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REVIEW

The utility of sputum eosinophils and exhaled nitric oxide for monitoring asthma control with special attention to childhood asthma

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Abstract
The monitoring of sputum eosinophils has received certain attention as a tool for improving asthma management both in children and in adults. The present paper reviews the technique and also the usefulness of induced sputum in the diagnosis and assessment of asthma, together with its ability to predict the response to treatment and to anticipate asthma exacerbations. Special attention is addressed to childhood asthma. The authors conclude that due to cost-effectiveness reasons derived from high labour costs, together with the unpleasantness of the technique and the failure to obtain adequate samples in a non-negligible percentage of children, this technique should be only used for research purposes.

Introduction

The Global Initiative for Asthma defines this disease as "a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role…".[1] The eosinophil is one of the most important of those cells and has been invariably related to asthma since this condition has been studied scientifically. More recently it has been possible to study the presence of eosinophils in the sputum, thus allowing the comparison of asthmatic individuals with normal ones and to try and establish a relationship of this presence and the clinical and functional situation of asthma. In the following lines we will describe the current knowledge on the usefulness of sputum eosinophils to monitor asthma control.

Eosinophil counts in induced sputum

The early reports on sputum eosinophils used smears of spontaneously produced sputum stained with May-Grunwald-Giemsa.[2] However, this technique has important limitations such as not obtaining adequate samples from some subjects,

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as in children[3,4]; not having enough sample quantity to allow a proper analysis[4]; or being a time consuming procedure. There have been two innovations that have made it possible to overcome those limitations.[5] The first one is the possibility of inducing sputum production by means of hypertonic saline inhalation using a standardised protocol, and the second is the use of dithiothreitol (DTT) to improve cell dispersion.[6,7]

The mechanism by which hypertonic saline induces sputum is not fully understood but it probably includes the increase of water influx across the airway epithelium thus reducing mucus viscosity and increasing tracheobronchial clearance and the volume of airway secretions. The direct stimulation of cough receptors by the hypertonic mist could be an additional help in the expectoration process.[5] Some authors have also suggested that the hypertonic; saline can directly stimulate the mucociliary escalator.[8]

The use of DTT to disperse cells in the sputum is quite recent. DTT is a sulphydryl compound that breaks the disulphide bonds which join glycoprotein fibres and maintains sputum in its gel form.[6,7] This treatment of the sputum allows a better cell differential count, making the results quicker and more reproducible. Furthermore, cell definition is improved in DTT treated sputum, enabling differential cell counts to be performed on Wright's stained cytospins (cell suspensions which have been centrifuged and then fixed onto a slide) without the need for further stains for eosinophils. The sputum supernatant can be used to measure molecular markers which reflect different aspects of airway inflammation, such as eosinophilic cationic protein (ECP), tryptase or cytokines.[5]

The exact protocol to induce sputum production and to process the samples is out of the scope of this review and can be consulted elsewhere.[5] What is important is to underline that with the current inducing protocols up to 76% of asthmatics are able to spontaneously produce adequate samples of sputum in their first attempt.[3] In children older than 6 years, the rate of successful sampling has been reported to be between 68% and 100%.[9]

The two main methods of processing the sputum–i.e. selecting the viscid components or processing the whole sample, including mucus and saliva- yield comparable results in terms of eosinophil differential counts, although they are not interchangeable.[10] Furthermore, and as reviewed by Kips et al.[11], the two ways of inducing the sputum production–either inhaling hypertonic saline at a constant concentration (4.5%) increasing the time periods of inhalation, or inhaling saline at increasing concentrations (3, 4 and 5%) over fixed time periods- do no affect the eosinophil counts. On the other hand, Saravia-Romanholo et al.[12] compared three different techniques of sputum analysis (without treatment with DTT, with treatment with DDT and with treatment with DDT and cytospin) in asthmatics with a wide range of disease severity and in controls, concluding that the three of them had comparable results, while the first two were quicker (32, 33 and 66 min, respectively) and cheaper in terms of the used materials ($1.64, $2.38 and $4.40, respectively).

