterfall plot of the absolute changes of mesothelin divided by clinically reported CT report (SD and PR versus PD). It demonstrated that the vast majority of patients with radiologically progressive disease had a rising mesothelin (with only 1 patient having a small fall in level).

Further subgroup analysis was performed depending on baseline mesothelin or histological subtype, see Table 6-3. Serial changes in mesothelin predicted radiological progression with 100% sensitivity in patients with a high baseline mesothelin (defined as >2nmol/l). There was a fall in sensitivity and specificity in patients with low baseline mesothelin or sarcomatoid disease, although sensitivity remained above 90% in the former group.

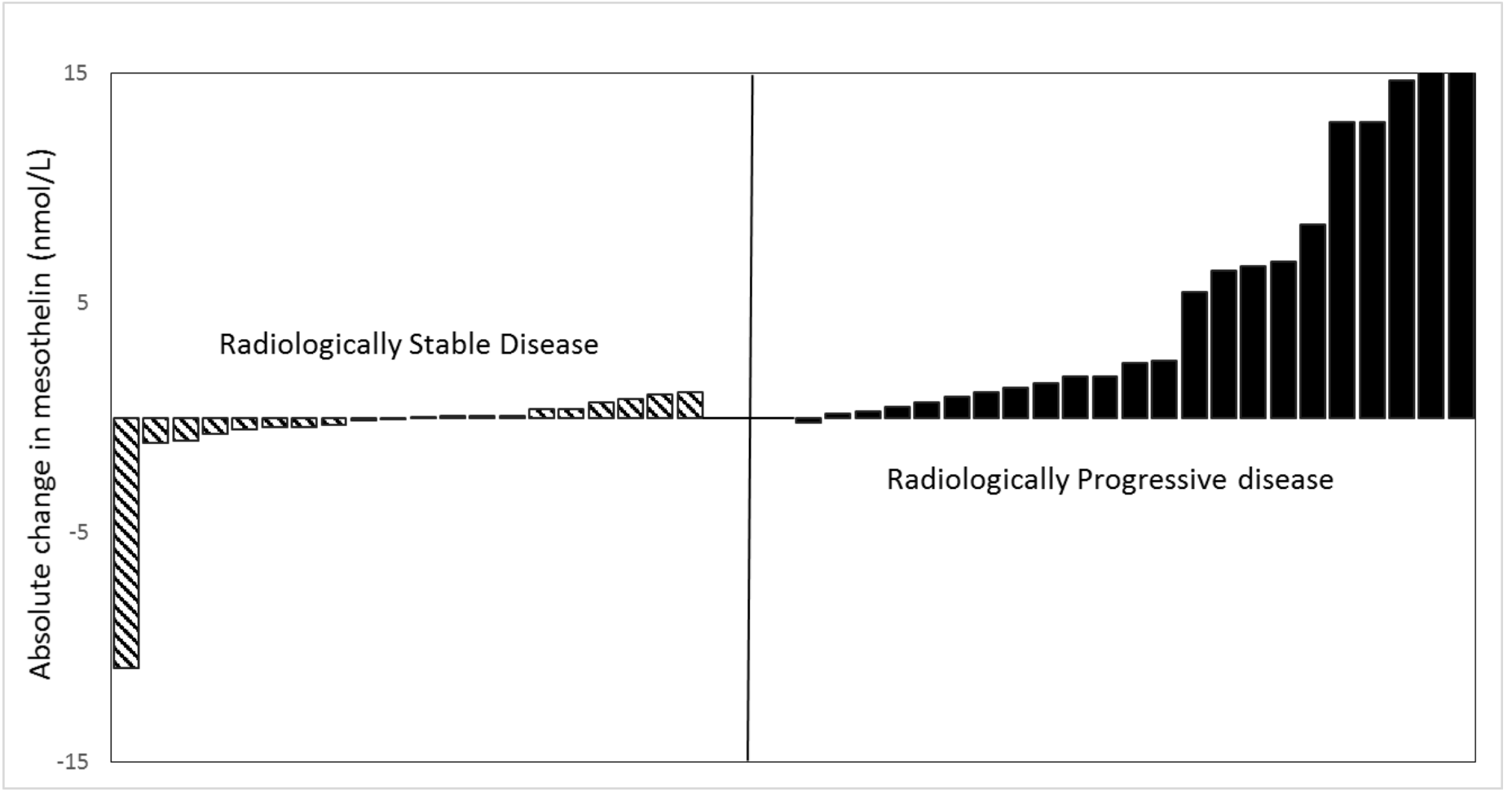

Figure 6-2: Waterfall plot showing changes in mesothelin between progressive disease and partially responded/stable disease groups (n=43)

	Baseline Mesothelin>=2 (n=19)	Baseline Mesothelin<2 (n=24)	Non-Epithelioid (n=9)
Progression on CT (Y/N)	13/6	11/13	5/4
Sensitivity	100 (75.3-100)	90.9 (58.7-99.7)	80.0 (28.4-99.5)
Specificity	83.3 (35.9- 99.6)	69.2 (38.5- 90.9)	75.0 (19.4- 99.4)
PPV	92.7 (68.4-98.7)	71.4 (51.9- 85.2)	80.0 (40.9-95.9)
NPV	100	90.0 (57.3-98.4)	75.0 (32.2-94.9)

Table 6-3: Subgroup analysis of ability of mesothelin to predict progression. (CT – computed tomography; Y - yes; n – no; PPV – positive predictive value; NPV – negative predictive value)

6.3.4 Change in mesothelin and overall survival

At 6 months, patients with a rising mesothelin (using a 10% cut-off) had a shorter median survival (from the date of sampling) compared to those with a falling or stable level (p=0.003). Those with a rising mesothelin had median survival of 175 days (IQR 80–211) compared to 448 days (IQR 321 to 554). Patients with radiologically progressive disease at 6 months (n=9) on clinically reported CT had a median survival of 170 days (IQR 72 - 223) from the date of CT scan compared to 433 days (IQR 255 to 581) in those with stable disease or partial response (n=11) (p=0.001). The majority of CT scans (10/21) performed at a 6-month time point were non-measurable using mRECIST criteria so these results were not analyzable.

In a multivariate cox regression analysis including age (≥70 vs <70), histological subtype (epithelioid vs non-epithelioid) and treatment modality neither change in mesothelin or clinically reported CT remained significant.

6.4 Discussion

This study assessed the ability of serum mesothelin to detect progression of MPM in patients not currently receiving active chemotherapy treatment. To our knowledge this is the first study to evaluate the role of mesothelin in disease monitoring in mesothelioma as opposed to mesothelin's role as a marker of treatment response during chemotherapy or surgery. No specific evidence exists on the optimal monitoring strategy for patients with MPM following first line chemotherapy. Based on expert opinion rather than published data, recent UK guidelines have suggested that following patients up every 3-4 month is good practice although what this follow up should involve was not specified [107].

There is considerable variation in UK practice [57], for two main reasons. Firstly, until recently many oncologists felt there was no data to support the role of second line chemotherapy in this setting [167] and there was limited access to clinical trials after first line treatment. There was, therefore, no perceived benefit from monitoring patients post first line chemotherapy or in those having BSC. However, given promising results from second line chemotherapy trials[110], as well as non-chemotherapeutic options [103] this perception is changing. Secondly, no radiological marker has shown the ability to accurately monitor disease progression [118]. A serum biomarker that could identify disease progression would be very useful to oncologists and physicians, with the additional benefit of reducing patient burden and healthcare costs.

Mesothelin is the most studied serum biomarker for mesothelioma with the majority of literature assessing its diagnostic potential. Although a raised level is fairly specific for MPM, many patients with non-epithelioid disease will have unrecordable levels even at an advanced stage. This has limited its utility as a diagnostic test, although recent studies

have attempted to combine it with more novel markers [168, 169]. The role of biomarkers in other malignancies has often begun as a putative diagnostic marker before becoming a marker of treatment response or recurrence (CA125, Prostate Specific Antigen) [170, 171].

Previous literature has shown that mesothelin can be used as a marker of treatment response when measured serially. The first example from Grigoriu and colleagues [126] took patients with a positive (>1nM/L) mesothelin at baseline receiving chemotherapy and immunotherapy (n= 40). They found that in patients with a 10% or greater rise in mesothelin there was a 75% chance of radiological progression using mRECIST as well significantly worse overall survival. The largest study of its type [96] correlated both CT scans (mRECIST) and PET (TGV and tumour volume) with change in mesothelin levels after treatment (using a 25% cut-off). In the chemotherapy group (n=55) the change in mesothelin significantly correlated with radiological response on CT ($p= 0.023$). Although only a minority of the cohort (n= 28) could be reassessed using PET imaging, due to prior pleurodesis or surgery, the change in both metabolic activity (TGV) and tumour bulk strongly correlated with change in mesothelin levels ($p<0.001$). Survival analysis demonstrated that the trend of mesothelin correlated with survival in a multivariate model that included age, sex, histology and treatment. Interestingly, this was not true when tumour volume on PET was added to the model, indicating that mesothelin was probably acting as a proxy for tumour bulk.

Across the literature we found several different thresholds for defining a clinically significant change in mesothelin level, including 10% [126], 15% [172] and 25% [96]. Wheatley-Price and colleagues [125] performed a post-hoc analysis of mesothelin values from a cohort of 42 patients using absolute (5mmol) and relative (10%) changes and their correlation with survival, finding that relative changes were more accurate. This current study assessed a variety of cut-offs, finding that a 10% cut-off reduced the specificity of a

rising mesothelin level but had a sensitivity of 96%. Given our aim was to assess the ability of mesothelin to detect disease progression we felt this reduction in specificity was justified with a false positive rate of 28% but a false negative rate of only 4%.

We demonstrated that disease progression could be detected using change in mesothelin of >10% with an accuracy of 86% and NPV of 94%. Unlike some previous literature [125, 126] this analysis did not exclude patients from the analysis with low baseline mesothelin or non-epithelioid disease. There has been uncertainty about the utility of serially measuring mesothelin in these patients. In this study, 44% (8/19) of patients with a mesothelin of less than 2mmol/L at baseline had an increased level (range 2.1-20.4) at later timepoints. Additionally, when the analysis was limited to patients with a baseline mesothelin of <2mmol there was only a limited reduction in sensitivity to predict progression. Given the small subgroup, no firm recommendations can be made regarding the utility of retesting mesothelin when the baseline is negative (<2nmol/l). This needs further assessment in other prospective studies.

This study has several limitations that could impact on the conclusions drawn. The cohort of patients was small, which has an impact on the conclusions of the multivariate analyses, but comparable to other studies of its type. This was an observational study alongside normal clinical practice and there were instances where a mesothelin was collected but the patient did not receive a scan within the pre-allocated 1-month period. Additionally, given the aggressive nature of MPM very few comparisons of timepoints over 12 months were available, but this is likely to be a pragmatic assessment of following up these patients in clinical practice. As previously mentioned other studies have monitored the change in mesothelin levels in response to systemic therapy. As a result, these studies had a proportion of patients where mesothelin levels fell considerably, which was correlated with radiological ‘partial response’. Worsening renal function has been shown to falsely

elevate mesothelin levels [173]. Although there was no difference in mesothelin sensitivity between patients with normal and abnormal renal function at baseline, there was no ongoing assessment during follow up. Given this study focused on patients not receiving treatment there were few instances of falling mesothelin. Finally, the difficulty with studies of this type is that the current 'gold standard' of monitoring (mRECIST CT) which mesothelin trends are compared to has been shown to be insensitive at detecting early progression. This analysis found that mesothelin correlated better with the clinically reported CT scan compared to mRECIST reporting, a finding of other similar studies [125]. Additionally, over half (25/43) of the CT scans used in the timepoint analysis were non-measurable by mRECIST criteria with a short axis diameter of less than 1 cm. This is another shortcoming of the mRECIST criteria that limits its sensitivity and applicability in clinical practice. It is for this reason that we assessed the impact of a changing mesothelin levels on survival as a means of validation, finding that a rising mesothelin at 6 months was a poor prognostic indicator. As with other cancer biomarkers [174] the reason for several 'false-positive' results (where a rising mesothelin occurred in the context of radiologically stable disease) could be because changes in mesothelin preceded radiological change.

In conclusion, we have demonstrated that a rising serum mesothelin is a sensitive marker of progression in the follow up of patients with MPM. Given the emergence of effective non-chemotherapeutic treatments and second-line agents, accurate disease monitoring is becoming increasingly important. This study has demonstrated that regular mesothelins could be used as a cost effective adjunct or alternative to serial CT scanning.

Footnote:

This chapter has been peer reviewed and published in the BioMed Central (BMC) Cancer online journal (February 2018). The mRECIST criteria used in this chapter are from version

1.0 as version 1.1 was not published at the time of CT reporting in this study. Hence the minimum measurable thickness is 1cm rather than 7mm as per the previous chapter.

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CHAPTER 7 FUTURE DIRECTIONS

Despite clinical guidelines with recommendations on the best management of pleural disease a number of issues remain unresolved. Asbestos related pleural disease is one such poorly researched area with low level evidence to inform guidelines. The diagnosis and management of malignant pleural mesothelioma (MPM) can be particularly challenging. This thesis explored the diagnostic conundrums and challenges of benign versus malignant pleural thickening. In addition, it examined aspects of treatment and monitoring of MPM.

7.1 Future directions:

- The DPT study confirmed patients without costo-phrenic angle obliteration could still have significant restrictive defect in their lung function. The IIDB now appreciates the limitations of relying solely on CXR appearances and have recently published a revised statement removing 'the costo-phrenic angle obliteration' requirement in order to be eligible for compensation and have recognised CT to be more sensitive for assessing DPT. The findings from this study will be included in any future iterations of their guidelines.
- Pleural pointillism is a promising simple visual assessment technique for differentiating benign from malignant pleural thickening on DWI MRI with high sensitivity and specificity. Currently only one study is published in the literature demonstrating its effectiveness. The study reported here is expected to be published in a peer reviewed journal in the near future. A large prospective multi-centre study with robust inclusion criteria is now needed to further assess its role, and the current study will be useful in providing the information required for the power calculation, for the grant application.

