

The use of sputum eosinophils in the evaluation of occupational asthma

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Purpose of review

The diagnosis of occupational asthma needs to be made objectively using as many criteria as possible. These include laboratory exposure tests with occupational agent(s), which are only available in specialized centres. Another approach is to monitor peak expiratory flow or methacholine airway responsiveness during periods at work and away from work. However, these measurements are open to misinterpretation when they are not performed optimally. Airway inflammation is one of the main characteristics of occupational asthma, but is not often assessed during its investigation. The purpose of this work was to review recent studies that have investigated and characterized the changes in sputum cell counts occurring in patients with occupational asthma, in order to evaluate the role of the analysis of sputum cell counts.

Recent findings

There is evidence that monitoring sputum eosinophils can help in the management of asthma. In the majority of cases of occupational asthma, the percentage of sputum eosinophils increases after exposure to occupational agents in the laboratory compared with baseline, but an increase in sputum neutrophils has also been observed. The changes in airway inflammation occurring at the workplace have been less investigated, but indicate that there are significant changes in airway inflammation and especially sputum eosinophils when workers are exposed to a sensitizer at their workplace compared with periods away from the workplace.

Summary

Induced sputum has successfully been used to manage patients with mild to moderate asthma. Its use is promising in occupational asthma, and its role in the investigation of occupational asthma needs to be better defined.

Keywords

induced sputum, eosinophils, occupational asthma, airway hyperresponsiveness

Abbreviations

BTS	British Thoracic Society
FEV₁	forced expiratory volume in 1 s
OA	occupational asthma
PEF	peak expiratory flow
SIC	specific inhalation challenge

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Introduction

The diagnosis of occupational asthma (OA) is a difficult diagnosis to make. A suggestive clinical history predicts a diagnosis of OA in only 63% of cases [1]. It is therefore important to confirm the diagnosis of OA by objective means. One method of diagnosing OA consists in serially monitoring peak expiratory flow (PEF) during periods at work and away from work [2–4]. Unfortunately, this method does not have an optimal sensitivity and specificity and has a number of pitfalls, such as low compliance, potential falsification of results, and underestimation of changes in airway calibre [5]. The addition of serial measures of airway responsiveness by methacholine or histamine challenge at the end of a work week and after a holiday period has also been advocated to provide a more objective measure of work-related changes [6].

The current gold standard method for diagnosing OA is the performance of specific inhalation challenge (SIC) [2], but this test is expensive, time consuming and requires a specialized sophisticated set-up. Consequently, it is performed only in a few specialized centres worldwide. The diagnosis of OA needs to be made objectively using as many criteria as possible. Therefore, there is a need to find an additional tool that can be easily made available to a large number of centres to overcome this difficulty. The purpose of this work was to evaluate the role of the analysis of sputum cell counts in the investigation of OA by reviewing studies published in English between 2001 and 2003 that investigated the changes in sputum cell counts occurring in patients with OA after exposure to occupational agents in the laboratory and at the workplace.

Sputum eosinophils in the management of asthma

The assessment of airway inflammation is most important in asthma because it reflects the disease directly,

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as opposed to spirometry and airway responsiveness, which are indirect functional markers of the condition. Several methods, such as bronchial biopsies, bronchoalveolar lavage and induced sputum, make it possible to assess airway inflammation. However, bronchial biopsies and bronchoalveolar lavage are too invasive to be used on a routine or serial basis.

Analysing induced sputum is a reproducible, valid, responsive and non-invasive method for studying airway inflammation [7]. This method consists of inducing sputum production by the inhalation of a hypertonic saline solution. Sputum is subsequently processed and cytopins are prepared and stained to obtain differential cell counts [7]. Immunocytochemistry [8] or in-situ hybridization [9] can also be performed on the slides. Inflammatory mediators can be measured in the sputum supernatants.

Eosinophils and eosinophilic cationic protein concentrations are increased by exposure to common allergens [10–13] and by a reduction in steroid treatment [14]. In contrast, they are decreased by treatment with corticosteroids [15,16]. Some studies have suggested that the presence of sputum eosinophilia may be a good predictor of the response to inhaled steroids [17], whereas others have shown that asthmatic individuals, in spite of the absence of sputum eosinophilia, have a satisfactory response to inhaled steroids [18]. Recently, Green *et al.* [19*] randomly assigned 74 mild to moderate asthmatic patients into two groups of asthma management, either by following the British Thoracic Society (BTS) asthma guidelines or by obtaining a normalization of the sputum eosinophilia observed in these patients. Over a period of 12 months, the patients were assessed on nine occasions. The patients in the sputum management group had fewer severe asthma exacerbations and hospital admissions than the BTS management group, but had a similar daily dose of inhaled steroids. Another study [20] suggested that changes in induced sputum are correlated with asthma control rather than with asthma severity.

