but no evidence of cancer was found after 1-year follow-up (high-risk). c) 54 healthy controls (HC) (mean age 51.1±15.3 years) who had no history or symptoms of LC or known respiratory disease. Sputum was self-expectorated and frozen immediately at −80°C, thawed in batches, mucolytics were added then samples centrifuged at 3000 rpm for 10 min to form pellets. FTIR was performed using the VERTEX 70 spectrometer (Bruker Optics Ltd, Banner Lane, Coventry, UK). Median absorbance values for each wavenumber for the LC and HC cohorts were compared, then principal component analysis (Abstract S38 Figure 1) and logistical regression identified the wavenumbers that provided the greatest accuracy in differentiating the two groups; the high-risk cohort was then applied to the predictive model to see if they could be correctly identified.

Results 126 light absorbance wavenumbers were significantly different between the LC and HC groups (each $p<0.05$). Two wavenumbers, 1031.7 cm^{-1} and 1409.7 cm^{-1} were used to develop a predictive model providing a sensitivity of 93% and specificity of 91%. This model then predicted 17 of the 24 high-risk cohorts as LC.

Conclusion FTIR spectroscopy can distinguish LC from HC with high accuracy but had reduced specificity when applying high-risk patients, tending to over-diagnose LC. Follow-up will determine if these 17/24 people are indeed false positives or have pre-cancerous molecular changes not identifiable by current methods.

REFERENCE

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S39 SUB TYPING OF NON SMALL CELL CARCINOMA IN EBUS-TBNA SAMPLES

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Introduction Differentiation and accurate classification of NSCLC (Squamous, Adenocarcinoma, Large cell carcinoma) is crucial in determining the prognosis and selecting targeted chemotherapy regimens. However, it is not always possible to subtype the tumours particularly if the biopsy samples are small and such undifferentiated tumour is referred as NSCLC not otherwise specified (NOS). It has been shown that 25% of bronchial biopsy specimens and 40% cytological specimens result in a diagnosis of NSCLC-NOS. However, the frequency of NSCLC-NOS with EBUS-TBNA samples is not known.

Methods We looked at the cytology reports of all patients with an EBUS-TBNA diagnosis of NSCLC over a period of 13 months. In patients with a diagnosis of NSCLC-NOS, we obtained further information on the details of the EBUS procedure and the cytological methods used.

Results Of the 243 patients who underwent EBUS-TBNA, 78 with a diagnosis of NSCLC were included. A confident initial cytological sub typing of NSCLC was possible in 68 (87%). Analysis of the remaining 10 patients with a diagnosis of NSCLC-NOS showed that biopsies taken from the lymph nodes were deemed adequate for cell block and immunohistochemistry (IHC) in all but one patient. Despite this, IHC was performed on 3 out of 9 samples. IHC was able to subtype the tumour in these cases. The Haematoxylin and Eosin (HE) and IHC profile of the 10 patients are shown in Abstract S39 Table 1.

Abstract S39 Table 1

Patients	HE	P63	CK5/6	TTF1	Final diagnosis
1	NSCLC-NOS	ND	ND	ND	NSCLC-NOS
2	NSCLC-NOS	ND	ND	ND	NSCLC-NOS
3	NSCLC-NOS	ND	ND	ND	NSCLC-NOS
4	NSCLC-NOS	ND	ND	ND	NSCLC-NOS
5	NSCLC-NOS	ND	ND	ND	NSCLC-NOS
6	NSCLC-NOS? Squamous	ND	ND	ND	NSCLC-NOS? Squamous
7	NSCLC-NOS? Adenocarcinoma	IT	IT	IT	NSCLC-NOS? Adenocarcinoma
8	NSCLC-NOS	++	++	–	Squamous
9	NSCLC-NOS	–	–	++	Adenocarcinoma
10	NSCLC-NOS	–	–	++	Adenocarcinoma

HE, Haematoxylin and Eosin; Squamous carcinoma markers – p63, cytokeratin 5/6, adenocarcinoma marker – Thyroid transcription factor 1, ND, Not done, IT, Inadequate tissue.

Conclusion Thus we have shown that adequate tissue samples can be obtained at EBUS-TBNA and the frequency of NSCLC-NOS is less (7/78=9%) compared to the histological bronchial biopsy samples. In cases, where morphological sub typing of NSCLC on HE is not possible, immunohistochemistry should be performed.

