Online depository 1.

Laboratory processing of pleural fluid and pleural biopsy samples.

Pleural fluid samples for cytology were collected into containers with added sodium citrate solution, to prevent clotting. On receipt in the laboratory, the specimens were centrifuged. A sample of the deposit was spread onto two glass slides or, if the deposit was scanty, two cytospin preparations were made. One slide was wet fixed for Papanicolaou staining and the other slide was air dried for MGG staining.

Cytolyt was added to the remaining deposit and the specimen was stored in the refrigerator. If immunohistochemical staining was required, a thrombin clot was prepared from the sample in Cytolyt, fixed in 10% buffered formalin and processed for histology.

Pleural biopsies for histology were fixed in 10% buffered formalin, embedded in paraffin wax and cut into sections 3μm thick. The sections were stained with Haematoxylin and Eosin.

Online depository 2

CT scans

Pleural phase contrast CT scans of thorax and upper abdomen were performed when indicated according to our usual departmental clinical protocol. Scans were reported by two radiologists providing a full clinical report in addition to a trial report commenting on the presence of Leung's criteria for malignant pleural thickening[1] and giving a categorised diagnostic conclusion (benign, infective, primary malignancy at a non-pleural site, suspicious of pleural malignancy or suspicious of mesothelioma).

1/. Leung AN, Muller NL, Miller RR. CT in differential diagnosis of diffuse pleural disease. Am J Roentgenol 1990:487 - 492

Online depository 3

Creatinine measurement and estimated glomerular filtration rate (eGFR) calculation:

Roche Cobas Creatinine plus ver.2 assay (Roche diagnostics ltd, UK). This is an enzymatic colorimetric method, which uses creatininase and creatinase to produce a coloured quinone imine chromagen.

The eGFR calculation used is the MDRD formula:

eGFR = 186 x (Creatinine umol/L x 0.011312)$^{-1.154}$ x (Age)$^{-0.203}$ x (0.742 if female) x (1.212 if Afro-Caribbean)

Online depository 4

Diagnostic criteria

Diagnostic category	Criteria
Malignant pleural mesothelioma	Definitive histological diagnosis from pleural biopsy specimen in the context of consistent radiology and clinical presentation. All reviewed at regional mesothelioma multi-disciplinary meeting with agreement regarding diagnosis amongst expert members.
Malignant pleural effusion	a/. Malignant pleural fluid cytology or pleural biopsy or b/.Histologically confirmed extra-pleural malignancy with radiographic evidence of pleural metastases or c/.Post mortem diagnosis.
Unconfirmed malignant pleural effusion	Radiographic evidence of pleural malignancy in the absence of diagnostic histology or cytology either local to the pleural or from a distant primary site.
Benign asbestos exposure related pleural effusion	Exposure to asbestos or radiographic evidence of pleural plaques and stable or improving CT appearances with radiographic clinical and CT follow-up for at least 12 months and benign pleural biopsy histology.
Pleural effusion due to a cardiac cause	History/examination features of cardiac disease/evidence of left ventricular failure/moderate-severe valve disease on echocardiogram/improvement in effusion with diuretic therapy in the absence of clinical, pleural fluid, serum and radiographic evidence of another cause.
Simple parapneumonic effusion	Clinical presentation suggestive of sepsis with appropriate chest radiology and pleural fluid which is gram stain and culture negative with a pH >7.2 and an absence of loculation on thoracic ultrasound and resolution of effusion on CXR after antibiotics or subsequent clinical progression to pleural infection.
Pleural infection	Clinical presentation suggestive of sepsis and pleural fluid pH ≤7.2 or pleural fluid loculation on ultrasound and follow up for 12 months inconsistent with pleural malignancy or pleural fluid gram stain or culture positive or frank pleural pus (empyema) or pleural infection confirmed by pleural biopsy histology and/or microbiological culture.
Pleural effusion due to connective tissue disease	Systemic features or known diagnosis of connective tissue disease and chest radiology (including CT imaging) showing benign features with at least 12 months follow-up and /or pleural biopsy negative for malignancy**.**
Pleural effusion due to pulmonary embolism	Evidence of pulmonary embolus on CTPA scan and no alternative explanation for the pleural effusion on CT or pleural fluid analysis.
Post CABG effusion	Effusion occurring within 3 months of CABG with no alternative cause demonstrated.
Non-cardiac transudate	Transudative effusion with biochemical evidence of hepatic or renal failure or hypoalbuminaemia in the absence of clinical, pleural fluid and radiographic evidence of another cause.
TB pleuritis	Culture or AAFB positive sputum, pleural fluid or pleural tissue or classical pleural tissue histology and resolution of pleural effusion with anti TB therapy at 6 month follow-up.
Idiopathic pleuritis	Pleural biopsy negative for malignancy and 12 months follow-up with interval CT scanning inconsistent with pleural

	malignancy.
Undiagnosed	None of the above criteria were reached despite exhaustive investigation and at least 12 months follow-up.

