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CASE STUDY

Serial sputum cell counts in the management of chronic airflow limitation

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Serial sputum cell counts in the management of chronic airflow limitation. K. Parameswaran, M.M.M. Pizzichini, D. Li, E. Pizzichini, P.K. Jeffery, F.E. Hargreave. ©ERS Journals Ltd 1998.

ABSTRACT: This case study illustrates the usefulness of serial induced sputum cell counts from cytospins to investigate the nature of airway inflammation in a patient presumed to have prednisone-dependent asthma for 30 yrs. She had bronchiectasis and chronic airflow limitation. Exacerbations of breathlessness were associated with an increase in chronic airflow limitation with little or no sputum.

Induced sputum showed elevated total cell and neutrophil counts at each exacerbation with no increase in the proportion of eosinophils. Pathogenic bacteria were cultured at each flare-up. The dose of prednisone was reduced progressively and each exacerbation was treated with an appropriate antibiotic without increasing the dose of prednisone, as was the case previously. The infections were associated with bronchiectasis of the right upper lobe which was removed. Examination of the specimen confirmed neutrophilic infiltration and did not show the usual airway structural changes of asthma.

These results provide further evidence of the value of sputum cell counts in practice, in this case to prevent overtreatment with prednisone in a patient with recurrent deteriorations in airflow which were due to recurrent infections.

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Bronchial asthma is defined by variable airflow limitation. Its pathogenesis is considered to include an eosinophilic airway inflammatory process [1, 2]. Eosinophilic airway inflammation with reversible airflow limitation are usually steroid responsive [3–5]. However, chronic airflow limitation can also result from inflammatory processes distinct from those of asthma, *e.g*. smoking and infections, which may also exhibit a degree of reversibility or variability and lead to a diagnosis of asthma. Such inflammation and variability of airflow limitation may not benefit from steroid treatment. The examination of inflammatory cells on cytospins of dithiothreitol-treated sputum has now become a reliable tool to evaluate the presence and assess the type and severity of airway inflammation [5]. In a previous report we illustrated how these were useful to direct successful treatment with prednisone in a patient with eosinophilic bronchitis (chiefly without features of asthma) who had not responded to treatment with high doses of inhaled steroid [6]. In the present we demonstrate the usefulness of induced sputum examination using cytospins in the management of a 56 yr old female previously presumed to have asthma and chronic airflow limitation. She had been treated with daily high doses of prednisone for 30 yrs.

Case report

A 56 yr old female with a previous diagnosis of "difficult asthma" was referred from another respirologist in October 1994. She had been considered to have rhinitis and allergy to dust, cats and dogs as a child. She had middle and lower lobectomies of the right lung in 1968 for bronchiectasis (confirmed by histological examination of the resected lobes) and a surgical correction of an atrial septal defect in 1980.

A diagnosis of asthma was made at ~26 yrs. Spirometry measurements had confirmed variable airflow limitation and improvement with inhaled salbutamol: her worst forced expiratory volume in one second (FEV_1) and vital capacity (VC) were 0.7 and 1.1 L, respectively (in 1980) and her best values were 1.7 and 2.5 L improving after inhaled salbutamol to 2.2 and 3.0 L (in 1983), when her predicted values were 2.6 and 3.3 L. She had been treated with prednisone 10–60 mg daily since the mid 1960s. At the time of referral, she was on prednisone 60 mg daily. Her other medications included budesonide (3200 μg) by Turbuhaler, salbutamol and ipratropium (four puffs) and cromoglycate inhaler (two puffs) each, four times a day. She had little or no sputum and no nasal symptoms. There was no suggestion of pancreatic malabsorption or similar illness in the family. She had a history of gastro-oesophageal reflux, osteoporosis and insulin-dependent diabetes mellitus. She had a 20 pack-yr smoking history and stopped smoking in 1985.

Clinical examination revealed a moderately breathless female with Cushingoid features. She was overweight (height 164 cm, weight 106 kg) and had dependent pedal oedema. Lenticular cataracts were noted in both eyes. Examination of the respiratory system revealed volume loss of the right lung compatible with the previous resection

Table 1. – Selected serial measurements of spirometry and sputum cell counts

Parameters	Oct '94	Exacerbation Mar '95	May '95	Exacerbation Jul '95	Nov '95	Exacerbation Jun '96	Jan '97
Symptoms	+	+++	++	+++	++	+++	+/-
FEV1/VC postbronchodilator	1.3/2.2	1.0/1.5	1.2/2.1	1.3/1.8	1.4/1.9	1.0/1.6	1.3/1.8
Sputum appearance	M	P	M	P	M	MP	no sputum
TCC × 10^6·mL^{-1} (normal <4.5)	9.0	41.6	9.1	26.5	1.46	40.3	no sputum
N % (normal <37%)	92	96	97	88	76	97	no sputum
Eo % (normal <2%)	0	1.0	0	0.3	2.0	0.3	no sputum
Prednisone mg·day^{-1}	60	10 alt. day	7.5	7.5 alt. day	2.5	7.5	7.5
Budesonide µg·day^{-1}	3200	3200	3200	3200	3200	3200	1600
Salbutamol puffs·day^{-1}	16–20	12	12	4	4	4	2–4
Sputum culture	-	normal flora	–	*P. aeruginosa*	–	S	–
Antibiotics	none	CLA	COT	CIP+CEF	TOB	ERY+PEN	CIP