Therefore, there is some evidence that this test may be useful in clinical practice. As eosinophilic inflammation is as in asthma a characteristic of OA, it is a logical step to consider sputum eosinophils in the evaluation of OA.

Use of induced sputum in the evaluation of occupational asthma

Changes in airway inflammation have been described in subjects with OA after exposure to occupational agents in the laboratory or at the workplace. We herein report the studies that described the changes in sputum cell counts in these two circumstances.

Evaluation of changes in sputum cell counts after exposure to occupational agents in the laboratory

In order to interpret the changes in sputum cell counts accurately after exposure to occupational agents, one needs to be sure that the exposure itself does not induce any change in airway inflammation, even in individuals that are not sensitized to the suspected agent. Obata *et al.* [21] found that some individuals with negative specific challenges to plicatic acid in red cedar, a low-molecular-weight agent, also had an increase in sputum eosinophils after exposure to this agent. Our group exposed three groups of subjects to occupational agents: subjects with possible OA, but with negative specific challenges to the suspected agents; subjects with OA confirmed by positive SICs; and asthmatic subjects without OA. The asthmatic controls were exposed to flour and cyano-acrylate [22]. Subjects with positive SICs had a significant increase in both sputum eosinophil and neutrophil numbers after exposure to the occupational agents compared with baseline. A clinically significant increase in sputum eosinophil numbers occurred in two out of 14 subjects with possible OA, but with negative SICs, and in 13 out of 17 subjects with positive SICs. The asthmatic subjects without OA showed no change in their sputum cell counts after exposure to occupational agents.

The majority of studies have evaluated the changes in airway inflammation after exposure to occupational agents in the laboratory. In the majority of cases, sputum eosinophilia has been observed after exposure to occupational agents. Sputum eosinophils have been shown to increase after exposure to both high- [22,23] and low-molecular-weight agents such as isocyanates [24], red cedar [21] or cyano-acrylate [25]. However, there are examples in which exposure to occupational agents can predominantly induce sputum neutrophilia. An increase in sputum neutrophils has been observed mainly after exposure to isocyanates [26,27*], but also after exposure to other agents; for example, metal working fluid [28] or grain dust [29]. The factors that influence the type of airway inflammation are unclear. We recently showed that the type of asthmatic reaction and the intensity of airway inflammation induced by exposure to isocyanates can be influenced by the concentration and the length of exposure to these agents [27*]. Isocyanate-induced asthma seems to be enhanced when isocyanates are generated at low concentrations for a long period of time compared with when they are generated at a higher concentration for a shorter duration of exposure. Other factors, such as the time of sputum collection after exposure to isocyanates, may also partly explain the diversity of the inflammatory response in individuals with isocyanate-induced asthma. After an acute exposure to

isocyanates, neutrophils may be attracted first, then followed by eosinophils. On the other hand, as reviewed by Douwes *et al.* [30^{*}], it is possible that an important proportion of cases of asthma are characterized by a neutrophilic type of inflammation rather than an eosinophilic type of inflammation. This may have important consequences for the management of asthma.

Evaluation of changes in sputum cell counts during investigation of occupational asthma at the workplace and away from the workplace

A few studies have investigated the changes in induced sputum at the workplace. A few years ago, we investigated individuals with OA and asthmatic individuals without OA working in the same environment [31]. Sputum induction was performed at the end of 2-week periods at work and away from work. The subjects with OA had an increase in sputum eosinophils when at work, which resolved when they were removed from their workplace. As far as asthmatic subjects without OA working in the same environment were concerned, they did not show any change in airway inflammation. However, the criteria chosen for defining OA were very strict. Therefore, it is likely that we selected individuals with clear-cut OA, who showed marked changes in sputum eosinophils. Another study looked at the changes in induced sputum in individuals with OA as a result of low molecular weight agents when working [32]. There was no comparison with periods away from work. Thirty-eight workers were investigated. Only 14 had sputum eosinophils greater than 2.2% when at work. However, the diagnosis of OA was not based upon the same criteria for all individuals. The authors reported that subjects had sputum neutrophilia (59% of neutrophils). However, the important variability in sputum neutrophils makes it difficult to consider 59% in sputum neutrophils as neutrophilia without having a comparison during periods away from work. An individual exposed to metal working fluid was reported to have a marked increase in neutrophils when at work, which resolved after periods away from work [28]. The sputum findings were mirrored by corresponding changes in spirometry and PC₂₀ methacholine.

Recently, we aimed to assess whether the monitoring of sputum eosinophils at the end of periods at and away from work combined with the monitoring of PEF and airway responsiveness could improve the diagnosis of OA. We enrolled individuals suspected of having OA. Serial monitoring of PEF was performed during 2 weeks at and away from work. At the end of each period, induced sputum was collected for cell counts and a methacholine challenge was performed.