Online depository 5

Mesothelin levels in patients with malignant pleural effusions

Malignant cell type	Number of patients	Serum mesothelin	Pleural fluid mesothelin
Epithelioid MPM	23	2.87 (0.83-10.05)	43.55 (23.18-32.38)
Sarcomatous/Biphasic MPM	5	1.00 (0.60-2.79)	11.84 (5.15-36.03)
Non-small cell lung cancer (NSCLC)	28	1.29 (0.95-2.00)	6.50 (4.02 – 22.34)
Small cell lung cancer	3	1.07 (0.90 – 2.20)	3.05 (2.65-5.06)
Breast carcinoma	9	0.70 (0.45-0.99)	3.70 (2.97-9.10)
Ovarian carcinoma	8	5.14 (0.95-19.51)	35.43 (15.41-65.24)
Gastrointestinal malignancy	3	1.04 (0.50-31.60)	14.02 (4.25 – 24.81)
Adenocarcinoma of unknown origin	4	1.25 (0.35 – 12.20)	13.35 (1.78-106.9)
Haematological malignancy	4	1.53 (1.04-2.2)	5.80 (3.73-11.40)
Renal cell carcinoma	4	1.14 (0.87-1.80)	3.34 (2.17 -12.33)
Other malignancy	11	1.10 (0.90 – 1.90)	12.50 (6.88 – 32.38)

Online depository 6

Clarification of non-diagnostic cytology results

223 patients underwent a diagnostic pleural fluid aspiration. 11 samples were frankly purulent, chylous or had positive bacterial MC and S results. 38 demonstrated malignant cells, 26 demonstrated atypical cells but were non-diagnostic for malignancy and 148 samples were benign following 2 diagnostic aspirations.

26 patients with atypical but non-diagnostic cytology included 13 with a final diagnosis of mesothelioma. In this group, serum mesothelin had sensitivity 61.5% (31.6 – 86.1),

Specificity 44.4% (13.7-78.8), PPV 61.5% (31.6-86.1), NPV 44.4% (13.7- 78.8) and PF mesothelin sensitivity 72.7% (39.0-94.0), Specificity 70.0% (34.6-93.3), PPV 72.7% (39.0 - 94.0), NPV 70.0% (34.7-93.3).

148 patients with repeatedly benign pleural fluid cytology included 15 with a final diagnosis of mesothelioma. Here, serum mesothelin had sensitivity 57.1% (28.8 – 82.3), specificity 68.6% (59.6-76.6), PPV 17.0% (7.6-30.8), NPV 93.4% (86.2 -97.5) and PF mesothelin sensitivity 64.3% (35.1 – 87.2), specificity 96.5% (91.2 -99.0), PPV 69.2% (38.6-90.9), NPV 95.6% (90.1-98.6).

Diagnostic characteristics of serum mesothelin in patients with benign pleural fluid cytology (excluding patients with unconfirmed malignancy and undiagnosed effusions).

Serum mesothelin	MPM	Not MPM	Total
≥ 1.5 nM	8	39	47
< 1.5 nM	6	85	91
Total	14	124	138

Diagnostic characteristics of pleural fluid mesothelin in patients with benign pleural fluid cytology (excluding patients with unconfirmed malignancy and undiagnosed effusions)

