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Analysis of bronchial biopsies in chronic cough

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ABSTRACT

Background: Chronic cough is commonly associated with asthma, gastro-oesophageal reflux disease and postnasal drip, but in a significant proportion, no associated cause can be found. We determined whether examination of bronchial biopsies would be useful in determining the cause associated with chronic cough.

Methods: 100 consecutive patients referred to a specialist cough clinic underwent a systematic assessment including a fiberoptic bronchoscopy for bronchial biopsies.

Results: In 38 patients, treatment of associated causes led to amelioration of cough ('explained') and in 62, there was no association or improvement ('idiopathic'). The latter group had a longer duration of cough, a lower FeNO levels and a more sensitive capsaicin cough response, with an increase in basement membrane thickness with no differences in goblet cell hyperplasia and seromucinous hyperplasia, and in lymphocyte, neutrophil and eosinophil counts. The duration of cough was inversely correlated with the degree of neutrophil infiltration.

Conclusion: We conclude that pathological examination of bronchial biopsies is unlikely to be useful in the diagnosis of chronic cough in non-smokers.

1. Introduction

Cough is one of the most frequent reasons for consultation with a family doctor, or with a general or respiratory physician. Patients with chronic cough account for 10–38% of respiratory outpatient practice [1]. The commonest conditions associated with chronic cough, in adults with a normal chest radiograph, include eosinophilic airway diseases such as asthma, cough variant asthma, and eosinophilic bronchitis, and conditions such as gastro-oesophageal reflux disease (GORD), and the postnasal drip syndrome or upper airway cough syndrome [1]. There is however a large group of patients where no causative factor can be found in chronic cough patients despite intensive investigations, or treatment directed at potential causative factors do not provide relief of cough [2–4]. This condition of chronic idiopathic cough or chronic cough of no known cause has been found to be present in a significant proportion of patients attending specialised cough clinics [1].

Guidelines on the management of patients with chronic cough advocate a diagnostic approach to finding the cause of chronic cough [5–9]. Use of non-invasive measurements of exhaled nitric oxide and induced sputum measurements have been advocated in the diagnostic pathway of chronic cough patients, mainly for the exclusion of asthma or other eosinophilic-associated conditions [10–12]. Although mucosal biopsies from patients with chronic cough have been examined previously [3,13,14], the value of this investigation in the management of patients with chronic cough remains unclear. Pathological examination of bronchial biopsies will allow an analysis assessment of inflammation and remodelling features that could point towards eosinophilic or neutrophilic conditions.

We therefore set out to examine the utility of examining mucosal biopsies by a routine histopathological service in 100 consecutive patients with chronic cough referred to our specialised

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Chronic Cough clinic. We also examined whether the pathological features in the biopsy could relate to the clinical features of chronic cough.

2. Methods

2.1. Subjects

Patients referred from general practice or from other pulmonary clinics were recruited from the Cough Clinic at the Royal Brompton Hospital. Current smokers or ex-smokers with greater than a 10 pack-year history of cigarette smoking were excluded. They had a chronic cough of at least 8 weeks' duration and underwent a diagnostic evaluation that included pulmonary function tests, chest radiography, capsaicin cough sensitivity testing, 24-hr oesophageal pH monitoring, methacholine challenge, skin prick tests, exhaled nitric oxide, high resolution computed tomography of chest and sinus and fibreoptic bronchoscopy for mucosal examination and mucosal biopsies. Patients gave written consent to participate in the study, which was approved by the Royal Brompton, Harefield and NHLI Research Ethics Committee.

Patients were divided according to whether or not there was an identifiable cause for their cough following a diagnostic pathway as recommended by the ERS guidelines for management of cough [5]. Patients in whom there were identifiable causes (e.g. asthma, gastro-oesophageal reflux, post nasal drip) for their cough and whose cough symptom had improved following such targeted therapy were classified as having chronic explained cough (CEC). Chronic cough caused by gastro-oesophageal reflux was diagnosed by 24-hr oesophageal pH monitoring and by response to proton pump inhibitors and dietary changes. Chronic cough was attributed to post nasal drip (PND) or upper airway cough syndrome when symptoms and objective diagnosis of PND were present and nasal topical anti-cholinergics and corticosteroids, and oral H1-antihistamines improved symptoms and cough. Asthma was diagnosed when there was a history of reversible airflow obstruction associated with airways hyper-responsiveness (PC_{20} < 4 mg/ml), diurnal variation of peak expiratory flow rate (>20%) or >15% increase in forced expiratory volume in 1 s (FEV_1) after β-agonist, and an improvement in cough following inhaled β-agonist and corticosteroid therapy. In patients in whom systematic investigation did not yield a specific diagnosis and who had failed trials of specific treatment were classed as having chronic idiopathic cough (CIC).