S40 EARLY EXPERIENCE OF ENDOBRONCHIAL ULTRASOUND-MINIPROBE (EBUS-MP) FOR INVESTIGATION OF PERIPHERAL PULMONARY MASS LESIONS

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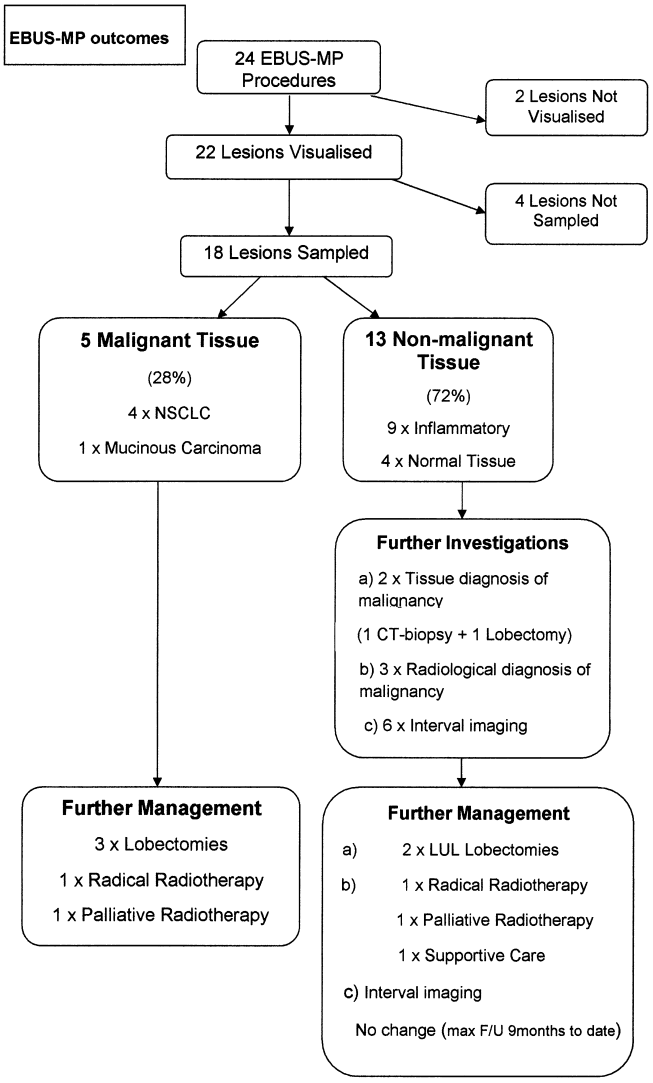
Introduction Peripheral pulmonary mass lesions are common findings in respiratory medicine. The frequency of detection of such lesions is rising with increasing availability of radiological imaging techniques. Their aetiology may need to be established by tissue sampling to facilitate appropriate management, for example, suspected malignancy. Traditional investigations include CT-guided biopsy, bronchoscopic biopsy, endoscopic ultrasound with fine needle aspiration (EBUS/EUS) and surgical intervention. Each modality has potential complications, for example, pneumothorax following CT-guided biopsy. Endoscopic ultrasound miniprobe is established as a valuable tool, particularly in the staging of early GI tumours and in extraductal visualisation of the biliary tract. EBUS-MP has been used for qualitative assessment of bronchial mural structures in lung transplant recipients but little is known about the role of EBUS-MP sampling of peripheral pulmonary mass lesions. The purpose of this paper is to demonstrate our experience with this technique to date.

Methods All EBUS-MP procedures were carried out over a 6-month period in a tertiary respiratory centre. Patients were referred for suspected malignancy. All procedures were undertaken by the same consultant bronchoscopist, assisted by a respiratory trainee. An Olympus UM-S20-17S 1.7 mm Miniprobe® was identify the target lesion. Samples (biopsies or endobronchial brushings) were then taken from the identified subsegmental bronchus. Each case was subsequently reviewed with respect to diagnostic rate, subsequent management, complications and potential alternative investigations to EBUS-MP.

Results 24 EBUS-MP procedures were performed on 22 patients (Age range 53–82 years (mean 70.4 years)). FEV1 ranged from 0.8 L to 2.9 L. 20 of 22 CT-identified lesions (14–60 mm) were visualised with EBUS-MP. No complications occurred in study population. Abstract S40 Figure 1 shows detailed outcomes for EBUS-MP.

Conclusions EBUS-MP is a novel technique in bronchoscopy. Our early experience has demonstrated some potential usefulness of the procedure, allowing good visualisation of lesions. No complications have occurred to date. We believe that EBUS-MP sampling may have



Abstract S40 Figure 1 EBUS-MP outcomes.

a role in investigation of peripheral pulmonary mass lesions. In a well selected cohort of patients it appears to be a safe alternative to CT-guided biopsy.