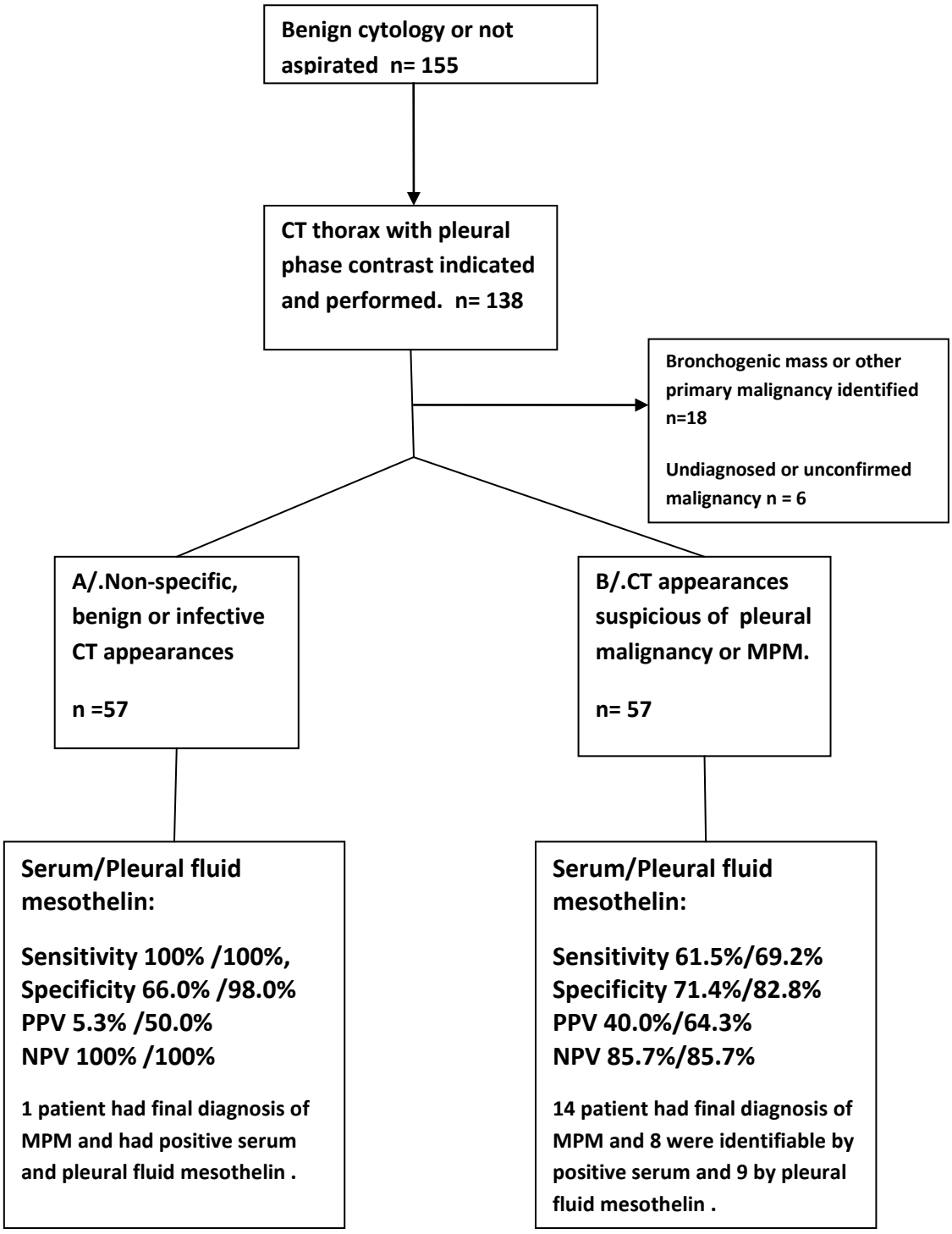
Pleural fluid mesothelin	MPM	Not MPM	Total
≥ 20.0 nM	9	4	13
< 20.0 nM	5	109	114
Total	14	113	127

Online depository 7.