- A project is currently being designed by the radiology researchers at Oxford with plans to collaborate with all UK centres performing DCE-MRI scans to assess pleural thickening, in order to develop software that may be able to analyse these scans using artificial intelligence (AI). The MRI data obtained from the current study will be shared with Oxford to help with their AI project.
- The results of the TARGET study are awaited with much interest. The full results of the study will be available by mid-2019, after completion of the 6-month follow-up period. The results are expected to be published in a peer reviewed scientific journal. Hopefully, the findings will point in the direction of whether or not PET-CT targeted pleural biopsies are justified in a difficult to diagnose cohort of patients with suspected pleural malignancy.
- The Zol-A unblinding is planned for late July 2018. The final unblinded results are awaited with anticipation, to determine if there was any effect in delivering ZA alongside chemotherapy. If the overall findings are positive, funding to be applied for a full phase III trial. The results will be published in a peer reviewed scientific journal.
- The association between MPM and mesothelin is very encouraging. Further large prospective studies are needed to validate our findings. Following on from the current study, mesothelin has been included in a prospective longitudinal cohort study conducted by the Academic Respiratory Unit at North Bristol Trust – the Assess-Meso study. This trial is currently recruiting mesothelioma patients from 3 centres across the UK and hope to open 12 more over the next 12 months. As a longitudinal cohort study, it will recruit and monitor mesothelioma patients over a 10-year period. The study protocol specifies 3 monthly follow-up appointments with serum mesothelin levels and CT scans, well placed to answer the question

whether CT scans could be replaced by a simple blood test in mesothelioma patients.

PUBLICATIONS ARISING FROM THIS THESIS

de Fonseka, D., et al., *The physiological consequences of different distributions of diffuse pleural thickening on CT imaging.* Br J Radiol, 2017. **90**(1077): p. 20170218.
I drafted the first manuscript which was reviewed and improved upon by D Edey and Professor Maskell.
de Fonseka, D., et al., *Randomised controlled trial to compare the diagnostic yield of positron emission tomography CT (PET-CT) TARGETed pleural biopsy versus CT-guided pleural biopsy in suspected pleural malignancy (TARGET trial).* BMJ Open Respir Res, 2018. **5**(1): p. e000270.
This is a protocol publication for the TARGET trial. The trial protocol was drafted by myself and reviewed by Dr Chris Rogers from Bristol CTEU and Professor Maskell. I authored this manuscript relating to the protocol publication.

de Fonseka, D., et al., *A prospective study to investigate the role of serial serum mesothelin in monitoring mesothelioma.* BMC Cancer, 2018. **18**(1): p. 199.
I drafted the first manuscript which was reviewed by Professor Maskell and the co-authors.

PRESENTATIONS RELATING TO THIS THESIS

TARGET trial – randomised controlled trial to compare the diagnostic yield of positron emission tomography computed tomography (PET-CT) TARGETed pleural biopsy versus CT-guided pleural biopsy in suspected pleural malignancy. **D De Fonseka**, N Maskell. Poster presentation at British Thoracic Oncology Group meeting in January 2016

A prospective study using serum mesothelin to monitor mesothelioma. D Arnold, **D de Fonseka**, L Stadon, et al. European Respiratory Journal 50; supple 61; 2017. Poster presentation at ERS September 2017. Project was carried out by myself and poster done by myself but was unable to attend meeting therefore presented by Dr D T Arnold.

Zol-A feasibility trial: Trial update and initial results. **D de Fonseka**, E Keenan et al on behalf of the Zol-A trial team. Presented at the iMig meeting in May 2018.

Patients' experiences of being diagnosed with mesothelioma & participating in research – qualitative interviews from the Zol-A study. **D de Fonseka**, AC Bibby, AJ Morley and NA Maskell on behalf of the Zol-A study team. Presented at the iMig meeting in May 2018.

I hereby declare that the statements of contribution given above are accurate to the best of my knowledge.

Professor Nick Maskell
PhD Supervisor
15/07/2018

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APPENDIX I – TARGET TRIAL NIHR RFPB GRANT APPLICATION

Research for Patient Benefit

Reference number	PB-PG-0214-33095
Lead Applicant	Dr Nick Maskell
Research Title	Randomised controlled trial to compare the diagnostic yield of Positron Emission Tomography Computerised Tomography (PET-CT) guided pleural biopsy versus CT-guided pleural biopsy in suspected pleural malignancy. (PET-biopsy trial)
Plain English Summary	Many patients with a history of asbestos exposure can develop irregular thickening of the lung lining (the pleura). Although in many cases the pleural thickening is not serious a small proportion of patients can develop cancer of the lung lining (mesothelioma). Chest X-rays and CT (computed tomography) scans of these patients can indicate a suspicion of mesothelioma but the only way to confirm the diagnosis is by obtaining a sample of the lung lining and examining the cells. Unfortunately, in a subgroup of patients, it can take a considerable time to confirm the diagnosis of mesothelioma with a tissue sample. This is due to the difficulty of obtaining a sample containing cancerous cells in some patients. These patients will often require repeated samples to be taken until a definitive diagnosis can be made. A rapid diagnosis is important as, although mesothelioma is incurable, life-extending treatments started early can be effective and diagnosis allows patients with confirmed mesothelioma to apply for compensation. Currently, pleural tissue samples are taken with guidance from a CT scan of the lungs. We believe that another type of scan (Positron Emission Tomography 'PET scan' is a whole body scan where radio-labelled glucose is taken up more avidly by cancerous cells) may be better at identifying the location of the cancerous tissue allowing a more targeted biopsy to occur, thus improving the diagnostic accuracy. PET scanners are expensive and in high demand so good evidence is required before they can be used routinely for this purpose. This study aims to determine how effective PET-scan targeted tissue sampling is compared to the conventional CT targeted sampling in patients requiring a second biopsy. We intend to conduct a clinical study comparing the results in two groups of patients who have been randomly allocated to

	one or the other method.
Total Research Cost	344,811.00

The Department of Health, National Institute for Health Research (DH NIHR) is the Data Controller under the Data Protection Act 1998 ('the Act'). Applicants for funding should be aware that information contained in this application might be shared with other DH NIHR bodies for the purposes of statistical analysis and other DH NIHR management purposes, including targeted communications with selected groups of researchers. Applicants may be assured that DH NIHR is committed to protecting privacy and to processing all personal information in a manner that meets the requirements of the Act

7. Patient and Public Involvement

In this section it is important that you describe, in as much detail as possible, how patients and the public have been involved in the development of the application as well as plans for involvement in the proposed research

Were patient and the public actively involved in either identifying the research topic/prioritising the research questions and/or preparing this application?
Yes

If Yes, please tick the appropriate boxes below
Involved in identifying the research topic/prioritising the research questions, Involved in preparing the Application

Please further describe how patient and public involvement has informed and/or influenced the development of the application and how patients and the public have been actively involved

Our research question and study protocol was put forward to a mesothelioma focus group made up of patients with the disease and their relatives/carers. We have redesigned our protocol and methodology as below, following the groups' feedback.

The research question was thought to be important and relevant as pleural malignancy (predominantly mesothelioma) can be difficult to diagnose. Patients undergoing investigations for mesothelioma would want a speedy diagnosis. Repeated investigations can be distressing. Negative results and further investigations can be frustrating to patients as well as leading to a delay in diagnosis. Therefore, PET scanning can be utilised as a quicker and more efficient way of diagnosing in a small group of patients where the diagnosis can be challenging.

There was concern regarding the extra travel costs for patients randomised to the PET arm. We have now costed in their travel expenses into the research costs.

The focus group highlighted an additional scan could incur a delay. In response to this we are now developing a standard operating policy (SOP) which would be adhered to across the sites, to ensure the PET-scan is done in the 2 week window while patients would normally be waiting to have the CT-guided biopsy.

We had originally planned a sub-study involving magnetic resonance imaging (MRI) in addition to PET scanning for those who would be randomised to the PET arm. The focus group was in the opinion that 3 scans would be too many for patients and that could deter them from entering the study. Therefore, MRI component has now been removed from the study.

Members of the focus group reviewed the lay summary on this application and advised to change some of the wording and technical terms that were originally used.

The Avon Mesothelioma Foundation is a charity made up of patients with mesothelioma and their relatives. They have supported our study as an important study which would help patients to get to their diagnosis quickly.

The original focus group and the North Bristol NHS Trust Research lay panel will be reviewing our patient information sheets to ensure they are easily understandable.

Please indicate the ways in which the public will be actively involved in the proposed research, by ticking all relevant boxes

Design of the research, Management of the research (e.g. steering/advisory group), Developing participant information resources, Contributing to the reporting of the study report, Dissemination of research findings

Please give more details, including how patient and public involvement will benefit the research, the reasons for taking this approach and arrangements for training and support
As mentioned above we have taken on board patients' and their relatives' views on the design of the study. Throughout the trial we will consult with the patient focus group on any changes made to the protocol and in preparation of the patients information sheet. This group together with the Research & Innovation Lay panel at North Bristol NHS Trust will be reviewing our patient information sheets. The opinions of patients and lay people are important as they may highlight issue that are not necessarily picked up by clinicians. We would like to have had a patient representative on our trial steering committee but unfortunately due to the nature of the disease (poor survival) it would be difficult to have a patient sitting on the trial committee. However partners of patients with mesothelioma are keen to support our research, therefore we have approached Anne Craig (a patient's partner) who has agreed to sit on our trial steering committee. We shall use one of the members from our focus group to help with the final write up of the research paper. Our research findings will be presented locally at an Avon Mesothelioma Foundation study day. These meetings are usually attended by patients with mesothelioma, their relatives and relatives of patients who have died of mesothelioma. At a national level we would apply to present our findings at a MesoUK study day. Members of the focus group we have used above will be informed of the end result in a final group meeting. The original focus group and the North Bristol NHS Trust Research lay panel will be reviewing our patient information sheets to ensure they are understandable.

8. Case for Support - Part 1

Aims and objectives

Describe the overarching aims of the research, outlining the research question which the work will address

Patients with suspected cancer of the lung lining (pleura) often have a biopsy of the pleura to confirm or refute the diagnosis of malignancy. Usually the biopsy is performed using radiological guidance (Computed Tomography or Ultrasound) or during thoracoscopy (a camera into the chest cavity through a small incision on the chest wall, which allows visualisation of the pleura and biopsy) . If the original biopsy does not give a definitive answer they would go on to have a second biopsy and this is usually under CT (Computed tomography) guidance.
Those patients who have already had one non-diagnostic biopsy, tend to have a low overall diagnostic rate for second and subsequent biopsies. Identifying an area where a high yield of cancerous cells is likely to be obtained at biopsy can be difficult from CT imaging alone. Positron Emission Tomography (PET) scanning can identify areas with high metabolic activity that occurs with malignancy. If patients have a PET scan first to identify areas of high metabolic activity then target the CT guided biopsy to these areas, the yield is much higher than just CT guided biopsy.
The overall aim of this study is to compare the effectiveness of PET targeted biopsy over CT guided biopsy in a population of patients with suspected pleural cancer, who have had one negative pleural biopsy.
Patients will be randomised to either have a PET scan followed by a targeted CT guided biopsy. Or a CT guided biopsy alone.
Our primary outcome measure is the number of pleural malignancies correctly diagnosed by PET-CT guided biopsy versus CT guided biopsy. All patients will be followed up for a year, during which time those patients who have cancer but have negative biopsies will become clinically and radiologically apparent, as their disease progresses.
As a secondary outcome measure we would like to look at the benefits of using PET-scan targeted biopsy in terms of improving diagnostic delays, number of hospital attendances for patients, number of invasive pleural procedures, survival and reduction in costs associated with health related resource use.

Mesothelin is a biomarker that can be raised in patients with cancer of the lung lining (Mesothelioma). At present the role of Mesothelin is unclear in the diagnostic pathway of mesothelioma. We would like to investigate the role of Mesothelin in patients with suspected mesothelioma by testing their levels at enrolment and at subsequent follow-up to evaluate its potential role in the diagnostic pathway for pleural malignancy.

Plain English Summary

Please summarise your proposed research in plain English

Many patients with a history of asbestos exposure can develop irregular thickening of the lung lining (the pleura). Although in many cases the pleural thickening is not serious a small proportion of patients can develop cancer of the lung lining (mesothelioma). Chest X-rays and CT (computed tomography) scans of these patients can indicate a suspicion of mesothelioma but the only way to confirm the diagnosis is by obtaining a sample of the lung lining and examining the cells.

Unfortunately, in a subgroup of patients, it can take a considerable time to confirm the diagnosis of mesothelioma with a tissue sample. This is due to the difficulty of obtaining a sample containing cancerous cells in some patients. These patients will often require repeated samples to be taken until a definitive diagnosis can be made. A rapid diagnosis is important as, although mesothelioma is incurable, life-extending treatments started early can be effective and diagnosis allows patients with confirmed mesothelioma to apply for compensation.

Currently, pleural tissue samples are taken with guidance from a CT scan of the lungs. We believe that another type of scan (Positron Emission Tomography 'PET scan' is a whole body scan where radio-labelled glucose is taken up more avidly by cancerous cells) may be better at identifying the location of the cancerous tissue allowing a more targeted biopsy to occur, thus improving the diagnostic accuracy. PET scanners are expensive and in high demand so good evidence is required before they can be used routinely for this purpose.

This study aims to determine how effective PET-scan targeted tissue sampling is compared to the conventional CT targeted sampling in patients requiring a second biopsy. We intend to conduct a clinical study comparing the results in two groups of patients who have been randomly allocated to one or the other method.

Scientific abstract of research

Please provide a structured summary which outlines the background to the research, the aims of the work, including the question to be addressed by this research, the plan of investigation and a summary of the potential benefits to patients and the NHS

Background

Asbestos was a commonly used material in the building industry in the 1950s. Due to the unknown deleterious effects to humans from asbestos exposure, it was used without any health and safety protection at the time. There was an increasing emergence of lung disease that was later found to be attributable to asbestos exposure.