SIC with the suspected agent was subsequently performed to confirm the diagnosis of OA. PEF graphs were drawn and interpreted visually by five independent observers as well as by using a computerized approach. When at work, subjects with positive SICs had a significant increase in sputum eosinophils, whereas the group with a negative SIC had higher neutrophils compared with the periods away from work. The mechanisms of neutrophilic inflammation are unclear, but it may be caused by an irritant effect of agents at the workplace in asthmatic patients. The long-term consequences of remaining at their workplace in these subjects who experience increased symptoms of asthma combined with a neutrophilic type of inflammation are unknown and need to be evaluated.

The addition of induced sputum cell counts to the monitoring of PEF substantially improved the sensitivity and specificity of this test compared with SICs. Indeed, the sensitivity was improved by an average of 8.2% (range 4.1–13.4%); and the specificity by an average of 18.0% (13.7–25.5%) when induced sputum was added to the PEF monitoring. The evaluation of any diagnostic tool has to be performed in comparison with a gold standard. The results of PEF monitoring and sputum induction were compared with SICs. Although SICs are reliable, these tests can be falsely negative when they are performed with the wrong agent, or when the subjects have been removed from exposure for a long period of time. Therefore, it is possible that some cases had been misdiagnosed in our study. At least two subjects with negative SICs had a high increase in sputum eosinophil counts when at work in addition to suggestive PEF monitoring. The study indicated that induced sputum may be an interesting tool to add to the investigation of OA (C. Lemière, 2003; personal communication).

Use of sputum eosinophils as early markers for occupational asthma or as prognostic factors in subjects with occupational asthma removed from exposure

One potentially interesting role for induced sputum is the early diagnosis of OA, before the occurrence of respiratory symptoms and pulmonary function changes. As in asthma, changes in induced sputum may precede functional changes. We examined whether changes in sputum differential cell counts, IL-5 and eotaxin might anticipate changes in functional parameters [forced expiratory volume in 1 s (FEV₁) and PC₂₀] after exposure to occupational agents in patients with OA [9]. We found an increase in sputum eosinophils, eotaxin and IL-5 on the day preceding the occurrence of a 20% fall in FEV₁, although there was no change in functional parameters (FEV₁ and

PC₂₀). This was confirmed recently after exposure to isocyanates [27*].

Induced sputum may also be beneficial for following workers after removal from exposure. Chan-Yeung *et al.* [33] showed that the percentage of sputum eosinophils was correlated inversely with FEV₁ and positively with the class of respiratory impairment. They also showed that sputum eosinophils were not correlated with airway responsiveness. Induced sputum may bring additional information beside FEV₁ and PC₂₀, and may be an asset for the follow-up of individuals with OA. More recently, we assessed 133 individuals with OA at a mean interval of 8.7 years (0.5–20.8) after the cessation of exposure by questionnaire, airway calibre and responsiveness to methacholine. Satisfactory samples of induced sputum were obtained from 98 individuals. We defined three groups of subjects: (1) cured: normalization of PC₂₀; (2) improved: increase in PC₂₀ by 3.2-fold or more, but PC₂₀ still abnormal; (3) not improved: no significant change in PC₂₀. Nine out of 28 subjects (32.1%) with no improvement versus six out of 56 (10.7%) with partial and complete improvements had sputum eosinophil levels equal to or greater than 2%, and 11 out of 28 (39.3%) versus 11 out of 56 (19.6%) showed sputum neutrophils equal to or greater than 61%. Levels of IL-8 and of neutrophil-derived myeloperoxidase were significantly more elevated in the sputum of subjects with no improvement. Those in the cured or improved groups had a significantly longer time lapse since diagnosis, and a higher PC₂₀ at the time of diagnosis. We concluded that failure to improve after the cessation of exposure to an agent causing OA is associated with airway inflammation at follow-up [34*].

Conclusion

Exposure to occupational agents induced marked changes in sputum in subjects with OA sensitized to those agents. Eosinophilic and neutrophilic airway inflammation have been observed in subjects with OA after exposure to occupational agents in the laboratory. The factors that influence the type of airway inflammation remain to be determined. When at work, individuals with OA show a predominant eosinophilic airway inflammation, which decreases or resolves after a period away from work. In contrast, neutrophilic inflammation has been observed in some asthmatic workers without OA who complained of a worsening of their asthma symptoms at work. The mechanisms of this neutrophilic airway inflammation need to be further investigated and understood. Although these findings need to be confirmed, the use of induced sputum is likely to be useful for the investigation, diagnosis and follow-up of OA. Its role in the early detection of OA also needs to be studied.

Acknowledgements

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