Induced sputum eosinophils in the diagnosis and assessment of asthma

It has been shown very consistently that the differential count of cells in sputum is a useful tool for the diagnosis of asthma and for the labelling of a specific asthmatic individual as suffering from an eosinophilic or non-eosinophilic asthma, with non-eosinophilic asthma accounting for a substantial number of cases (25–55%).[13,14] This differentiation could have important implications for the response to treatment and for the prognosis of the disease, as it has been shown that eosinophilic asthma responds better to inhaled corticosteroids (ICS)[13,15–18]; this can be especially true in the case of infants and preschoolers in whom viral wheeze elicits mainly a neutrophilic response.[19] Non-asthmatic individuals have less than 1% of eosinophils in sputum,[20,21] whereas eosinophilic asthmatics have higher counts which depend on the underlying inflammation: more severe asthmatics usually have higher percentages.[22] Park et al. found that a differential eosinophil count of $\geq$5% had a sensitivity of 85.4% and a specificity of 92.6% for diagnosing asthma in 68 patients with respiratory complaints.[23]Furthermore, patients with chronic non-productive cough responsive to anti-asthma therapy characteristically have eosinophilic airway inflammation, which may play an important role in the development of the condition: the evaluation of airway inflammation by examination of induced sputum may be useful for diagnosis and deciding on therapeutic strategies in these patients.[24]

Sputum eosinophils relate consistently with the clinical and functional asthma situation. In one of the first reports addressing this point, Pizzichini et al.[25] examined 19 asthma patients and compared them with 20 controls. Asthmatic patients had higher levels of sputum and blood eosinophils and sputum ECP; however, blood ECP was comparable in the two groups. Among the control individuals, smokers had lower counts of sputum eosinophils, although the difference with non-smokers was not statistically significant (0.5% vs. 0.3%). The inflammatory marker that was best inversely correlated with symptoms score, % predicted FEV1 and methacholine PC20 among the asthmatic subjects, was sputum eosinophils. Sputum ECP was only inversely correlated to symptom scores. The area under the ROC curve showed that sputum eosinophils were; more accurate markers of asthma than blood eosinophils and serum ECP (although differences were not statistically significant). Other studies have found similar results.[22,26]

Not only do sputum eosinophils correlate with the clinical and functional situation of asthma, also but vary across asthma severity. In a group of 71 occupational asthmatics to Western cedar, those who had left exposure had lower levels of sputum eosinophils than those who had not. Among these, the sputum marker showed a significant inverse correlation with FEV1 and a positive one with the class of respiratory impairment according to the American Thoracic Society.[27] These findings have also been found in non-occupational asthma.[28,29] Furthermore, after eliminating the effect of confounding factors such as age, sex, ethnicity and the use of ICS, the eosinophil percentage in induced sputum is independently associated with lower FEV1 and lower methacholine PC20.[30] Conversely, the levels of exhaled nitric oxide (eNO) showed only weak correlations with sputum eosinophils in asthmatic subjects taking and not taking ICS[31] in one of the largest studies performed so far. Other studies have found strong correlations between the two markers.[32,33]

When using a clinical (symptoms), functional (FEV1) and inflammatory (sputum eosinophils) score system to quantify asthma control Boulet et al.[34], found that none of the scores was related to each other, leading the authors to the conclusion that the assessment of asthma severity according to clinical and functional findings only partially reflects the inflammation in the airway. It is possible that the way these authors built their score systems interfered with the relationship between the clinical, functional and inflammatory markers. However, it should be underlined that the variability of FEV1, for example, is not fully explained by the variability of the percentage of sputum eosinophils: the explanation, although statistically significant, is only between 15% and 25%.[25,29] The results of the study by Boulet et al.[34] probably reflect this fact.