Malignant pleural mesothelioma is cancer of the lung lining occurring as a consequence of asbestos exposure. It is an aggressive and fatal tumour that is still on the increase in many parts of the world. Due to its long latency period from exposure, patients may not develop mesothelioma up to 30 to 40 years from exposure. Unfortunately, once diagnosed the average life expectancy is 9 to 15 months, as no curative treatments are available for mesothelioma.

Diagnosis of mesothelioma can be challenging in the absence of pleural fluid (fluid in the chest cavity), to perform a thoracoscopy for direct visualisation and biopsy of the pleura. Computed Tomography (CT) or Ultrasound (US) guided biopsy of the pleura are two of the commonest techniques used in this situation but the diagnostic rate is low.

Diagnostic imaging in pleural malignancy remains a significant challenge. PET scanning has proved itself a useful tool in diagnosing and staging lung cancer. It identifies areas of high metabolic activity, which is a feature of malignant disease, by highlighting areas of uptake of the radio labelled glucose analogue Fluorodeoxyglucose (FDG).

We hypothesise that targeting the CT guided biopsy to these highlighted areas on PET may increase the diagnostic yield.

Aims

This study will assess whether a PET-CT guided biopsy is superior to a standard CT guided biopsy when obtaining pleural tissue in suspected pleural malignancy, in patients who have already undergone one non-diagnostic pleural biopsy.

At present there are no studies comparing the two approaches. Pilot data from our institution has shown a 66% sensitivity with PET for repeat biopsies as opposed to 20% sensitivity with a repeat CT guided biopsy.

Plan of investigation

This multi-centre randomised controlled study will recruit patients from 6 respiratory departments over a 24 month period or until 78 patients have been recruited. Patients will be randomised either to receive a PET targeted biopsy or a standard CT guided biopsy using an online randomisation tool provided by the Bristol Clinical Trial Evaluation Unit (CTEU). The experimental group will undergo a PET scan which will be reviewed by a local radiologist to identify the most suitable area for biopsy, then a CT guided biopsy targeted to the afore highlighted area. The standard CT group will have a repeat CT and a biopsy from a site identified as per the local radiologist. Standard Operating Procedures (SOP) will be in place to minimise variation.
The diagnostic yield for the two biopsy methods in identifying malignancy will be compared using the chi-squared test for proportions.
Final analysis will occur once all recruited patients have undergone a second pleural biopsy and a tissue diagnosis confirmed or 12 months follow-up occurred.

Potential impact

If this superiority study is proven successful, it would shorten the patient's cancer journey and reduce the number of invasive investigations they undergo.
An early diagnosis may allow more patients to have life prolonging chemotherapy that they may not be able to have at a later stage in disease if they are too unwell.
Mesothelioma patients have a very poor survival and often some of this time may be spent in hospital. An early diagnosis would allow patients more time to enjoy the financial benefits of the compensations they receive following a diagnosis of mesothelioma.

Background and rationale

What is the problem being addressed?

Describe the background to the research, describing the limitations identified in the evidence base that the research is trying to address

Asbestos was a commonly used material in the manufacturing and building industry since the late 19th century for its high tensile strength, heat resistance and affordability[1]. Due to the unknown deleterious effects to humans from asbestos exposure, it was used without any health and safety protection at the time. There has been an increase in emergence of lung disease that was later found to be attributable to asbestos exposure[2].

Patients who have been exposed to asbestos can develop thickening of the lung lining (pleura). Although in most cases the pleural thickening is benign in nature, a small proportion of patients can go on to develop cancer of the lung lining (malignant pleural mesothelioma) secondary to the asbestos exposure.

Malignant pleural mesothelioma is an aggressive and universally fatal tumour, the incidence of which is increasing in many parts of the world and currently kills one person every 4 hours in the UK[3]. Despite accounting for 1% of malignant disease in this country, it remains under researched and is currently incurable.

The vast majority of patients require a pleural biopsy in order to confirm the diagnosis. When lack of pleural fluid makes thoracoscopy difficult or when pleural thickening is the only abnormality on CT, pleural tissue is usually obtained using CT guided pleural biopsy with a Tru-cut needle[4] or with ultrasound guidance. However, the yield remains low (70-75%) as only one small area of the pleural thickening is biopsied, leading to occasional false negative biopsy results. This means patients often undergo multiple diagnostic procedures in order to establish a histological diagnosis.

Diagnostic imaging in pleural malignancy remains a significant challenge and a topic of international debate[5]. Tumour growth is unlike that of solid tumours due to its circumferential expansion and hence tumour may conceal itself within areas of pleural thickening. This, along with secondary pleural effusion and atelectasis make precise delineation of the tumour volume and radiological staging difficult. In addition, the appearances of benign pleural thickening and pleural malignancy on CT may be similar and hence other imaging modalities have been evaluated in order to improve the diagnostic pathway for patients.

Positron Emission Tomography (PET) scanning has proved itself as a useful tool in the diagnosis and staging of lung malignancy. It identifies areas of tissue with the highest metabolic turnover by highlighting areas of uptake of the radio-labelled glucose analogue - Fluorodeoxyglucose (FDG). Initial results in pleural malignancy have been encouraging[6], but no study has yet looked at using this modality to target biopsies.

We hypothesise that targeting the CT guided biopsy to the 'hot' areas on PET may improve the diagnostic yield. This would reduce the number of biopsies required to make a diagnosis (with their associated risks and costs).

Audit data in our institution recorded that only 3 out of 15 (20%) repeat pleural biopsies for suspected pleural malignancy (all later confirmed to be mesothelioma) were positive. This compares to 6 patients for whom special funding was obtained to undergo a PET-CT guided pleural biopsy targeting the area of highest metabolic activity instead of a repeat CT guided biopsy. A positive histological diagnosis was made in 4 of the 6 patients (66%). All 6 were eventually confirmed as having malignancy. This data reflects PET-CT scanning and biopsy is far superior to CT guided biopsy alone.

This has highlighted a potentially significant role for PET scanning in this group of patients, which warrants further investigation.

In this study we will also compare the use of the different PET-CT interpretation methods, Standardized Uptake Value (SUV) and Total Glycolytic Volume (TGV), different methods of interpretation of PET-CT findings, to evaluate their role as non-invasive methods of predicting a subsequent diagnosis of malignant disease.

Identification of a potential biomarker for mesothelioma is also a subject of current research. Mesothelin levels have been shown to be elevated in 80% of patients with mesothelioma at presentation but its role is yet to be firmly established in routine clinical practice[7].

A literature review using PubMed and the keywords malignant mesothelioma, fluorodeoxyglucose-positron emission tomography biopsy and computed tomography biopsy did not identify any randomised controlled trials directly comparing the two groups of PET versus CT guided biopsy for histological confirmation of malignant

mesothelioma. Published literature has identified PET scanning as a highly sensitive imaging method to diagnose and stage mesothelioma[8] but histological confirmation is still required with a biopsy of the pleura for the final diagnosis.

This trial will be the first to address targeted biopsies in patients with suspected pleural malignancy using PET scanning and to evaluate the role of these other novel tests in the diagnostic pathway.

If this proves successful, it could alter the investigation pathway for patients with this condition and help to expedite a diagnosis. By doing so, we would expect fewer repeat procedures required to make a diagnosis and hence a reduction in associated risks and costs. In addition, by expediting the diagnosis, more patients may be fit enough to receive chemotherapy, which has been shown to have a survival advantage[9].

Why is this research important in terms of improving the health of the public and/or patients and the NHS?

If our study proves that PET scanning and targeted biopsies are superior to current standard care of CT guided biopsy, for patients who have already had one non-diagnostic biopsy, then PET scanning can be incorporated into the diagnostic pathway of suspected mesothelioma patients. At present, although PET-scanning is widely used in cancer diagnosis and staging, it has a very limited role in mesothelioma.

Literature suggests PET-scanning as a method of identifying areas of high metabolic activity that is invariably seen with mesothelioma. Therefore utilising this technique to target areas for biopsy would increase the chances of a positive biopsy.

There are several reasons for trying to obtain an early diagnosis for patients with suspected mesothelioma:

1. Mesothelioma is an incurable disease and patients have a median life expectancy of 6 months[9] from diagnosis, without palliative treatment such as chemotherapy. Chemotherapy can prolong life to 8 to 14 months.[10] Patients cannot have chemotherapy without histological confirmation of mesothelioma. Therefore it is vital to confirm the diagnosis by biopsy if patients are to have chemotherapy.
2. The longer patients wait for a diagnosis the higher the chances their disease could progress and can become 'unfit' for chemotherapy due to general deterioration in health. Therefore, an early diagnosis could lead to more patients being suitable for palliative treatment and prolongation of life.
3. Patients diagnosed of mesothelioma are eligible for compensation. If at the time of diagnosis patients are fit and healthy they are able to enjoy these financial benefits more, compared to a later stage of diagnosis where they are likely to be unwell from the disease.
4. More chances of a positive diagnosis with PET-scanning means patients are less likely to under go repeated invasive investigations. Fewer hospital trips and more likely to avoid surgery for a biopsy which would require an inpatient hospital stay and a general anaesthetic for surgery.

A reduction in the number of investigations and saved bed days would be favourable for the NHS from a health economic point of view.

Novel bio-markers play an important role in monitoring disease activity. Mesothelin is one such marker that is elevated in mesothelioma. We would like to investigate the role of mesothelin in the diagnostic and monitoring pathway of mesothelioma, whether mesothelin can be used to as a surrogate marker of disease response to chemotherapy and in disease progression.

9. Case for Support - Part 2

Research Plan

Describe the proposed research plan, providing descriptions of the overall research design and a strong justification of sampling strategies, methods of data collection and analysis.

It is vital to add as much detail as possible on design and methodology, including justification of sample size, power calculations and sample selection and exclusion criteria where applicable.

Study design: A multi-centre parallel group randomised controlled trial (RCT) to compare PET-CT guided pleural biopsy versus standard CT guided biopsy for the diagnosis of mesothelioma.

Population: Patients with suspected pleural malignancy

Setting: Respiratory departments in 6 hospitals in the UK.
The lead centre will be North Bristol NHS Trust. The other centres that have agreed to recruit patients to the study are: Oxford Centre for Respiratory Medicine, Oxford; Kings Mill Hospital, Mansfield; Great Western Hospital, Swindon; Royal United Hospitals, Bath and Musgrove Park Hospital, Taunton

Inclusion Criteria: Patients eligible for the trial must meet all of the following criteria at randomisation

- Undiagnosed pleural thickening on CT suspicious of pleural malignancy
- Have had a pleural biopsy (either by thoracoscopy or under radiological guidance) which was non-diagnostic for cancer
- Lung Cancer Multi-disciplinary team (MDT) meeting decision to perform further CT-guided biopsy to pursue a diagnosis

Exclusion Criteria: Patients will not be eligible if any of the following apply:

- Unfit for a radiological biopsy (coagulopathy, severe underlying lung disease)
- Expected survival of less than 1 month
- Unable to give written informed consent
- Pregnancy or lactation
- Age <18 years
- Prior talc pleurodesis

Interventions: Eligible patients who consent to participate will be randomised in equal proportions to receive a second biopsy, either with PET scanning and targeted CT biopsy or a standard CT guided biopsy.

Image acquisiton will be standardized as much as practically able, across the sites. All sites are members of the PET/CT research network, which undertakes regular quality assurance measures (under the supervision of the NCRI PET core lab based at St. Thomas' Hospital in London) to ensure the PET centres have standards to deliver high quality PET data for multicentre trials.

Participants allocated to the PET group will first undergo a PET-scan. This will take place at the recruiting centre where these facilities are available locally, or at the regional PET centre.

The pleural biopsy will then be performed with CT guidance but targeted to areas highlighted on the PET-scan. The PET scan is to take place within the 2 week window period while patients are awaiting the CT guided biopsy.

The other group will have a standard CT guided biopsy.

If the results of this second biopsy (whether PET targeted or standard CT) are also negative for cancer the participants may undergo further investigations at the discretion of the treating clinician or according to MDT decision.

All patients regardless of which arm they are randomised to, will have serum Mesothelin levels performed at baseline and at each follow up visit (1, 3, 6 monthly and at 12 months from recruitment).

Primary outcome: A definitive diagnosis of cancer on the second biopsy.
Secondary outcomes: Secondary outcomes will include

- Total number of invasive procedures (video-assisted thoracic surgery [108] or radiology guided biopsies) undertaken following randomisation to confirm the diagnosis
- Time from randomisation to cancer diagnosis (those not diagnosed with cancer will be censored at last follow-up)
- Time from randomisation to death (survivors will be censored at last follow-up)
- Total number of hospital attendances following randomisation to confirm the diagnosis
- Procedure related adverse events
- Fitness for chemotherapy following a positive diagnosis (assessed using objective published criteria)
- Uptake of chemotherapy following a positive diagnosis, in the 12 months following recruitment
- Health status as measured using EQ-5D
- Estimated costs associated with health-related resource use from randomisation to diagnosis
- Serum Mesothelin levels measured at baseline and 6 months
- PET scan parameters (TGV, maximum and mean SUV) (PET-CT group only)

Participant follow-up: Participants will have clinical follow-up at 1, 3, 6 and 12 months. At each follow-up appointment participants will have a chest X-ray and a clinical examination of the chest as a minimum. They will also have serum mesothelin levels repeated at each follow-up visit. Active participation in the trial will end at 12 months after randomisation or death, whichever is sooner. Unidentified underlying malignancies will become apparent during follow-up year, due to radiological and clinical deterioration.

Sample size: The study size has been set at 78 participants, 39 allocated to the CT guided biopsy group and 39 to the PET-CT guided biopsy group. A study of this size will have 80% power (5% statistical significance) to detect a difference of 30% in the proportion of participants with a positive cancer diagnosis after the second biopsy (20% in CT group vs. 50% in PET-CT group). This difference represents a large effect size, but is consistent with our audit data, and would be sufficiently compelling to lead to a change in practice. A study of this size will also have 80% power to detect a doubling of the “hazard” (hazard ratio of 2) for time to diagnosis or survival, assuming 5% dropout.

Recruitment rate: Recruitment will take place over a 24 month period across 6 institutions (average 6 to 7 patients per year per site). Our audit data suggest these numbers are achievable.