2.2. Spirometry and exhaled NO

Spirometry (FEV_1 and forced vital capacity, FVC) was measured using a dry wedge spirometer (Vitalograph, Buckinghamshire, UK). Exhaled nitric oxide (eNO) level was measured using an online chemiluminescence analyser (NIOX, Aerocrine, Stockholm, Sweden) at a constant expiratory flow of 0.05 L/s.

2.3. Capsaicin cough challenge

Capsaicin challenge was performed as previously described [15]. Coughs were counted for one minute after single-breath inhalations of 0.9% sodium chloride and capsaicin solutions of increasing concentrations (0.98–500 μm) generated from a dosimeter (P.K. Morgan Ltd, Gillingham, UK) set at a dosing period of 1 s. The concentration that caused more than 5 coughs was recorded as C5, and the data was analysed as $\log_{10}$C5. The Leicester Cough Questionnaire [16] was used to assess the impact of chronic cough on the patients' quality of life.

2.4. Bronchial biopsy and biopsy quantification

Bronchoscopy was performed under sedation with intravenous midazolam (3–6 mg) and following topical anaesthesia to upper and lower airways with lignocaine. A fibreoptic bronchoscope (Olympus BF10; Key-Med, Herts, United Kingdom) was passed through the nasal passages into the trachea. Three to five biopsies were taken from the segmental and subsegmental bronchi of the right lower lobe. Biopsies were fixed in 10% formalin and embedded in paraffin wax.

Five μm sections were cut and stained with haematoxylin and eosin to assess morphology. A semi-quantitative score was used to assess histological parameters, which was shown to be reproducible amongst practising pulmonary pathologists [17]. The following parameters were assessed: goblet cell hyperplasia in the epithelium; lymphocyte, neutrophil and eosinophil counts in the section; reticular basement membrane thickness and seromucinous gland hyperplasia. Each of these parameters were scored on a scale of 1–3, with 1 indicating no or mild, 2 moderate and 3 severe by two experienced airway histopathologists (LF and AN).

2.5. Statistical analysis

Statistical analyses were performed using the Statistical Package of the Social Sciences (SPSS for Windows (version 12.0: SPSS Inc, Chicago, IL, USA)) and Graph Pad Prism (version 5, Graph Pad Prism, San Diego, CA, USA). Data of capsaicin C2 and C5 were log transformed for statistical analysis. Data were expressed as mean ± standard error of mean (SEM). Correlations were measured using Spearman rank correlation. Comparisons of continuous variables between groups were made using Mann–Whitney *U* test and Kruskall-Wallis test with Dunn's correction where appropriate. Chi-squared tests were used for the comparison of categorical data. A two-tailed p value < 0.05 was considered as statistically significant.

3. Results

One hundred patients were recruited to the study. Their characteristics are summarised in Table 1, with the patients divided into two groups, CEC and CIC. Nearly two thirds had CIC. Out of the thirty eight patients that had CEC, 12 had asthma, 3 eosinophilic bronchitis, 6 post-nasal drip, 13 gastro-oesophageal reflux disease, and 4 bronchiectasis. There was no difference in age, gender, smoking or atopic status between CEC and CIC patients. Compared to CEC, the CIC group had a longer duration of cough (9.6 versus 5.3 years, $p < 0.001$), higher exhaled NO (32 versus 13 ppb, $p = 0.04$) and lower FEV_1/FVC ratio (0.79 versus 0.76, $p = 0.03$). However, the CIC patients had increased capsaicin cough sensitivity (log C5: 0.61 versus 1.03, $p = 0.02$).

Table 2 shows the distribution of the scores for various histopathological features seen on the biopsy. There was at least a mild degree of change in all the biopsies. Basement membrane thickness was increased in the CIC group compared to the CEC group. However, there was no significant difference in the scores of cellular inflammation and seromucinous hyperplasia between the 3 groups.

There were positive correlations between eosinophil infiltration and goblet cell hyperplasia ($r = 0.33$, $p = 0.001$), basement membrane thickening ($r = 0.26$, $p = 0.009$), lymphocyte infiltration ($r = 0.331$, $p = 0.001$) and neutrophil infiltration ($r = 0.31$, $p = 0.002$) (Table 3). There was a positive correlation between basement membrane thickening and goblet cell hyperplasia ($r = 0.24$, $p = 0.016$). There was a positive correlation ($r = 0.42$, $p = 0.007$) between LCQ score and capsaicin log C5 (Fig. 1). Cough duration tended to be longer in patients with higher LCQ scores ($r = -0.28$, $p = 0.06$), but there was no association with FEV_1. Cough