Drugs influencing eosinophil counts in induced sputum

Corticosteroids are the most powerful drugs in reducing the number of sputum eosinophils in asthmatics. In an early report, Claman et al.[35] compared six days of prednisone treatment (0.5 mg/Kg/day) with that of placebo in a randomised, double blind study of 24 asthmatics. Sputum eosinophils fell from 14.1% at baseline to 1.8% after treatment with prednisone, whereas in the placebo group eosinophils raised slightly from 10.3% to 11.1%. The change difference was statistically significant.

Similarly to oral corticosteroids, ICS have also proven their efficacy to reduce sputum eosinophils, thus reflecting their anti-inflammatory properties. After 4 weeks treatment with budesonide (800 mcg daily) of 10 steroid-naive mild asthmatic patients there was a significant reduction of sputum eosinophils: from 3.8% at baseline to 1.3% at the end of the treatment period.[36] However, lower doses of budesonide (100 mcg/day) for four weeks did not reduce the number of eosinophils significantly.[37] In this study, 400 mcg/day and 1600 mcg/day reduced the number of those cells in a somewhat dose-response fashion. Again in steroid-naive asthmatic patients (mild to moderate), three months of budesonide treatment (200 mcg twice daily) lowered the number of eosinophils in sputum (from 14.8% to 1.3%), the decrease being higher in sputum than in blood.[26] In a randomised, placebo-controlled crossover study[38] the combination of terbutaline (1000 mcg four times daily) and budesonide (400 mcg twice daily) had similar effects on sputum eosinophils than budesonide (400 mcg twice daily) alone in patients with chronic persistent asthma. Fluticasone propionate (500 mcg b.i.d for a period of 2 or 4 weeks) also decreases sputum eosinophils significantly (from 2.85% to 0.68% 2nd week and to 0.44% 4th week) in patients with mild asthma as compared to placebo in a parallel, double blind study.[39] Furthermore, as compared to salbutamol, fluticasone propionate (500 mcg/daily for 12 weeks) significantly decreased sputum eosinophils (from 12.8% to 0.3% with fluticasone vs 5.3% to 4.2% with salbutamol).

Leukotriene receptor antagonists (LTRAs) have also been shown to have a significant action in reducing sputum eosinophils. In fact, as in the case of ICS, the reduction of these cells in induced sputum has been a secondary endpoint in several clinical trials both in adults and in children. However, there is at least one study in which the ability of montelukast to reduce airway eosinophilic inflammation was the primary endpoint: 10 mg montelukast once daily for 4 weeks reduced sputum eosinophils from 7.5% to 3.9% in mild to moderate asthmatics not on ICS during the prior month. Conversely, patients treated with placebo had their eosinophils increased from 14.5% to 17.9%.[40]

Apart from corticosteroids and LTRAs, several drugs have shown a lowering effect on sputum eosinophil counts in asthmatic patients. A single dose of salmeterol (50 mcg) decreased the amount of eosinophils in sputum in a group of 11 asthmatics who were allergen challenged in a double-blind, placebo-controlled crossover study. The median of the percentage of eosinophils was 6% after the challenge and rose to 31% among the patients who received placebo while for the patients treated with salmeterol the corresponding figure was 12%.[41] However, as compared to beclomethasone dipropionate (500 mcg twice daily), the ability of salmeterol (50 mcg twice daily) to reduce sputum eosinophils in a more prolonged treatment (4 weeks) is lower (from 12.0% to 1.0% with beclometahsone dipropionate vs 18.1% to 7.0% with salmeterol).[42] Theophylline also has some effect on sputum eosinophils: as compared to baseline, treatment with this drug once daily (200–600 mg/day) for 4 weeks reduced the sputum eosinophil percentage significantly (from 41.3% to 23.6%). As in other studies with ICS, peripheral blood eosinophils did not change significantly.[43]