Recruitment: Potential study patients will be identified by principal investigators (PIs) at each site at the local lung cancer multi-disciplinary team meetings (MDTs). A member of the local research team (trial investigator or research nurse) will send study information to eligible patients, before meeting them at their next hospital visit, when they will discuss the trial and answer any questions. Patients willing to take part will then be asked to provide written informed consent. Detailed screening logs of all referrals, including reasons for ineligibility, non-approach and non-consent, will be kept.

Randomisation: Randomisation, via a secure internet based randomisation system ensuring allocation concealment will occur after consent and collection of baseline data. Allocations in a 1:1 ratio to CT or PET-CT guided biopsy will be computer generated with varying block sizes, and stratified by centre.

Evaluation of PET scans: The PET scans will be reported by local radiologists using a standard protocol. The Standard Uptake Value (SUV) and Total Glycolytic Volume (TGV) will be calculated using validated software in routine research use, in the participating centres. According to SUV and TGV values areas will be highlighted for biopsy when patient returns for their subsequent CT-guided biopsy.

Evaluation of CT scans: All CT components of the scans will be reported using a volumetric assessment. The modified RECIST criteria are the standard radiological criteria currently in use to stage mesothelioma[11]. These criteria will be used for reporting and staging patients with mesothelioma in our study.

Pleural biopsies: All pleural biopsies will be performed by one radiologist at each recruiting centre to minimise operator variability. All radiologists will follow a standard protocol to ensure similar biopsy site selection. The radiologist will not be blinded to the treatment group for safety reasons. Audit data suggests that the radiologists all achieve comparable numbers of cancer positive biopsies.

To minimise variability further for participants in the PET-CT guided group, the area selected for biopsy will be the area with the maximum SUV, taking into account possible changes due to previous biopsy and regions of pleura that are safely accessible to percutaneous biopsy. If no area of increased SUV is identified the most accessible and suspicious area on the CT will be biopsied.
For participants in the CT-only guided group the radiologist will select an accessible area with suspicious malignant features. Biopsy results will be captured in the study database and discussed in the MDT.

Evaluation of biopsy specimens: The histopathologists interpreting the biopsy specimens will be blinded to the treatment group and clinical details.

Blood sampling: Participants will have blood tests at recruitment and at each follow up visit at 1 month, 3 months, 6 months and at 12 months from recruitment the biomarker mesothelin.

Data collection: Data collected for the study will be entered into a purpose-designed database, developed by the clinical trials unit collaborating on this trial. The database will be hosted in the NHS and accessed via the NHS clinical portal (password controlled). It will include facilities to upload PET, CT and biopsy reports directly into the database. The database will include real-time data queries for missing or erroneous data, to ensure data quality.

Statistical analysis: The analyses will be by intention to treat. All randomised participants will be included, unless consent is withdrawn. The number of positive biopsies will be compared, as a proportion of the participants recruited and as a proportion of those with a confirmed diagnosis of mesothelioma in the 12 months following randomisation using logistic regression. Other binary outcomes will be analysed similarly. Time-to-event outcomes will be compared using survival methods. Quality of life data will be analysed using mixed regression, accounting for the correlation between repeated measures on one individual and will be modelled jointly with survival. Differences between groups will be quantified and reported with 95% confidence intervals. If the data are sufficient to allow parameter estimation, we will adjust for centre as a random effect. The ability of the serum Mesothelin levels to predict a positive diagnosis (sensitivity, specificity, positive and negative predictive values, area under Receiver Operating Curve) will be assess for the study cohort as a whole. Similar analyses of the value of the PET scan parameters to predict a positive diagnosis will be restricted to participants in the PET-CT group. The analyses will take place at the end of the study after all queries have been resolved and the database locked; no interim analyses are planned.

10. Case for Support - Part 3

Projected outputs and Dissemination

Dissemination

Please describe your plans for disseminating the findings of this research

We hope to publish the results of this study in a high impact respiratory journal such as Thorax or Lancet where respiratory and general physicians who are managing pleural malignancies have access to. To simplify the diagnostic process of mesothelioma we propose a '**mesothelioma diagnostic pathway**' be incorporated into the publication with emphasis on where PET- guided biopsy sits on this pathway. We hope the study results will help inform the next BTS pleural disease guidelines.

Presentation at international research conferences such as British Thoracic Society (BTS) Winter meeting and European Respiratory Society (ERS) annual meeting, again with emphasis on the pathway to simplify the diagnostic process.
Our mesothelioma focus group who assisted us with the design and feedback during the initial preparation of this application will be informed either in writing and at a group meeting.
A lay summary of the results will be presented at an Avon Mesothelioma group meeting and at a MesotheliomaUK study day to ensure patients and relatives who supported this project are informed of the results.

Expected Output of Research/Impact

Please describe how the outcomes of this research could be translated into the NHS and wider healthcare community to provide improvements in service delivery, patient health and/or wellbeing

Findings from this study would be made easily accessible to respiratory specialists who are at the forefront of decision making regarding initial investigations. We aim to publish the results in a high impact journal such as the Lancet.
We hope to collaborate with British Thoracic Society (BTS)/National Institute of Clinical Excellence (NICE) to introduce the mesothelioma diagnostic pathway for patients with suspected pleural malignancy, which would simplify and ensure uniformity across diagnostic investigations across the country.
Principal investigators from each of the sites will be informed of the results along with the proposed mesothelioma diagnostic pathway as above.

If PET-CT is proven to be superior in this study, it is an easily implemented investigation as PET-scanners widely available with the increasing use of this modality in cancer investigations. Our patient population is clearly defined and the number of patients who would be going down pathway are small. Therefore implementing this investigation as a routine for the clearly defined population is inexpensive. Furthermore in the longer term due to the fewer investigations these patients are having, likely to be more cost-effective than the traditional method of repeated CT guided biopsies.
If this study is positive, future patients would gain the benefits of less invasive pleural investigations in total with less complications. More patients are likely to start life prolonging treatments and claims for mesothelioma cases are more likely to settle in the patient's lifetime.
Furthermore, a positive outcome and inclusion into the investigation pathway of PET scanning might provide other valuable information for patient care such as prognostication using baseline PET - TGV (total glycolytic volume). All PET scans will be double reported and TGV calculated and compared to overall patient survival.

Relevant expertise and experience

Please outline the individual role of each member of the research team, highlighting the skills and experience of the team that make them well placed to carry out the work

The Bristol Pleural Trials Unit has extensive experience in conducting randomised studies in patients with pleural diseases, with over 500 patients enrolled over the last 7 years. Most of these studies have been multicenter and conducted in over 20 UK centres.
The Oxford CTU also has an excellent track record with high data quality delivery to Oxford based multi-centre randomised trials. Results of these trials have been published in high profile journals (NEJM 2011;365(6):518-26, NEJM 352(9):865-74, JAMA.13;307(22):2383-9, AJRCCM 170(4):377-82) and entered standard national treatment guidelines (Thorax. 2010 65(8):667-9, Thorax. 2010 2:ii32-40).
Dr N Maskell is a Reader in Respiratory Medicine at University of Bristol and runs a tertiary pleural service, holding 3 grants to conduct randomised trials in other pleural diseases and has substantial expertise in trial design and delivery.
Najib Rahman has conducted 3 randomised studies in pleural disease (n=650 total), two of which are published with first or senior authorship (NEJM 2011, JAMA 2012). He leads the Oxford Pleural Service and ORTU, and has experience of methodology and design of clinical trials, with an MSc in Clinical Trials (LSHTM).
Professor Fergus Gleeson has an international reputation in respiratory radiology disease, and provides expertise in radiological outcomes. Professor Iain Lyburn runs the regional PET service in Cheltenham Cobalt Centre and has published widely in this field. Dr Anthony Edey, Consultant radiologist at North Bristol NHS Trust has a specialist interest in pleural based imaging and sits on the regional Mesothelioma MDT.
Dr. Chris Rogers is co-director of Clinical Trials Evaluation Unit (CTEU) and a senior statistician. She has experience of leading several NIHR funded multi-centre trials. She will provide methodological leadership to the trial.
Ms Sarah Smith is a specialist nurse in lung cancer and mesothelioma with over 10 years' experience working in this field. She is also a member of the Avon Mesothelioma Group and will work closely with patients and relatives for their feedback and input.
Dr D de Fonseka is a senior respiratory registrar with an interest in pleural disease. She would work closely with the other members of the team as above and members of the clinical trials unit to ensure the trial runs smoothly.

11. Management and Governance

Research timetable

Please provide an overview of the research plan which includes specific milestones and deliverables

Year 1:

- Set up phase (1st 3 months):
  - Ethical and regulatory approvals
  - Protocol finalisation and CRF design
  - Database design, testing and validation
  - Recruitment of trial specific staff to respiratory trials unit
  - Sign off of trial related documents at first TSC meeting
- Initial recruitment phase (9 months) to include:
  - 9 months of recruitment (at a rate of 3-4 patients per month)
  - TSC meeting 6 months into recruitment

- Statistical analysis plan written and signed off byTSC

Year 2:

- Further recruitment phase (12 months) to continue recruitment (upto 78 patients, at a rate of 3-4 patients per month)

- Data entry, verification and checking to occur "live" during recruitment phase
- TSCat 6 monthly intervals to review progress
- Follow up of all patients for minimum of 12 months to obtain final clinical diagnosis (gold standard)

Year 3: (12 months)

- Further 3 months of recruitment to complete 24 months (78 patients in total)
- Follow-up of all patients for minimum of 12 months to obtain final clinical diagnosis (gold standard)

Year 4: (3 months)

- Final TSC meeting
- Final phase (3 months of year 4) to ensure all patients have completed 12 months follow-up. Data analysis, report writing, presentation of results and publication (middle of 4th year)

Research management arrangements

Please explain the practical arrangements for managing the research and its constituent components

Inolvement of the Clinical Trials Evaluation Unit (CTEU) at Bristol has enabled us to ensure good trial management is achieved according to good clinical practise guidelines, for the duration of this trial. A trial manager based at Bristol CTEU will ensure smooth running of the trial across the recruitment centres as well as be a first port of call for trial related issues arising from other centres.

Trial manager will ensure regular 6 monthly trial steering committee meetings to identify and resolve any practical issues that may hamper the progress of the trial.

Trial manager will ensure any new changes are relayed to the other recruiting centres and regular progress updates including recruitment targets achieved or not, are made available to all members of the research team. Research teams at other recruiting centres are overseen by the prinicpal investigator (PI) at each site, who in themselves are established researchers.

Has any work relevant to this proposal already commenced?	No

Success criteria and barriers to proposed work

Please set out the measurements of success you intend to use and also the key risks to delivering this research and what contingencies you will put in place to deal with them

Measurements of success – achieving the recruitment rate set out at 3-4 patients per month.

If recruitment is significantly lower than the anticipated rate of 3-4/month in the initial 6 months since starting recruitment, 2 contingency plans are in place.

1. Invite other sites to enrol in the trial to improve recruitment rates (we have four extra sites expressing interest if needed)
2. Extend recruitment period from 24 months to 30 months.

As the follow-up period is clinical follow up that would happen as a part of routine clinical practice anyway, extending the recruitment period by a further 6 months and hence delaying follow-up by a further 6 months would not incur extra expenses. It would however, delay the data analysis.

Does the proposed research raise ethical issues?	Yes

If yes, discuss how these issues will be addressed.

Ethics may question that with such a high sensitivity why PET-scanning is not offered as the first line investigation in this group of patients. Our pilot data is small and there is some selection bias in the patients who had biopsy following PET-scanning. Therefore, our data is not robust enough for a direct comparison against CT, which would be best achieved with a randomised controlled study as we have suggested.

Ethics may also highlight the extra radiation dose received by the PET scanning but as these are a terminal group of patients, the longterm effects of the extra radiation is insignificant here.

Please detail how and when you intend to get ethical review completed

We hope to apply to Bristol REC in 8 weeks for ethical approval.

Have any appropriate regulatory bodies already granted the necessary approvals?	No

Involvement of Clinical Trials Units

Is Clinical Trials Authorisation required?	Yes

Is a Clinical Trials Unit (CTU) involved with this research proposal?	Yes

If yes, what is the name of the CTU?

Please provide the name of the CTU involved.

Bristol Clinical Trials Evaluation Unit

Does the CTU hold a UKCRC registration number?	Yes

If yes, please provide the CTU Registration Number?

11

Please describe how you have worked with the CTU in developing your application and what support they will provide if funding is approved
Dr Chris Rogers co-director of CTEU has appraised the research proposal, calculated the sample size for the study in discussion with the clinical investigators and has advised on the study design and statistical analysis. We would be relying heavily on CTEU throughout the trial. CTEU will provide the necessary research management support and staff for this project, such as a part-time trial manager, statistician and database manager. The trial manager will oversee the trial and attend trial steering committee meetings to ensure smooth running of the trial. A 24 hour web-based centralized randomization service is provided by the CTEU for randomization purposes. Database design, set up and management is provided by CTEU although data entry will be undertaken by the research fellow. All statistical analysis will be undertaken by the CTEU, in line with the analysis plan that they will write in collaboration with clinical colleagues.

13. Involvement with NIHR Infrastructure and Other Partner Organisations

Network involvement

Please describe links to NIHR networks, identifying, if appropriate, any benefits that have already accrued from working with networks

We have established links with with members of the Avon Mesothelioma Foundation in preparation of this application. Particular input in the patient and public involvement section.
We hope to disseminate our data at a Mesothelioma UK study day.
Our study will be publicized in the British Thoracic Society(BTS) newsletter. If in the event recruitment opens to new centres other than those mentioned previously, we would advertise in the BTS newsletter.
We hope to work closely with UKCRN

Research Design Services (RDS) Involvement

Please indicate, if applicable, which organisations (e.g. NIHR Research Design Service) you have contacted in the course of preparing this application

South West

Please describe the RDS's input

Significant input from an RDS statistician, concentrating particularly in the methodology section but also help reviewing the rest of the application. Helpful input into how to improve the case for support and patient and public involvement sections of the application.