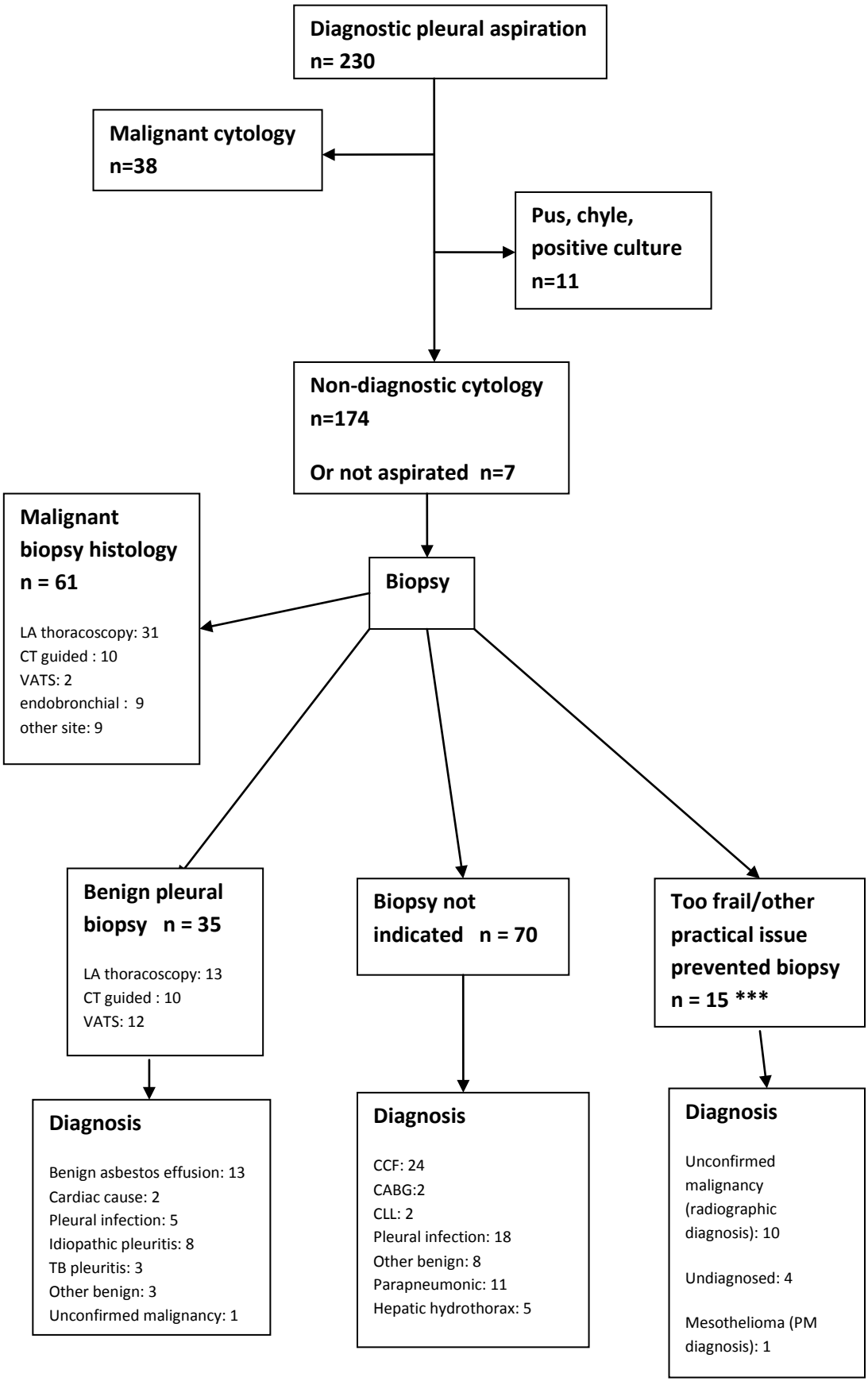
CT thorax information in patients with suspicious pleural fluid cytology

All 26 patients with suspicious/atypical pleural fluid cytology underwent a CT. 4 patients had unconfirmed malignancy at the end of the trial and 5 had bronchogenic or other clear primary sites of malignancy on CT. Of the remaining 17 patients, 2 had benign or non-specific CT reports but both had a final diagnosis of mesothelioma, one with a positive serum mesothelin and both with negative pleural fluid tests. The final 15 patients had CT appearances suspicious of pleural malignancy and amongst them, the diagnostic characteristics of the test for serum and pleural fluid were as follows: Sensitivity 63.6% (30.8-89.1)/88.9% (51.8-99.7), Specificity 25.0% (0.63-80.6)/50.0% (67.6-93.2), PPV 70.0% (34.8-93.3)/80.0% (44.4-97.5), NPV 20.0% (0.5-71.6)/66.7% (9.4-99.2).

CT scan results and diagnostic characteristics of mesothelin in 138 patients with benign pleural fluid cytology who underwent CT.



Online depository 8: The investigation pathway leading to final diagnosis



Online depository 9. Benign asbestos related effusions (BAPE)

5/13 patients had a positive serum mesothelin and 2/10 for whom fluid was available had a positive pleural fluid test. All had stable or improving CT appearances on serial scans over at least 12 months.

5 positive serum results occurred in 1 patient with a post mortem confirmation of BAPE, 1 patient who had undergone a benign VATS biopsy, 2 patients with repeated benign CT guided biopsies and 1 patient who had undergone a CT guided biopsy containing minimal pleural tissue. In this final patient, malignancy remains a possibility.

2 positive pleural fluid results occurred in patients with benign CT guided biopsies.