Sputum appearance: M: mucoid; P: purulent; MP: mucopurulent; TCC: total cell count; N: neutrophils; Eo: eosinophils; Antibiotics: CLA: amoxycillin + clavulanic acid; COT: cotrimoxazole; CIP: ciprofloxacin; CEF: cefaclor; TOB: inhaled tobramycin; ERY: erythromycin; PEN: penicillin; *P. aeruginosa*: *Pseudomonas aeruginosa*; S: *Streptococcus pneumoniae*; FEV1: forced expiratory volume in one second; VC: vital capacity; alt. day: alternate days.

and reduced breath sounds bilaterally. There was no clinical evidence for pulmonary hypertension. Spirometry demonstrated a mixed obstructive-nonobstructive pattern with an FEV1 and VC of 1.1 and 1.8 L, respectively. This improved to 1.3 and 2.2 L, respectively, after inhaled salbutamol (200 µg). Skin-prick tests with extracts of the common aeroallergens, including *Aspergillus fumigatus*, gave no wheal and flare responses in spite of a positive histamine control. The same result was obtained later when the skin tests were repeated when the patient was on 7.5 mg of prednisone on alternate days. A serum precipitin test with *A. fumigatus* was negative. Total serum immunoglobulin (Ig)E was normal.

Sputum was induced and processed by the methods described by PIZZICHINI *et al*. [7, 8]. The induction procedure was made safer using a modified protocol for severe asthmatics [5]. The sputum was mucoid and had a total cell count of 9 million·mL^{-1} (normal <4.5) of which 92% were neutrophils (table 1). There were no eosinophils and so the dose of prednisone was progressively reduced to 7.5 mg daily over 5 months with no reduction in spirometry and she was commenced on regular therapy with cotrimoxazole. Over the next eight months, she experienced worsening of her symptoms almost every month, with cough, breathlessness and reduction in FEV1 by 400 mL. Sputum was examined at each exacerbation. Eosinophil counts were never elevated (normal <2%) and there were no free eosinophil granules. However, each exacerbation was associated with an increase in total cell count and the proportion of neutrophils. *Pseudomonas aeruginosa* was cultured on two occasions and the patient was treated with the appropriate antibiotic. The prednisone dose was further reduced to 2.5 mg on alternate days but this had to be increased to 7.5 mg daily to prevent hypoadrenalism.

Once it was realized that her exacerbations were due to recurrent infections, computed tomography of the thorax was performed in November 1995. This identified localized bronchiectasis in the remaining upper lobe of the right lung and a spiculated soft tissue nodule (1 cm) at its base. The left lung appeared normal. Further investigations for a cause of recurrent bronchial infection included sinus radiographs, a full work-up for immunodeficiency, sweat chloride test and genotyping for cystic fibrosis mutations were normal. Ciliary motility studies were not performed. She had a resection of her right upper lobe because this was considered to be the cause for her recurrent infections which could not be prevented and were disabling.

Multiple tissue blocks of the resected lung specimen were examined histologically and by immunohistochemistry for inflammatory cells using antibodies directed against activated eosinophils (EG-2), neutrophils (elastase) and mast cells (tryptase) and for evidence of structural changes. The pseudostratified columnar epithelium of the large and small airways was intact. The epithelial reticular basement membrane of both the large and small airways was never thickened. The intra-epithelial inflammatory cell infiltrate was predominantly neutrophilic in both the large and small airways. Subepithelial infiltration was predominantly mononuclear (lymphocytic) in both the central and the peripheral airways and there were prominent lymphoreticular aggregates. There were a few eosinophils and mast cells but these were outnumbered by neutrophils, which were frequent (fig. 1). The histological picture was therefore not compatible with the usual findings of asthma. It was compatible with a follicular type of bronchiectasis.

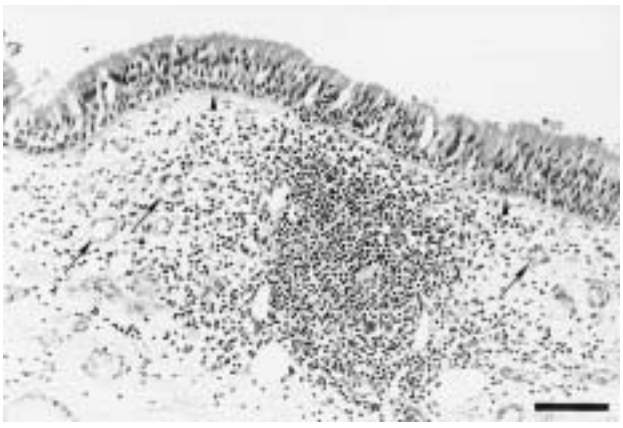

Fig. 1. – Haematoxylin and eosin stained section of airway mucosa to show intact pseudostratified ciliated columnar surface epithelium, a thin reticular basement membrane (arrow heads), frequent appearance and prominence of bronchial vessels (arrows) and a subepithelial lymphoreticular aggregate of lymphomononuclear cells, some of which are migrating into the surface epithelium. (Internal scale bar = 80 µm).