Immunity and fibrosis in chronic asthma

S41 EXPRESSION OF FUNCTIONAL RECEPTOR ACTIVITY MODIFYING PROTEIN (RAMP)-1 BY AIRWAY EPITHELIAL CELLS WITH DYSREGULATION IN ASTHMA

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Introduction and Objectives Epithelial cell expression of calcitonin-gene-related peptide (CGRP) is a feature of provoked asthma. Receptor activity modifying protein 1 (RAMP1) and the calcitonin-receptor-like receptor (CRLR) combine to form the CGRP1 receptor. We determined whether functional RAMP1 is expressed by airway epithelial cells and if there are alterations in asthma at baseline and after allergen challenge.

Methods BEAS-2B and A549 cells lines were studied by RT-PCR, confocal microscopy, a quantitative immunofluorescence assay and ELISA. Bronchial biopsies from normals and asthmatics were examined by immunohistochemistry and in situ hybridisation.

Results Inflammatory cytokines induced CGRP release and CGRP mRNA in BEAS-2B and A549 epithelial cell lines. RAMP1 was highly expressed by resting, unstimulated BEAS-2B and A549 cells. CGRP induced internalisation of RAMP1 and IL-6 production, both of which were inhibited by the CGRPR antagonist, CGRP8-37. Activation of BEAS-2B and A549 cells by inflammatory cytokines induced CGRP secretion, binding of CGRP to RAMP1 and RAMP1 internalisation which was blocked by CGRP 8-37. RAMP1 immunoreactivity and RAMP1 mRNA expression in bronchial biopsies from asthmatics was significantly lower than in normal subjects ($p=0.002$ and $p=0.007$, respectively). Inhalational challenge of atopic asthmatics with allergen-derived peptides produced a significant decrease in the numbers of RAMP1-positive epithelial cells in responders ($p=0.027$) but not non-responders.

Conclusions RAMP1 was expressed both by airway epithelial cells in culture and in bronchial biopsies from normal subjects and internalised after epithelial cell activation through autocrine feedback of CGRP. There is an apparent dysregulation of RAMP1 in asthmatic epithelium suggesting continuous stimulation of pathways involving CGRP.

S42 AIRWAY EPITHELIAL TOLL RECEPTOR EXPRESSION IN ASTHMA AND ITS RELATIONSHIP TO DISEASE SEVERITY

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Introduction Asthma is classically considered a Th2 mediated disease. However, severe and treatment-resistant disease is more heterogeneous and often associated with airway neutrophil recruitment. This may be related to an altered airway bacterial colonisation. Bacteria express pathogen associated molecular patterns (PAMP's) that are recognised as non-self by pattern recognition receptors (PRRs). These PRRs represent an essential component of the innate immunity and an important family of PPRs are the Toll-like receptors (TLR). These are expressed on a range of innate immune cells including epithelial cells. TLR-1, -2, -4, -5 and -6 are located on the cell surface membrane and respond to bacterial cell wall components. This study has investigated the expression of mRNA for TLR-2, -4 and -5 in airway epithelial cells in asthmatics and healthy volunteers.

Methods Epithelial brushings were obtained from the large central airways at fibre-optic bronchoscopy from 18 healthy non-asthmatic volunteers (8 female and mean age 26 years) and 34 asthmatic volunteers (25 female, mean age 43 years). The asthmatic group comprised 7 non-steroid treated and 27 steroid treated asthmatics. The brushings were placed in Trizol and the RNA subsequently extracted and converted to cDNA, prior to real time TaqMan RT-qPCR analysis for the Toll receptors, TLR2, TLR4 and TLR5, as well as IL-8.

Results Gene expression for TLR-2 ($p=0.008$) and TLR-4 ($p=0.012$) was significantly increased within the epithelial brushing sample from the asthmatics compared to the healthy control subjects whilst that for TLR-5 did not differ significantly. Interleukin 8 mRNA was also increased within the epithelial brushing sample in the asthmatics ($p=0.007$) compared to that in the healthy control subjects. These significant differences from the healthy population were also individually present in both the mild and severe asthmatic groups, with no significant difference being evident between mild and severe asthma.

Conclusions These findings reveal up regulation of epithelial gene expression for members of the Toll receptor family relevant to bacterial responses within the airways. Additionally there is enhanced IL-8 gene expression. These features are indicative of on-