Attending the grant application support workshop was very helpful.

Involvement with other partners

What, if any, other organisations will partner this research?

n/a

APPENDIX II - PET-CT IMAGING PROTOCOL FOR THE TARGET TRIAL

Preparation

Patients should not eat any food for 6 hours prior to their appointment time, however it is important that they remain hydrated – water only. Light meal on the night prior to the PET scan and refrain from consuming alcohol. Medication can be taken as prescribed providing that it is not required to be taken with food.
All patients must avoid exercise for at least 6 hours prior to their examination.
Patients with diabetes:
Type II diabetes
PET study should preferably be performed late morning
Patients must comply with the fasting rules above
Type I diabetes
Ideally an attempt should be made to achieve normal glycaemic values prior to the PET study, in consultation with patient and his/her attending medical doctor
The PET study should be scheduled for late morning

The patient should eat a normal breakfast at 7am and inject the normal amount of insulin. Thereafter the patient should not consume any more food or fluids, apart from the prescribed amount of water - adequate pre-hydration with 1 litre of water in the 2 hours prior to injection of contrast.
Parenteral nutrition and IV fluids containing glucose should be discontinued 4 hours prior to the PET examination.
****IMPORTANT - Prior to arrival of the research patient please ensure that there is a signed copy of the patients consent form, an ARSAC certificate for the particular trial, ethics approval has been granted and you have read and understood the study protocol noting any variations such as FDG uptake time, which may be in variation to the protocol described below.**

1. On arrival take the patient through to the PET suite, explain procedure to patient and complete PET data acquisition form. Ask the patient if they have understood and whether they have any questions they wish to ask. Ask the patient to sign the data sheet to show that you have explained the procedure and they are happy to continue.
2. Establish the patient's weight and height.
3. Enter the patient's weight and height data into the automatic dispenser interface.
4. Either ask the patient to lie supine on the couch in the Patient "hot" rest room and establish intra venous access using a cannula or insert a cannula whilst the patient is seated in the interview / preparation room. Insert the cannula into the forearm/antecubital fossa/hand on the opposite side to the site of pathology, with a triple-channel system (three-way tap) to enable saline flushes and flushed with 10 ml of 0.9% sodium chloride to ensure patency.

NB: Ensure that the injection site is chosen carefully so as not to interfere with any area on the scan which may be critical

5. If the cannula is inserted in the interview room ask the patient to lie supine on the couch in the Patient "hot" rest room once IV access has been established.

6. Take approx. 0.5 mls blood for a pre-scan glucose measurement (normal fasting value = 3-5 mmol) and, if you are not going to use it immediately, keep the line patent by flushing with 10 ml of 0.9% saline.

7. Once the automatic system has completed dispensing, inject the activity into the patient using the 'Radinject' system and then record the exact activity and time it was measured on the Data Acquisition form, then remove the IV access.

8. If dispensing manually then calculate the required volume in order to administer the required dose (for the purpose of TARGET trial this should be less than 400MBq) of ^{18}FDG by using the standard protocol. When you have drawn up the required volume of FDG record the exact activity and time it was measured on the Data Acquisition form, then inject it followed by 10 ml of 0.9% saline flush, then remove the cannula.

9. Make sure the patient is warm enough and allow him/her to rest quietly for the 60 minute uptake period.

10. At the end of the uptake period ask the patient to go to the toilet to empty his/her bladder.

11. Position the patient on the scanning couch (supine, head first) with his/her head resting on the headrest. Try to ensure that the patient's head is not turned to one side or the other and explain the importance of keeping the head straight.

12. If the patient is able, ask him/her to raise their arms above their head and place them on the arm support provided. If required and the patient agrees, the arms can be placed within the Velcro arm straps / supports. Explain to the patient that the scan will take about 15-20 minutes in this position. If the patient is unable to hold their arms up above their head or thinks they might struggle to keep still, get them to hold their hands together and rest them anteriorly over the pelvis.

13. Ensure that the patient is comfortable and warm enough. Explain to the patient exactly what will happen during their examination and explain the requirements on them to remain still. Explain to the patient that we can hear them at all times and will be watching them throughout the scan to ensure their safety.

14. Using the gantry controls, raise the bed to a height of -150 and move the patient into the gantry. Ask the patient to close their eyes whilst you position and turn on the laser lights. Position the internal laser light just above the patient's vertex.

15. Allow the patient to open their eyes if they wish and explain again that they need to remain still.
16. Return to the control room and set up the pre – scan information. Select the patient from the scheduler and input their height and weight details, referrer details, FDG / Radiotracer details including time measured and time injected and blood glucose measurement details.
17. Patient can be scanned using the standard clinical scanning protocol used at the each centre. Load the protocol and click 'OK' and wait for the control panel yellow scan button to flash. Press this button once.
18. Adjust the length of the scan as required (i.e. lower orbits to mid-thigh), using the left hand mouse button to select length / move options / boxes on the scout view that has been produced. Click again on load and wait for the control panel white move button to flash. Press this button continuously until the yellow scan button starts flashing. Press the yellow button once to start the low dose CT acquisition.
19. The white move button on the control panel will start flashing, press once and the PET acquisition will commence.
20. Once the scan has completed the acquisition boxes will become active again. Click on the CT 'free' box (empty recon box) and position the guidelines on the half body image to outline the Lungs. Click on recon and ok. The lung images will then be reconstructed using a lung CT window.
21. On the left hand screen go to the image tag at the top of the window and select 'create ranges' which will run in the background. On the right hand screen click on the close scan icon and then ok. Release the patient from the scanner and explain any aftercare required.

    Once the images have reconstructed archive them to the PACS system and send them to the relevant reporting station. Complete all of the dose information on RIS and within the patients data sheets and send the patients folder to the reporting station. Please Note: at the end of the trial we will import the scans electronically, to the lead centre

<u>Scanner Settings for Half Body Examinations.</u>

Attenuation CT Scan Parameters

KV	120
MAs/slice	80 (max – actual will vary according to dose saving software)
Rotation Speed	0.5
Collimation	16x1.5mm
Pitch	0.6
Dose Right	Yes
Slice Thickness	5 mm
Increment	5mm
FOV	600

Incidental findings on PET-CT

Incidental, non-pleural based high uptake areas on the PET-CT scan is to be investigated at the attending clinician's discretion.

If the PET-CT scan detects a more accessible high up take area, which may be extra-pleural (for example a liver metastases that is easily accessible), and the radiologist feels this is a better area for biopsy than the pleural lesion, the extra-pleural lesion can be biopsied. Please ensure the biopsy proforma (form C2) specifies the area biopsied.

APPENDIX III – ZOL-A NIHR RFPB GRANT APPLICATION

NHS
National Institute for Health Research

Research for Patient Benefit

Reference number	PB-PG-1014-35052
Lead Applicant	Dr Nick Maskell
Research Title	Zoledronic acid in the management of malignant pleural mesothelioma - a feasibility study (Zol-A Study)
Plain English Summary	Malignant mesothelioma is an incurable cancer of the lung lining that usually develops as a result of previous asbestos exposure. This is an incurable disease with an average survival of 9 to 14 months from diagnosis. Treatment proven to prolong life is limited to one form of chemotherapy which only extends life by a few months, on average. Zoledronic acid (ZA) is a licensed drug that is commonly used for the treatment of bone disease, such as thinning of the bone (osteoporosis) and in cases where bone deposits occur from other cancers. This drug has been is use for a number of years with a good safety record. Animal studies in mice have shown ZA may have a role in the treatment of mesothelioma by slowing the disease. It is not a curative treatment but it may have a role in prolonging life. In animal studies where ZA was used in combination with certain chemotherapy regimens there appears to be an even greater effect. Unfortunately at this stage the drug is not licenced in mesothelioma, mainly due to the lack of human studies. We propose a preliminary study to assess the practicality of running a trial to establish the role of ZA in patients who have mesothelioma and are undergoing or eligible for chemotherapy. We plan to assess the benefit of giving ZA in addition to chemotherapy in half the patients and compare this with chemotherapy alone in the other half of the patients. Neither the patients nor the investigators will know what treatment they have been allocated until the end of the study. In addition, patients who decline to have chemotherapy will be offered the ZA drug alone to assess whether there is any benefit to having this ZA on its own.
Total Research Cost	287,465.00

The Department of Health, National Institute for Health Research (DH NIHR) is the Data Controller under the Data
Protection Act 1998 ('the Act'). Applicants for funding should be aware that information contained in this
application might be shared with other DH NIHR bodies for the purposes of statistical analysis and other DH NIHR management purposes, including targeted communications with selected groups of researchers. Applicants may be assured that DH NIHR is committed to protecting privacy and to processing all personal information in a
manner that meets the requirements of the Act.

1. Research Details

Research title	Zoledronic acid in the management of malignant pleural mesothelioma - a feasibility study (Zol-A Study)

Host organisation (which will administer any award)
North Bristol NHS Trust

Research duration	30

Proposed start date if grant awarded	01/10/2015

Application type	Feasibility Study

Themed call name	Mesothelioma

If your application is related to one of the past NIHR themed calls, please indicate which call here.
(Select)

7. Patient and Public Involvement

In this section it is important that you describe, in as much detail as possible, how patients and the public have been involved in the development of the application as well as plans for involvement in the proposed research.

Were patient and the public actively involved in either identifying the research topic/prioritising the research questions and/or preparing this application?
Yes

If Yes, please tick the appropriate boxes below
Involved in identifying the research topic/prioritising the research questions,Involved in preparing the Application

Please further describe how patient and public involvement has informed and/or influenced the development of the application and how patients and the public have been actively involved.
<u>Identification of the research topic</u> The UK government in conjunction with the NIHR recently funded a James Lind Alliance (JLA) Priority Setting Partnership (PSP) to identify research priorities in mesothelioma. The PSP comprised of patients, carers, health professionals and organisations involved in the care of patients with mesothelioma. The Lead Applicant (Dr Maskell) was a member of the PSP and the steering committee. Over 800 responses were received from patients, carers or health care professionals. These were then ranked and the role of Zoledronic acid (ZA) in the treatment of mesothelioma appeared in the top 50 questions that needed answering. In addition to this the final top 12 ranked questions were published in December 2014 at http://www.lindalliance.org/top-tens.asp. This highlighted two other questions pertinent to our research: Question Rank 11. Can PET-CT scans help to aid the assessment of response to treatment? Question Rank 12. How can the level of mesothelin be best incorporated in the diagnosis, response and progression of mesothelioma? **<u>PPI involvement in study design and development of the application</u>** During development of the research plan and preparation of this application, patients, carers and patient groups have been consulted. Drafts of the application have been presented to a focus group from the Avon Mesothelioma Foundation (a local mesothelioma charity and support group). This was made up of eight mesothelioma patients and 2 carers. We have amended the study design to reflect their views including their preference to have the ZA infusion on a different day to their chemotherapy and the addition of a further non-randomised observational arm of patients who declined chemotherapy but still wanted to receive ZA. As the numbers are likely to be small and our follow-up time constraints we have not randomised this arm, although this data will help with planning of a randomised non-chemotherapy group in the full study. The trial involves extra out-patient visits. Patient/carer feedback unanimously agreed that extra clinic visits would not be an issue, in fact they felt these visits would give them further opportunities to ask questions and to have more contact with the specialist hospital team.

Please indicate the ways in which the public will be actively involved in the proposed research, by ticking all relevant boxes below.
Management of the research (e.g. steering/advisory group),Developing participant information resources,Contributing to the reporting of the study report,Dissemination of research findings

Please give more details, including how patient and public involvement will benefit the research, the reasons for taking this approach and arrangements for training and support.
In addition to the input in the research design process outlined above, we plan to involve patients and carers during the set up and running of the trial as highlighted below. Patient/carer group will be involved in preparing the lay summary and patient information sheet for the study. In addition, we will be asking them for any further comments on the final protocol including identifying any practical issues that could be addressed to help support patients participating in this trial. The research team will also consult with the focus group periodically before any changes to the study protocol or any new literature is given out to study participants.

Unfortunately due to the terminal nature of the disease and rapid deterioration it is difficult for a patient with mesothelioma to sit on the trial steering committee (TSC) for the duration of the trial. We have therefore approached a carer who will join the TSC.
We intend to request help from the focus group in overseeing the writing of the final results of our research. The results will be presented to our focus group mentioned above. On a regional scale results will be presented at an Avon Mesothelioma Foundation study day, which is attended by patients and carers of mesothelioma in the South West region.
We have also requested involvement of the Research & Innovation (R&I) team's generic lay panel to review our patient information sheets and other literature that patients would come across. This would allow for an opinion from lay public who do not have much involvement with mesothelioma normally.

8. Case for Support - Part 1

Aims and objectives

Describe the overarching aims of the research, outlining the research question which the work will address

Malignant pleural mesothelioma (MPM) is an incurable cancer, which affects over 2500 people each year in the UK. It is an under-researched area and therefore has been given recent NIHR priority for funding. The aim of our research programme is to determine the role that Zoledronic acid (ZA), licenced for other conditions but not for mesothelioma currently, could have in the management of MPM. Zoledronic acid will be administered intravenously alongside chemotherapy. We also hope to address the question of whether there is a role for ZA alone in patients who decline chemotherapy.

We ultimately plan to undertake a multi-centre, randomised controlled trial (RCT) of ZA and Pemetrexed/Cisplatin chemotherapy versus placebo and Pemetrexed/Cisplatin chemotherapy to determine if the addition of ZA increases progression free survival (PFS) and overall survival in this population, compared to chemotherapy alone. Furthermore, the future trial will be used to assess a number of secondary but important outcomes relating to tumour growth, biomarkers, imaging and quality of life, in relation to ZA, with and without adjunct chemotherapy.

