Induced sputum examination: diagnosis of pulmonary involvement in Fabry’s disease

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Abstract

Fabry’s disease is a rare inherited metabolic disorder caused by a deficiency in the enzyme α-galactosidase A. It can affect almost every organ, including the lungs. Confirmation of lung involvement has depended on invasive bronchial biopsy specimens or brushings to confirm the presence of typical lamellar inclusion bodies within bronchial epithelial cells. We report a patient with known Fabry’s disease in whom these inclusion bodies were identified by examination of induced sputum.


Keywords: Fabry’s disease; induced sputum; electron microscopy

Fabry’s disease is a rare X-linked metabolic disorder in which the primary defect is an inherited deficiency of the enzyme α-galactosidase A.1 This results in an abnormal accumulation of the glycosphingolipid ceramide trihexoside in vascular smooth muscle throughout the body, particularly in vessels of the skin, kidneys, heart, pulmonary vascular system, and neurological system.2 Patients with lung involvement can present with varying symptoms including dyspnoea, wheeze, haemoptysis, pneumothorax, frequent respiratory infection, and airflow limitation.1,3 To date, the diagnosis of lung involvement in Fabry’s disease has depended on the demonstration of the typical lamellar inclusion bodies within bronchial epithelial cells in bronchial biopsy specimens, brushings, or lavage fluid.1,3 We describe a woman with Fabry’s disease in whom lung involvement was confirmed relatively non-invasively by the examination of induced sputum.

Case report

A woman aged 34 years presented with a five year history of increasing effort dyspnoea, intermittent chest tightness, wheezing, and dry cough. She was breathless on performing light workloads, normal heart rate response, and increased ventilation at 45% predicted. A stage 1 exercise test showed a reduced power output (45% predicted) with increased ventilation at all workloads, normal heart rate response, and no oxygen desaturation or post-exercise bronchoconstriction. Allergy skin prick tests with 19 allergen extracts were negative.

Sputum was induced, selected from the expectorator, and processed with dithiothreitol as described by Pizzichini et al.1 It was mucoid, squamous cell contamination was <5%, and the cell viability was 84%. The total cell count and proportion of neutrophils were raised (16.4 × 10⁶ cells/mg and 82.5%; normal <9.7 × 10⁶ cells/ml and <64.4%); eosinophils were borderline increased (2.2%; normal <1.1%); macrophages were 14.8% (normal <86.1%), and lymphocytes were 0.5% (normal <2.6%). The sputum cell suspension was centrifuged at 290 g for four minutes, the cell pellet fixed in 2% glutaraldehyde in 0.1M cacodylate buffer at 4°C for two hours, and then post-fixed in cold 1% osmium tetroxide in cacodylate buffer. After dehydration in ethanol, the pellet was embedded in Spurr’s epoxy resin. Ultrathin sections were stained and examined with a Philips 300 electron microscope, confirming loss developed at 18 years, and more recently she had noted intermittent abdominal pain with associated nausea, vomiting, and diarrhoea. Investigation confirmed autonomic nervous system involvement, peripheral neuropathy, mild diastolic dysfunction, and mild proteinuria. A clinical diagnosis of Fabry’s disease was confirmed by deficient plasma α-galactosidase levels at the age of 27. Subsequent testing confirmed that one sibling and her twin sons also had the condition. She had been treated with carbamazepine and phenytoin for the peripheral neuropathy, and omeprazole and metoclopramide for abdominal pain.

On examination she was obese with a weight of 104 kg and height 162 cm. Breath sounds were decreased and there was a diffuse expiratory wheeze on auscultation. Abdominal tenderness was present on palpation and there was evidence of a sensory peripheral neuropathy involving predominantly her lower limbs. Cardiovascular and skin examination were normal.

Her chest radiograph was normal. Spirometric values, lung volumes, and carbon monoxide transfer factor (TLCO) showed moderate but not significantly reversible airflow limitation, a moderate non-obstructive defect, and normal gas transfer coefficient (KCO). Her forced expiratory volume in one second (FEV₁) and slow vital capacity (VC) were 1.71 and 2.71 l, respectively (predicted 3.31 and 3.9 l). A stage 1 exercise test showed a reduced power output (45% predicted) with increased ventilation at all workloads, normal heart rate response, and no oxygen desaturation or post-exercise bronchoconstriction. Allergy skin prick tests with 19 allergen extracts were negative.

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the presence of lamellar inclusion bodies typical of Fabry’s disease within all ciliated bronchial epithelial cells examined (fig 1). These inclusion bodies were not present in macrophages and were therefore not consistent with surfactant bodies. Sputum culture was negative for common respiratory pathogens.

Discussion

This report shows that lung involvement in Fabry’s disease can be demonstrated by examination of induced sputum and is associated with a sterile neutrophilic inflammatory response. Presumably the source of these abnormalities is the airways since the lamellar inclusions were observed in airway epithelial cells and have previously been demonstrated in bronchial biopsy specimens and brushings. In addition, the inflammatory response was associated with mild to moderate chronic airflow limitation, which is a common functional abnormality in Fabry’s disease and, in this patient, was not associated with any other cause. We have examined sputum from five subjects without Fabry’s disease and were unable to find similar inclusions. The pathogenic significance of these inclusions is uncertain, and it is unknown whether they are present in patients with Fabry’s disease without clinical manifestations of pulmonary involvement.

This report adds to the increasing evidence that examination of induced sputum is clinically useful. Sputum induction itself can be very successful and safe when performed by experienced technologists. The processing of the sputum for cell counts and some fluid phase measurements can be reliable, valid, and responsive—the qualities of good measurements. Induced sputum examination was introduced to diagnose pulmonary infection and bronchial carcinoma. More recently it has identified the occurrence of eosinophilic bronchitis without asthma and has been used to support the diagnosis of occupational asthma and poor compliance with steroid treatment. There is increasing evidence in patients who present with chronic cough, asthma, or chronic airflow limitation that sputum eosinophilia predicts a beneficial effect of steroid treatment and that the lack of sputum eosinophilia predicts a lack of response, at least to short term treatment. Recent studies also raise the possibility that the presence of haemosiderin in macrophages in sputum might be used as the surrogate marker of heart failure in breathless patients and that the presence of lipid-containing macrophages correlates with reflux to the level of the upper oesophageal sphincter. Consideration therefore needs to be given to making available reliable sputum examination for indications other than just the diagnosis of lower respiratory infection.

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