Following the surgery, she was monitored on the same dose of prednisone and the dose of budesonide was reduced to 1600 μg. In the following nine months, she had five exacerbations of infective bronchitis which were treated with courses of antibiotics. Her best FEV_1 and VC have since have been 1.3 and 1.8 L, respectively.

Discussion

This case report highlights the use of serial measurements of sputum cell counts to identify that exacerbations of symptoms and airflow limitation were associated with neutrophilic infective bronchitis without an increase in the proportion of eosinophils. It was possible to treat these successfully with an antibiotic and not with added prednisone. For the first time in 30 years the patient was able to reduce regular treatment with prednisone which had caused considerable side-effects. In addition, a comparison of sputum cell counts with the histology of large and small airways illustrated that the inflammatory cell profiles were similar. The results illustrate that sputum cell counts can be useful to identify the overuse of prednisone.

The patient had been considered to have asthma because she had variable airflow limitation. She had risk factors for the development of asthma, such as an onset of symptoms when she was young and possible atopy. However, she also had a previous right lower and middle lobectomy for bronchiectasis. She also had chronic airflow limitation which has been defined as "abnormal tests of expiratory airflow that do not change markedly over periods of several months of observation" [9]. This abnormality can be linked with smoking, bronchiectasis, and asthma associated with eosinophilic airway inflammation. The patient was a smoker and had right upper lobe bronchiectasis. The bronchiectasis was not clinically obvious because there was no sputum between exacerbations and only scanty sputum at the time of exacerbations. The cause of bronchiectasis in this patient was probably childhood infection. However, the purpose of this case report is not to illustrate the aetiology of her bronchiectasis, but to demonstrate that the episodes of clinical deterioration were always neutrophilic and did not require increased doses of prednisone. Serial sputum examinations enabled the dose of prednisone to be reduced to the minimum to prevent the effects of adrenal failure.

The different conditions associated with chronic airflow limitation respond differently to treatment. For example, Brown [3] and Shim *et al*. [4] have observed that sputum eosinophilia predicts a good response to treatment with prednisolone in patients with chronic airflow limitation associated with chronic asthma and chronic bronchitis, respectively. This observation is supported in recent reports using improved methods of sputum examination [5, 10]. In the patient in the present report, sputum examination identified that the exacerbations were associated with neutrophilic airway inflammation due to infection and not with eosinophilic inflammation. As a result of this, it was possible to treat the exacerbations with an antibiotic, to avoid increasing treatment with prednisone and to progressively reduce the regular prednisone dose.

The use of smears to obtain sputum cell counts in clinical practice in the past was recognized to be unreliable [11]. The improved methods of sputum examination used in this patient have been shown to be highly reliable [7], valid and responsive to changes with exacerbating factors and treatment [12–14]. When compared with bronchial biopsies, bronchoalveolar lavage (BAL) and washings, sputum cell counts show differences between the different compartments sampled [15–17]. For example, eosinophils and neutrophils are higher in sputum compared with BAL, which contains more macrophages and lymphocytes. In the present case report, we have been able to compare sputum cell counts with the histology in large and small airways. The inflammatory cell infiltration was similar. The histology showed an absence of the usual features of asthma [18]. Specifically, there was no disruption of airway epithelium, no hyaline appearance and thickening of the reticular basement membrane and no tissue infiltration by activated eosinophils. These results therefore further identify the usefulness of sputum cell counts; they strengthen the construct and criterion validities of sputum examination.

It could be argued that the long period of prednisone therapy may be responsible for the absence of airway eosinophilia and the usual structural changes associated with asthma [19]. This is unlikely for eosinophilia because exacerbations with prednisone reduction in prednisone-dependent asthma are associated with eosinophilia [20]. Prednisone treatment might have reversed the thickening of the reticular basement membrane [21].

The use of induced sputum examination for the determination of airway inflammation is still in its research stage. The procedure of induction is relatively noninvasive and can be performed safely. The processing of sputum is relatively simple and short. However, while this can be performed by a hospital haematology laboratory, it requires dedicated staff and regular quality control checks to maintain reliability. This report illustrates the value of sputum cell counts to identify the type of airway inflammation during exacerbations in a patient considered to have prednisone-dependent asthma, but who had chronic airflow limitation and recurrent infective bronchitis which did not require prednisone treatment.

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