Prior to undertaking an adequately powered Phase III trial, however, there are a number of uncertainties that need to be addressed. The purpose of the current study is to establish the feasibility of the full trial and to assist in its design. In particular we need to obtain accurate information on:

1. Presentation, recruitment, consent and randomisation rates of MPM patients at each site.
2. Acceptability of recruitment procedures, consent and randomisation, and data collection methods.
3. Acceptability of ZA in MPM patients, and the optimal timing and location for ZA administration.
4. Qualitative assessment in a subgroup of 15 patients to evaluate patients experience in the randomisation and recruitment process
5. Quantification of drop-out and data completeness rates
6. Estimates of outcome event rates eg. survival times, measures of mean response and outcome variance (continuous variables such as quality of life) and confidence intervals around estimates of proportions, categorical variables such as recruitment rates)to use for calculating full trial size and number of sites.
7. Measure of variation between patients and between sites and information required to assess the intra class correlation coefficients to be used to estimate clustering effects.

We hope the feasibility study we propose would give us the necessary information to design a robust phase III trial to evaluate the role of ZA in mesothelioma.

Scientific abstract of research

Please provide a structured summary which outlines the background to the research, the aims of the work, including the question to be addressed by this research, the plan of investigation and a summary of the potential benefits to patients and the NHS

Background:
Malignant pleural mesothelioma (MPM) is an incurable primary pleural malignancy with an average survival of 9-14 months and a 3 year survival rate of only 8%. Currently only one form of treatment (Pemetrexed and Cisplatin chemotherapy) has been shown in an RCT to give a survival advantage. But even with this treatment only a further 10-12 weeks of life is gained on average. Due to the toxic side effects of this regime and the co-morbidites of those who are diagnosed with the disease a significant proportion of patients are not eligible for this regimen.
Zoledronic acid (ZA) is an n-bisphosphonate that has been used for a number of years to treat bone related pathology such as osteoporosis, Paget's disease and bone metastases in certain cancers such as breast and prostate cancer. Studies in animal models inoculated with mesothelioma cells have been promising showing a survival advantage in those receiving ZA. Animal studies have also demonstrated a synergistic effect between ZA and certain chemotherapy agents such as platinum based chemotherapy regimes in mesothelioma.

Despite the encouraging results from animal studies there remains a lack of human trials investigating the benefit of ZA in mesothelioma. This is a drug that is well tolerated wiht minimal side effects and is widely used currently
in other disease entities.

Aims:

We propose a double blind randomised controlled multi-centre feasibility study to investigate the practicality of the trial prior to embarking on a phase III trial. We aim to investigate recruitment rates, suitability of the research plan and extrapolate numbers needed to achieve a clinically and statistically significant difference in the primary endpoint (should a difference exist).

Plan of investigation:

Patients with a histocytologically confirmed diagnosis of mesothelioma who are offered chemotherapy, with a World Health Organisation (WHO) performance status of 0 or 1 will be our patient population. Those who are undergoing chemotherapy will be randomised to ZA or placebo in a 1:1 ratio. Those who decline chemotherapy will be offered ZA on its own. These patients will form a third non-randomised group.
We propose a feasibility study to recruit 50 randomised patients over 1 year.
Randomised patients will either receive 4mg of ZA in 100ml of 0.9% saline or a placebo in 100ml of 0.9% saline over a 15 minute period. Patients will receive the infusion within a 24-48 hour window prior to their chemotherapy. Non-randomised patients who accept ZA will have 4mg in 100ml of 0.9% saline every 3 weeks.

Potential impact:
This feasibility study will have direct bearing on the proposed full study, which is a large scale multi-centre double blind randomised controlled study to investigate the effects of ZA in mesothelioma. The feasibility study will provide valuable information regarding recruitment methods and rates, consent procedures, randomisation success and acceptance, patient derived qualitative information and numbers required to recruit for a statistically significant primary outcome, tolerability of the drug and the robustness of our research plan.
The full study will have a signifcant impact in that if successful and a survival benefit with ZA is demonstrated, this would be a fairly safe drug that can be easily administered alongside chemotherapy with minimum side effects, to extend life in mesothelioma patients.

Background and rationale

What is the problem being addressed?

Describe the background to the research, describing the limitations identified in the evidence base that the research is trying to address.

Malignant pleural mesothelioma (MPM) is an aggressive and fatal tumour of the pleura that usually develops as a result of previous asbestos exposure. Although the import of asbestos was banned over two decades ago, mesothelioma remains a major clinical problem in the UK. Health and Safety Executive data for 2012 shows that mesothelioma caused 2,535 deaths in the UK [1]. Current treatment for MPM is very limited and average prognosis from diagnosis remains poor at 9 to 14 months [2]. Pemetrexed and Cisplatin chemotherapy is the only treatment proven to extend patients' life expectancy [3]. The survival benefit is a modest 10-12 weeks on average and approximately 40% of patients are not fit enough to tolerate this treatment at presentation. Despite the chemotherapy treatment life expectancy remains poor with a 3 year survival rate of only 8% [4]. Hence the desperate need for other treatments that could improve the length and quality of life in these patients.

Bisphosphonates are a synthetic analogue of naturally occurring pyrophosphate. Bisphosphonates are commonly used in the treatment of osteoporosis and other bone disorders such as Paget's disease, due to their action on inhibiting osteoclast mediated bone resorption [5]. Nitrogen containing bisphosphonates (n-bisphosphonates) have been shown to inhibit various epithelial cancer cells in vitro, by inhibiting the mevalonate pathway [6]. Potential anti-tumour activity of bisphosphonates include reduced tumour angiogenesis, reduced tumour cell proliferation, migration, invasion and adhesion, increased tumour cell apoptosis and increased cytotoxicity of gamma-delta T cells, which subsequently leads to reduced tumour vascularization [7].
Several studies using n-bisphosphonates, particularly Zoledronate have shown a survival benefit in patients with breast cancer [8, 9]. In vivo studies on mice inoculated with mesothelioma cells, treated with bisphosphonates have shown a significant survival advantage [10], supporting the direct anti-cancer properties of bisphosphonates in Mesothelioma. Similar results have been seen in other in vivo studies of murine models inoculated with small

cell and non-small cell lung cancer, both showing a reduction in tumour burden and increased survival in mice treated with n-bisphosphonates [11, 12].
Zoledronic acid (ZA) is known to be a potent nitrogen containing bisphosphonate which has bone independent anti-tumour activity. In addition, when combined with certain chemotherapy agents such as Paclitaxel, Etoposide, Cisplatin and Irinotecan in lung cancers, it was shown to have an even greater synergistic effect in induction of apoptosis in vitro [11].
As human studies investigating the synergistic effect between ZA and chemotherapy do not exist, the optimum timing of ZA in relation to chemotherapy is still unknown. Murine models using subcutaneously injected breast cancer cells have shown the greatest effect on increasing apoptosis, reducing proliferation and neovascularization was seen when the cytotoxic drug was given 24 hours after ZA [7].
There remains an absence of evidence in the literature regarding the effects of ZA in mesothelioma in humans. Our institution recently completed a small pilot study investigating the effect of ZA in patients with advanced malignant pleural effusions, who were not on any other form of treatment (currently in submission to PLOS one). This small study included a few patients with mesothelioma and although it failed to show a significant treatment effect with ZA for all malignant pleural effusions, two of the six patients with mesothelioma given ZA showed a reduction in tumour bulk according to modified RECIST criteria, thereby highlighting the need to investigate the effect of ZA further in a study with exclusively mesothelioma patients. In addition, there are reports of ZA being used 'off licence' by certain centres on a patient to patient basis[13].
A double blind multi-centre RCT would be best placed to investigate the hypothesis that treatment with n-bisphosphonate ZA in addition to the standard chemotherapy (Pemetrexed and Cisplatin) confers a survival benefit to patients with MPM compared to chemotherapy alone. We propose a feasibility study prior to undertaking a full study to capture the data needed to inform the definitive trial. In our feasibility study a non-randomised third group will consist of patients who are fit for chemotherapy but have declined to have chemotherapy. These patients would be offered ZA in isolation.
Soluble mesothelin related peptide (SMRP or Mesothelin) is a glycoprotein found on the surface of mesothelial cells that can be over expressed in mesothelioma. Studies have shown significant correlation between mesothelin levels in serum and tumour burden in mesothelioma [14]. We propose using serum Mesothelin as a biomarker for monitoring tumour response state in the study to assess its value in the full trial.
Positron emission tomography computerised tomography (PET-CT) has proved itself a useful diagnostic and staging imaging modality in mesothelioma, due its ability to highlight areas of increased metabolic activity by uptake of 18-Fluorodeoxyglucose (18-FDG), which is used as a nuclear tracer for this form of imaging. Total glycolytic volume (TGV) has been shown to be a more robust measurement of total mesothelioma activity [17] and our centre has expertise in measuring the PET TGV because of our previous work on the SWAMP trial (currently in submission to British Journal of Cancer). A baseline PET-CT scan and a repeat PET-CT scan after 3 cycles of chemotherapy will be performed and evaluated using PET response criteria in solid tumours (PERCIST) or methodology analogous to this to assess treatment response or not and if it should be used in the main trial [18].

Why is this research important in terms of improving the health of the public and/or patients and the NHS?

Malignant pleural mesothelioma (MPM) is an incurable cancer, which is becoming more prevalent in the UK and represents a major public health issue. It is estimated that over 1% of men born in the UK in the 1940's will die from mesothelioma and currently over 2500 new cases are diagnosed in the UK each year. Despite this, high quality research in this field is lacking. This has resulted in the UK Government commissioning the James Lind Alliance (JLA) to set up a Priority Setting Partnership to identify research priorities in mesothelioma [15]. After extensive consultation with the public, carers, patients and healthcare providers, 50 key questions were identified that required urgent research. This included the question 'What is the role of Zoledronic acid in the management of mesothelioma?'.
Our study will, by helping in improving the design of the full trial, also address two further JLA questions that were ranked in their top 12 research priorities in mesothelioma. These are:

1. Can PET-CT scans help to aid the assessment of disease response?
2. How can the levels of mesothelin be incorporated in the diagnosis, response and progression of mesothelioma?

Zoledronic acid (ZA) is used in a number of different cancers for a variety of its properties. Animal studies with mice inoculated with mesothelioma cells who were treated with ZA have shown a clear survival benefit. Furthermore, ZA and certain chemotherapy agents such as Cisplatin appear to have a synergistic and additive effect.
This research addresses an important question regarding the role of ZA in patients with mesothelioma. Apart from a small study in USA looking at the role of ZA in advanced mesothelioma (expected to report early 2016) there are no other trials investigating the treatment effect of ZA in mesothelioma, particularly in patients with a

good functional status who are able to have other life prolonging treatment as well. Despite the lack of any evidence in mesothelioma patients, this drug is used in a number of centres off licence, on a patient by patient basis. Therefore access to ZA across the UK is currently unequal. We hope this feasibility study will pave the way to a phase III trial that can answer the question 'What is the role of ZA in mesothelioma?' and if it is found to be of benefit it will provide robust evidence for the NHS to consider its routine use in this setting across the UK. As ZA has a good safety profile and is generally well tolerated it might also prove useful in those patients not wanting first line chemotherapy.

9. Case for Support - Part 2

Research Plan

Describe the proposed research plan, providing descriptions of the overall research design and a strong justification of sampling strategies, methods of data collection and analysis.

It is vital to add as much detail as possible on design and methodology, including justification of sample size, power calculations and sample selection and exclusion criteria where applicable.

Study design:
We propose a multi-centre double blind randomised controlled feasibility study to compare standard first line chemotherapy (Cisplatin and Pemetrexed) plus Zoledronic acid (ZA) versus chemotherapy and placebo, in patients with histocytologically confirmed Malignant Pleural Mesothelioma (MPM).
A third non-randomised group to this feasibility study would consist of patients with MPM who are fit enough to receive chemotherapy but have declined. They would be offered ZA (open label) on its own. We have included this arm to the study as per PPI feedback, that patients who decline chemotherapy should still have access to a potential treatment for MPM. We anticipate the number of patients in this third group will be small and hence we have not included a fourth placebo-without-chemotherapy group. Depending on the feasibility outcomes particularly recruitment rates to this arm, when designing the Phase III trial we can consider a four group trial.

Population:
Patients with a histocytologically confirmed diagnosis of MPM with a WHO performance status 0 or 1.

Setting:
Three hospitals in the South-West region of the UK; North Bristol NHS Trust (NBT) which would be the lead centre, Bristol Royal Infirmary (BRI) and Royal United Hospital (RUH), Bath. Patients will be recruited and managed by respiratory and oncology departments at the above hospitals.
Inclusion criteria:
-Histocytologically confirmed diagnosis of MPM
-fit enough to receive chemotherapy (WHO performance status 0-1)
-measurable disease on CT as per modified RECIST criteria (tumour thickness >5mm)
Exclusion criteria
-Not fit for chemotherapy due to performance status or other co-morbidities
-Previous chemotherapy for MPM
-IV bisphosphonates in the 3 months preceding randomisation
-significant renal disease (calculated as a creatinine clearance of < 40 ml/min as per Wright's formula (ref))
-Hypocalcaemia on baseline blood tests (defined as a level less than 2.20 mmol/L)
-Pregnancy or lactation
-Age < 18 years
-Known allergy to bisphosphonates or excipients of its preparation
-Severe untreated dental caries

Patient identification and trial interventions:
Patients will be identified from the local lung cancer and mesothelioma multi-disciplinary team (MDT) meetings and respiratory/oncology out-patient clinics. Patients who have a confirmed histological or cytological diagnosis of mesothelioma who are eligible for chemotherapy will be identified as potential candidates.
When patients attend the out-patient clinic for their diagnosis following biopsy, they are usually given information about further management. These may very occasionally include surgical options, but usually consist of palliative chemotherapy or best supportive care. Those who are candidates for palliative chemotherapy and willing to proceed with chemotherapy will be approached by a member of the research team with a patient information leaflet regarding the trial.
Patients are next seen when they attend to see the lung cancer/mesothelioma nurse specialists. The research nurse will again approach the patient to discuss the trial at this appointment. If the patient is happy to take part and meet the eligibility criteria they will be consented at this appointment. Following consent they will have their baseline investigations as below. A baseline PET-scan will be arranged between this appointment and their first cycle of chemotherapy.
It is anticipated the patient's next visit to hospital would be for chemotherapy therefore, patients will be randomised before this visit.
Patients who decline chemotherapy following discussion with the respiratory physician or oncologist will be approached by the research team with information regarding the open labelled third arm of the study. Those who

are interested in the trial will be given information pertaining to the third arm only.
Baseline Assessment (before randomisation):
-Baseline bloods (Serum mesothelin, FBC, Urea & Electrolytes, Calcium, Phosphate, Magnesium)
-Baseline breathlessness Visual Analogue Scale (VAS) score
-Baseline PET-CT scan
Randomisation:
Patients who are proceeding with chemotherapy, will be randomised to receive either ZA or placebo, using software developed at Bristol Clinical Trials Evaluation Unit (CTEU). Randomisation will be stratified by centre and minimised by histology; epithelioid or other.
Patients declining chemotherapy but wishing to take ZA only arm will not be randomised as described earlier.

Trial interventions:
Randomised patients will receive either ZA or placebo with each cycle of chemotherapy. Prior to each cycle of chemotherapy all patients will have routine pre-chemotherapy bloods (FBC, U&Es, clotting screen) and trial bloods (Calcium, phosphate, Magnesium, Serum Mesothelin level).
The dose of ZA will depend on the estimated creatinine clearance, as calculated using the Wright equation [16]:

Estimated creatinine clearance (ml/min) as calculated by the Wright Equation	Dose of Zoledronic acid (mg)
≥60	4
≥50- <60	3.5
≥ 40-<50	3.3

Zoledronic acid in 100ml of 0.9% sodium chloride or placebo in 100ml of 0.9% sodium chloride will be prepared ready for use as necessary depending on the randomised allocation.
The drug will be delivered within a 24-48 hour window before each cycle of chemotherapy as a single dose, delivered intravenously over a 15 minute period.
Patients in the non-randomised group who are not having chemotherapy would have ZA every 3 weeks, in the same manner as above. They will have routine and trial bloods checked 2-4 days prior to each cycle of ZA.
Patients, clinical and research staff will be blind to allocation (except for patients in the open labelled third arm).
Patients who experience a fall in serum calcium, magnesium or phosphate will be supplemented as necessary with oral agents. If necessary the next infusion maybe delayed or missed.
If electrolytes remain persistently low despite supplementation, no further ZA or placebo will be administered and the patient included in an intention to treat analysis. Failure to complete the full course of treatment will be documented.
All patients will have a repeat PET-CT scan after their third cycle of chemotherapy.
Patients will have routine chest CT scans as per their usual care following 3 cycles of chemotherapy and post-completion of chemotherapy (usually after 6 cycles). Patients in the non-randomised 3rd arm will have CT scans after 3rd and 6th cycles of ZA.

Outcome measures
The feasibility of this study will be assessed using following criteria:

1. Presentation, recruitment, consent and randomisation rates of MPM patients at each site.
2. Acceptability of recruitment procedures, consent and randomisation, and data collection methods.
3. Acceptability of ZA in MPM patients, and the optimal timing and location for ZA administration.
4. Qualitative assessment in a subgroup of 15 patients (5 patients from the randomised group, 5 patients from the non-randomised group and 5 patients who declined to participate in the study) to evaluate patients experience in the randomisation and recruitment process
5. Quantification of drop-out and data completeness rates
6. Estimates of outcome event rates eg. survival times, measures of mean response and outcome variance (continuous variables such as quality of life) and confidence intervals around estimates of proportions, categorical variables such as recruitment rates)to use for calculating full trial size and number of sites.
7. Measure of variation between patients and between sites and information required to assess the intra class correlation coefficients to be used to estimate clustering effects.

Information gathered from the feasibility study will be used to design the definitive phase III trial and inform the choice of primary and secondary endpoints.
Anticipated potential end points for the full trial are as below:

- Rate of progression of MPM, as measured by modefied RECIST criteria on CT, after 3 cycles of chemotherapy (Pemetrexed/Cisplatin)

- Rate of progression in MPM, as measured by modified RECIST criteria on CT, after 6 cycles post-chemotherapy.
- Progression free survival
- Overall survival
- Tumour metabolic activity as determined by Total glycolytic volume (TGV) on PET-CT scans, after 3 cycles of chemotherapy
- Value of TGV or standard uptake value (SUV) in the assessment of disease response/progression
- Serum mesothelin as a useful biomarker for monitoring disease and detecting treatment response
- Quality of life determined by dyspnoea Visual analogue scale (VAS) scores?

Sample size:
We propose a sample size of 50 randomised patients (25 per group) recruited from three sites.
The third non-randomised arm will be recruited in parallel and we anticipate 10-15 patients during the 12 months of recruitment.
A study of this size would allow an assumed 40% consent rate (125 eligible, 50 recruited) to be estimated with a confidence interval of +/- 9%.

Recruitment:
Recruitment will be from three large NHS hospitals in the South West region; North Bristol NHS Trust (NBT) Bristol Royal Infirmary (BRI) and Royal United Hospital (RUH), Bath. North Bristol NHS Trust will act as the host and the lead recruiting centre.
Detailed screening logs of all referrals including reasons for ineligibility, non-approach and non-consent will be kept.

Recruitment Rate:
A recruitment rate of 4-5 patients per month from the 3 centres is anticipated over the 12 month period. This would allow us to recruit our target of 50 randomised patients in a 12 month period.

Follow-up:
Patients will have routine follow up with oncology as per their usual care, which is normally prior to each cycle of chemotherapy. Appropriate case report forms (CRF) will be filled at each visit.
Patients in the ZA only non-randomised arm will have regular clinical follow up as per their usual care.
All participants will have trial follow-up at 6 and 12 months from recruitment.
CT scan evaluation:
All CT scans will be evaluated using volumetric assessments. The CT scans will be reported using modified RECIST criteria as per current accepted staging criteria used in mesothelioma.

PET-CT scan evaluation:
These scans will be evaluated by a PET-CT specialist radiologist. Standard Uptake Value (SUV), TGV and PET Response Criteria in Solid Tumours (PERCIST) or methodology similar to this will be used for evaluation of the PET scans.
Blood sampling:
In addition to the routine pre-chemotherapy bloods patients would have as a part of their usual care, they will have additional bloods taken for extended electrolytes such as calcium, magnesium, phosphate and serum mesothelin levels. The additional bloods will be taken at the same time as their pre-chemotherapy bloods.
Patients in the open labelled third arm will have the same bloods taken 3 weekly, prior to their next cycle of ZA to ensure electrolytes remain stable.

Data Collection:
Data will be collected on the appropriate CRFs at each research visit. All data collected as a part of the study will then be entered into a purpose-designed database developed by CTEU in Bristol. The database will include real time data queries for missing or incorrect data, to ensure data quality.

Statistical analysis:
As this is a feasibility study it is not powered to compare the outcomes between groups.
Study outcomes will be described according to the allocated group (ie. by intention to treat). Binary outcomes (eg. recruitment and compliance rates) will be reported as a number and percentage and continuous outcomes will be reported as mean and standard deviation (or median and interquartile range if skewed). Survival rates will be estimated using the Kaplan-Meier method. Rates will be reported with 95% confidence intervals. These data will be used to inform the sample size calculation of the definitive phase III trial.
Statistical input will be provided by a statistician based at CTEU Bristol.

10. Case for Support - Part 3

Dissemination

Please describe your plans for disseminating the findings of this research.

1. Our findings will be presented at national and international research conferences such as the British Thoracic Oncology Group (BTOG), British Thoracic Society and International Mesothelioma Interest Group (IMIG) to quickly inform the scientific and clinical community of any potential benefit this drug may have.

2. We hope to publish the results of this study in an open access oncology journal such as Lancet Oncology so that oncologists managing patients with mesothelioma will have easy access. In addition once the protocol is finalized we plan to submit this to the 'BMJ Open' which is interested in publishing the protocols for NIHR funded studies.

3. We will also present our findings at regional support group meetings such as the Avon Mesothelioma Foundation meetings and inform national support groups such as Mesothelioma UK, Clydeside Action on Asbestos and the British Lung Foundation.

4. The focus group who helped in developing this application will also be notified either by writing or at another focus group meeting.

Expected Output of Research/Impact

Please describe how the outcomes of this research could be translated into the NHS and wider healthcare community to provide improvements in service delivery, patient health and/or wellbeing.

The expected output of this feasibility study is the production of a dataset which will inform the planning and design of a full phase III multi-centre randomised controlled trial. This study will provide us with robust data to inform the application for a Health Technology Assessment (HTA) grant to support the full Phase III trial.
The current treatment options for patients with newly diagnosed mesothelioma are severely limited. Zoledronic acid animal studies have looked promising but this has not been translated into human studies in patients with mesothelioma. The findings from this feasibility study will be published in a peer reviewed open access oncology journal providing rapid access to all healthcare professionals caring for patients with mesothelioma.
In addition, this feasibility RfPB grant will help evaluate the role of serial mesothelin measurements and TGV PET-CT values in assessing possible response rates in patients with mesothelioma.
One of the outputs of this study, the qualitative assessment data will provide us with essential patient derived information upon which the Phase III trial can be further improved on.
The output of this study will prove invaluable in the design of the phase III study. It will identifypotential pitfalls and issues that we have not yet realised. It will inform us whether there is a need for a 4th arm to the Phase III study so that patients not having chemotherapy can be randomised to ZA or placebo.
We anticipate that this work (feasibility study and subsequent full study) will inform future NICE guidelines on the management of MPM.

Relevant expertise and experience

Please outline the individual role of each member of the research team, highlighting the skills and experience of the team that make them well placed to carry out the work.

The academic respiratory unit at North Bristol NHS trust/University of Bristol, has extensive experience in conducting randomised controlled trials (RCT) in patients with pleural diseases, a number of these trial are large UK multicentre trials. The unit has enroled over 500 patients with pleural disease into trials in the last 5 years.
Dr Maskell is a Reader in Respiratory medicine at University of Bristol and runs a tertiary pleural service at North Bristol NHS Trust. He a leading pleural disease / mesothelioma researcher in the UK, currently holding 9 grants in this area. He currently sits on 11 Trial Steering Committees and has substantial expertise in multi-centre RCT design and delivery. He is the chair of the regional mesothelioma multi-disciplinary meeting and also co-chairs the British Thoracic Society (BTS) Mesothelioma Guideline group.
Professor Iain Lyburn runs the regional PET service in the Cheltenham Cobalt Centre and has published widely in this field. He has previously participated in multi-centre mesothelioma RCTs and has extensive experience in

reporting PET-CT scans.
Dr Anthony Edey is a consultant radiologist at North Bristol NHS Trust with a specialist interest in pleural based imaging and sits on the regional Mesothelioma MDT. He is also a member of the BTS Mesothelioma guideline group and has experience in participating multi-centre RCTs.
Dr Dangoor is an oncology specialist with a specialist interest in mesothelioma. He will be providing oncological input into this trial as well acting as the principal investigator for one of the research sites.
Dr Chris Rogers is a Reader in Medical Statistics and the co-director of the Clinical Trials Evaluation Unit (CTEU) in Bristol. She will be providing us with methodological and statistical support in this trial. She is a co-applicant on several NIHR funded studies and has successfully conducted several multicentre RCTs in the past.
Ms Sarah Smith is a specialist nurse in lung cancer and mesothelioma with over 10 years' experience working in this field. She is a founder member and a trustee of the Avon Mesothelioma Foundation and will work closely with patient support groups.
Dr de Fonseka is a clinical research fellow working towards a PhD in pleural disease. She has extensive experience in mesothelioma, helping to run the mesothelioma tertiary clinics and deputising on behalf of Dr Maskell at the regional mesothelioma MDT meetings. She also sits on the BTS Mesothelioma guideline group.

11. Management and Governance

Research timetable

Please provide an overview of the research plan which includes specific milestones and deliverables.

Research timetable:

Year 1
Set up phase - 3 months

- Protocol finalisation
- Ethical and regulatory approvals
- CRF design
- Database design, testing and validation
- Recruitment of trial specific staff to respiratory trials unit
- Sign off trial related documents at first TSC meeting

Initial recruitment phase – 9 months

- 9 months of recruitment at a rate of 4-5 patients per month
- TSC meeting 6 months into recruitment
- Data entry, verification and checking to occur 'live' during the recruitment phase

Year 2
Recruitment phase (3 months)

- Continue recruitment for another 3 months at a rate of 4-5 patients per month
- Continue data entry, verification and checking
- TSC meetings at 6 month intervals to review progress

Follow-up phase (9months)

- Follow up of all patients up to 12 months from recruitment

Statistical plan written and signed off by the TSC

Year 3
Follow-up phase (3 months)

- Follow-up until all recruited patients have had 12 months follow-up (should complete at 3 months)
- Data entry, verification and checking

Analysis phase (3 months)

- Data analysis
- Writing up of results for publication
- Final TSC meeting
- Dissemination

Research management arrangements

Please explain the practical arrangements for managing the research and its constituent components.
The study is to be delivered by an experienced research team with a proven track record. The Chief Investigator (CI), Dr Maskell, has successfully conducted and completed several randomised controlled trials, some of which are NIHR funded. Dr Maskell will oversee the trial as the chief investigator but the trial co-ordinator will be responsible for the day to day running of the trial including liaising with other sites on a regular basis to ensure recruitment targets and project milestones are met. Any issues arising will be discussed with the CI as necessary and addressed in conjunction with the rest of the research team. Monthly progress meetings within the research team and 6 monthly TSC meetings will take place to ensure satisfactory progress of the trial. Trial steering committee will comprise of the co-applicants, principal investigators, patient representative and an independant member. We will work closely with the Clinical Trial Evaluation Unit (CTEU) to conduct a streamlined trial. We have worked closely with the director of the CTEU who has given input in the design of the study and we will liaise with her throughout the study to ensure the trial is conducted smoothly and robustly.

Has any work relevant to this proposal already commenced?	No

Success criteria and barriers to proposed work Please set out the measurements of success you intend to use and also the key risks to delivering this research and what contingencies you will put in place to deal with them.
As this is a feasibility study, the main purpose is to identify issues that may arise prior to designing a phase III study. The primary success criterion will be achieving the anticipated recruitment rates. Due to the nature and progression of the disease, limited numbers of patients take up chemotherapy. Some maybe opposed to the idea of a further drug, therefore numbers recruited maybe slow and small. Patients may be deterred by the idea of extra visits to hospital for the drug and scans which again may lead to slow recruitment. Although relatively safe, we are unaware of the side effects of the ZA alongside chemotherapy, which may lead to a number of patients dropping out of the trial early. Any issues identified from our pilot study would be used inform the design of the full phase III study so these can be anticipated and contingencies put in place prior to commencing the trial.

Does the proposed research raise ethical issues?	Yes

If yes, discuss how these issues will be addressed.
We do not anticipate significant ethical issues with regards to the running of this trial. Although this is a new drug in the mesothelioma arena, ZA has been around for a number of years with a good safety profile. It has been used in a number of metastatic malignancies as well non-malignant diseases such as osteoporosis with good tolerance. However, as the patients are NHS patients, as per the research governance framework we will obtain ethics approval and R&D approval at all research sites prior to the start of the trial. All research staff will be trained as per Good Clinical Practice (GCP) criteria. One ethical issue that was raised at the PPI meeting and has now been addressed is that patients who decline chemotherapy should not be deprived of the drug. The proposed third non-randomised group patients who have declined chemotherapy will be offered ZA open label.

Please detail how and when you intend to get ethical review completed.
We have allowed 3 months prior to the start of the trial for protocol finalisation and relevant approvals. All patient literature and the finalised protocol will be submitted to the Bristol Research & Ethics committee for approval. We will be working closely with our R&D department as well our sponsor North Bristol NHS trust when seeking ethical approval.

Have any appropriate regulatory bodies already granted a favourable opinion?	No

Involvement of Clinical Trials Units

Is Clinical Trials Authorisation required?	Yes

Is a Clinical Trials Unit (CTU) involved with this research proposal?	Yes

If yes, what is the name of the CTU? Please provide the name of the CTU involved.
Bristol Clinical Trials Evaluation Unit (CTEU)

Does the CTU hold a UKCRC registration number?	Yes

If yes, please provide the CTU Registration Number?
11

Please describe how you have worked with the CTU in developing your application and what support they will provide if funding is approved.
The director of the CTEU, who is also a co-applicant on this application, was approached at a very early design stage of this application. She has given us input in developing the study and with feasibility outcomes we should be aiming for. The CTEU is hosting the database and providing us with a database manager for this project. Furthermore we would be using electronic randomisation software developed at CTEU. Statistical input will also be provided by the CTEU

APPENDIX IV – SEMI STRUCTURED INTERVIEW THEMES

1. Diagnosis
- Patient understanding
- Diagnostic methods
- Information received about the diagnosis
- Impact on quality of life (patient and family)

2. Symptoms
- Pre-diagnosis
- Post-diagnosis
- Impact on quality of life??

3. Management
- Chemo/no-chemo and reasons behind their decision
- How they tolerated treatment and associated side effects
- Radiotherapy treatment
- Palliative care input

4. Zol-A trial
- Which arm do they think they are in (for non-randomised patients)
- Their understanding of randomisation.
- Randomised patients – do they think they received the treatment
- Their thoughts on trial related procedures such as PET scans and extra visits for bloods
- Did they have any concerns about having the PET scan which involves a radioactive dye?

5. Prognosis and dying
- Change in their outlook of life since the diagnosis
- Thoughts about actual prognosis – how long to live
- Finalising other arrangements such as making a will, funeral arrangements etc.

APPENDIX V – ZOL-A STATISTICAL ANALYSIS PLAN

1. INTRODUCTION TO SAP

1.1 Scope

This statistical analysis plan (SAP) details information regarding the statistical analysis of the Zol-A randomised controlled feasibility trial (RCT) and covers all analyses of trial data outlined in the study protocol, with the exception of the qualitative elements of the project.

1.2 Editorial changes

Any changes made to this SAP after approval must be clearly justified and documented as an amendment at the end of this document. The SAP should then be re-approved.

1.3 SAP document approval

The Chief Investigator should authorise this document.

2. STUDY BACKGROUND AND OBJECTIVES

2.1 Study background

Zol-A is a multi-centre double blind parallel-group feasibility RCT comparing zoledronic acid (ZA) and placebo for the treatment of with malignant pleural mesothelioma (MPM) alongside chemotherapy. The study also includes an 'open treatment group' of patients who decline randomisation and chemotherapy but consent to receiving ZA.

2.2 Study objectives

The aim is to establish the feasibility of the full multi-centre trial and to assist in its design. Specific objectives are to obtain accurate information on:

- Presentation, recruitment, consent and randomisation rates of MPM patients at each site.
- Acceptability of recruitment procedures, consent and randomisation, and data collection methods.
- Acceptability of ZA in MPM patients, and the optimal timing and location for ZA administration.
- Qualitative assessment in a subgroup of up to 150 patients to evaluate patients experience in the randomisation and recruitment process
- Quantification of drop-out and data completeness rates
- Estimates of outcome event rates e.g. survival times, measures of mean response and outcome variance (continuous variables such as quality of life)
- Measure of variation between patients and between sites and information required to assess the intra class correlation coefficients to be used to estimate clustering effects.

2.3 Outcomes

No primary or secondary outcomes have been explicitly defined. Quantifiable feasibility outcomes are defined as follows:

- Number of patients screened, approached and consented to the RCT or the open treatment group.
- Timing of drug administration
- Location of drug administration
- Drop-out rate
- Data completeness rates
- Estimates of outcomes to inform the design and size of a full trial, i.e.

- progression as assessed by modified RECIST criteria on CT)
- standard deviation of quality of life measures (dyspnoea Visual analogue scale (VAS) scores and EQ5D and EQ5D VAS);
- Tumour metabolic activity as determined by Total glycolytic volume (TGV) on PET-CT scan
- Value of TGV or standard uptake value (SUV) in the assessment of disease response/progression
- Serum mesothelin levels

Additional outcomes not listed explicitly in the protocol that will be reported on are:

- Adherence to study treatment and reasons for non-adherence
- Rate of unblinding

2.4 Changes to the study objectives during the course of the study (if required)

Primary feasibility outcome was changed from 12 to 13 months. Some of the eligibility criteria changed slightly (but this may not be a change to the objectives)

3. STUDY POPULATION

The study population is adult patients with a new diagnosis of mesothelioma.

The planned sample size for the trial is 50 randomised patients (25 ZA and 25 placebo) plus 20 in the open treatment group over 12 months

3.1 Flow of participants

Participants are randomised into the RCT and undergo up to 6 cycles of chemotherapy plus study treatment. The baseline CT scan used in this study was the clinical scan performed in the immediate run up to diagnosis (within 8 weeks of trial entry). They have CT scans at 3 cycles post treatment and again at 6 months from randomisation. They have a PET-CT scan at baseline and again after 3 cycles and are followed up at till 6 and 12 months after randomisation. Patients consenting to open treatment receive 6 doses of ZA without chemotherapy. Otherwise the assessment pathway is the same.

3.2 Characteristics of non-trial non-randomised patients

Characteristics of patients who declined randomisation but consented to the open label treatment group will be compared to those who consented to randomisation.

3.3 Randomisation

Patients are randomised (1:1 ratio) to ZA or placebo. The allocation will be blocked using varying block sizes and stratified according to histological sub-type.

3.4 Protocol deviations

The following types of protocol deviation will be considered:

- Trial patient received the alternative treatment to that allocated.
- Trial patient did not meet the study eligibility criteria but was treated in the study.

- Trial patient did not receive study treatment according to the protocol, i.e. study treatment was not administered in approximately 3 weekly cycles, coinciding with chemotherapy. Note: Patients who delay chemotherapy delay study treatment. Patients who stop chemotherapy stop study treatment at that time.
- Chemotherapy treatment was not in line with the study protocol, i.e. not combination treatment with Pemetrexed and Cisplatin/Carboplatin for a maximum of 6 cycles.
- Concomitant medication not in line with study protocol, i.e. supplementary calcium tablets (AdCal D3 one tablet twice a day) not prescribed. Note: patients who develop hypercalcaemia should stop taking the Adcal D3.
- Calcium level not checked with each pre-chemotherapy blood test
- Treatment allocation unblinded not in response to a request for unblinding or safety grounds

Note it may be possible for patients to be classified as a protocol deviation for more than one reason.

The frequency of each type of deviation will be tabulated by treatment allocation (reference Table T2) with full details given in separate listings (reference Table T3).

3.5 Withdrawals

Patients (or clinicians on their behalf) can withdraw from the study at any time post-randomisation. In some cases patients were happy for data collection to continue, and therefore such patients will be included in the study analyses on an intention to treat basis (ITT), see section 3.6.

Data on all withdrawals is captured on a specific case report form (CRF), and will be tabulated by treatment allocation (reference Table T4) with full details given in separate listings (reference Table T5).

3.6 Analysis population

The analysis population consists of all randomised patients excluding

- Patients who died after randomisation but prior to any data collection.
- Patients withdrawn who were unwilling for data collected to be used.

The trial data will be presented by allocated group (ITT).

3.7 Safety population

The safety population will consist of the analysis population. Safety data will also be presented by allocated group (ITT).

4. DERIVATIONS

4.1 Outcomes

New variable	Rules
Proportion of patients eligible	Number of patients meeting eligibility criteria / number of patients screened
Proportion of patients consenting to RCT	number of patients consenting to be randomised / number of patients meeting eligibility criteria and being approached for the trial
Proportion of patients consenting to open treatment group	number of patients declining randomisation but consenting to receive ZA treatment / number of patients meeting eligibility criteria and being approached for the trial
Timing of drug administration	Date of treatment – data of randomisation

New variable	Rules
Time to death (randomised patients)	If death = yes then date of death – randomisation date Else date of last follow-up – randomisation date
Time to death (open treatment group)	If death = yes then date of death – consent date else date of last follow-up – consent date
Survival indicator	If death = yes then indicator = 1 Else indicator = 0
Time to disease progression	If progression = yes then date of progression – randomisation date Else if progression = no and death = yes then date of death – randomisation date Else if progression = no and death = no date of last follow-up – randomisation date
Disease progression	To be defined (based on *modified RECIST criteria on CT)*
Time to disease progression (open treatment group)	If progression = yes then date of progression – consent date Else if progression = no and death = yes then date of death – consent date Else if progression = no and death = no date of last follow-up – consent date
Progression free survival indicator	If progression = yes then indicator = 1 Else indicator = 0
EQ-5D	Data from the EQ5D questionnaire will be used to derive a five digit 'state' from the mobility, self-care, usual activities, pain/discomfort and anxiety/depression scores using the following: State = 10000*mobility score + 1000*self-care score + 100*usual activities score + 10*pain/discomfort score + anxiety/depression score Each state will then be assigned a single summary index score according to standard scales. These index scores are numerical and range from -0.59 to 1.00, with a score of 1.00 denoting perfect health. If any of the five scores are missing, the state score will be missing.
Adherence	If chemotherapy = yes and study treatment = no then adherence = 0 Else if chemotherapy = yes and study treatment = yes then adherence = 1

4.2 Other variables

New variable	Rules
Age	(Operation date – DOB)/365.25
BMI	Weight (kg) / Height $(cm)^2$ * 10,000
Number of cycles of chemotherapy received	
WHO performance status	0-2
Laterality of disease	Right / Left
Mode of diagnosis	LAT/Image guided/VATS/cytological
Histological subtype	Epithelioid/Sarcomatoid/Biphasic/Mesothelioma NOS/Other

New variable	Rules
Previous Pleurodesis	Yes / No
IPC in -situ	Yes / No
CT staging of disease	Ia, Ib, II, IIIa, IIIb, IV
PET-CT parameters	Upper, mid and lower SUVmax
Baseline Mesothelin	Mesothelin level (nmol/L)
Baseline Neutrophil/Lymphocyte ratio	Neutrophil count divided by lymphocyte count at baseline
Baseline EQ5D VAS score	

5. STATISTICAL ANALYSES

5.1 Baseline data

Baseline (i.e. patient demography and past history) characteristics will be described by treatment group for patients in the analysis population. Tables T6 to T7 will be used as templates for this.

Continuous variables will be summarised using the mean and standard deviation (SD) (or median and inter quartile range (IQR) if the distribution is skewed), and categorical data will be summarised as a number and percentage. The summary statistic headings given in Tables T6 to T7 are those we expect to use based on a-priori knowledge of the clinical measurements gained from previous studies. However, if distributional assumptions are not valid, changes will be made.

Any imbalances in the characteristics of the patients at the start of the study will be described but statistical tests for baseline imbalance will not be carried out.

Characteristics of patients in the open treatment group will also be described.

5.2 Outcomes

5.2.1 Adjustment in models

As this is a feasibility study and not powered to test research hypotheses, no statistical comparisons between groups will be made.

5.2.2 Analysis models

- All outcomes listed in the study protocol will be presented in template tables. Details specific to each outcome are described as appropriate.
- Binary or categorical outcomes will be presented as numbers and percentages of patients in each treatment group. Rates will be presented with 95% confidence intervals (95% CI).
- Continuous outcomes will be summarised by the mean and SD in each treatment group, if distributions are approximately normal. If distributions are non-normal data will be summarised by the median and IQR or geometric mean (GM) if a logarithmic transformation provides an approximately normal distribution.
- Time to event outcomes will be summarised by the median and IQR in each treatment group estimated using the Kaplan Meier method.
- Continuous longitudinal outcomes will be summarised as means and SDs (or medians and IQRs if distributions are skewed) at each time point. Statistical significance

5.2.7 Missing data

In all tables missing data will be indicated by footnotes.

5.3 Safety data

Adverse events occurring in the study period for all patients in the safety population will be tabulated.
Expected adverse events listed in the study protocol, with events that meet the serious criteria will be summarised. Such events are captured via the study CRFs.
Unexpected serious adverse events (SAEs), i.e. events that are not listed in the study protocol that meet the serious criteria will be summarised separately. Such events are captured via separate SAE report forms and full details will also be given as listings, with events that are classified as possibly, probably or definitely related highlighted
No formal comparisons between treatment groups will be made, as numbers of events are expected to be small.