Sputum eosinophils to predict response to treatment

The percentage of eosinophils in sputum has been shown to predict asthma response to treatment (control achievement) and to anticipate asthma exacerbations (loss of asthma control). The study by Little et al.[15] included 39 asthmatics with no exacerbations during the preceding month who attended for two visits separated by a two-week course of oral prednisolone (30 mg/day). A positive response to treatment was defined as an increase of at least 15% in the FEV1 value in the second visit over the first baseline visit. The authors used a cut-off point for sputum eosinophilia of 4%. The positive predictive value of responding to treatment in subjects with $\geq$4% sputum eosinophils was 64%; and the negative predictive value of those with $<$4% for not responding to it was 68%. Therefore, in this study, the ability of eosinophils in induced sputum to anticipate treatment response to corticosteroids was not very high. However, almost 2/3 of the patients had an eosinophil percentage under 4% before treatment with prednisolone and the authors do not offer information on the prior treatment of the patients included in the study, thus being possible that some of those patients did not have true eosinophilic asthma, and/or the room for FEV1 improvement was very thin. So, the conclusion would be that in well-controlled asthmatic patients (no exacerbations in the last month) sputum eosinophils are not very good markers of the response to an oral corticosteroid trial as a surrogate of the reversibility of lung function. Further studies with better characterised and less-controlled

patients would be needed to better know the usefulness of sputum eosinophils to predict the response to anti-asthmatic treatment.

Sputum eosinophils for predicting asthma exacerbations

It has been shown quite consistently that high counts in sputum eosinophils is a marker of loss of asthma control. Giannini et al.[44] studied a group of 30 moderate stable asthmatics on low-doses of ICS and tested the usefulness of sputum eosinophils to predict the recurrence of asthmatic symptoms once ICS were withdrawn. Asthma recurrence was defined based on a symptom diary. Patients had their sputum analysed at the beginning and at the end of a three month period, or when symptoms appeared. Twenty patients were treated with placebo and 10 continued with their usual ICS. The study was double-blind and randomised. Among placebo-treated patients, those whose symptoms recurred had a higher percentage of sputum eosinophils at the beginning of the study than those who did not have symptoms in spite of being treated with placebo (8.2% vs 0.9%). Patients who recurred also had a higher increase of sputum eosinophil percentages at the end of the placebo period than those patients who did not recur. Changes of sputum eosinophils were parallel to changes in the maximum amplitude of peak expiratory flow rate (PEF). The authors conclude that the withdrawal of treatment induces a quick recurrence of asthma symptoms (2 to 4 weeks) in one third of the asthmatics subjects, particularly in patients with sputum eosinophilia, in spite of the good functional and clinical control. Thus, sputum eosinophilia could be a useful tool to decide which patients can reduce or discontinue treatment.

With slight modifications, Jatakanon et al.[28] have replicated the same results in a group of stable asthmatics on a medium to high dose of ICS when switched to a low dose. The endpoint here was the appearance of a mild exacerbation of asthma defined either by a decrease of morning PEF, or the existence of night time symptoms or the need to use rescue medications. Patients who suffered a mild exacerbation within the study period of 8 weeks had a higher percentage of sputum eosinophils than patients who did not (13.6% vs. 0.2%). There was a strong correlation between the increase of sputum eosinophils and the decrease of lung function parameters. A very interesting finding of this study is the fact that patients who developed an asthma exacerbation did not have higher values of eNO at the time when the ICS dose was dropped.

Also among the individuals included in the Salmeterol or Corticosteroids (SOCS) trial[45] -who were on ICS prior to the randomisation to triamcinolone acetonide 400 mcg/day; salmeterol 42 mcg/12 hrs; or placebo- the increase of sputum eosinophils 2 weeks after the start of the study also predicted an asthma exacerbation (sensitivity 90% and specificity 65% for a cut-off point of 0.8%) in the placebo group as compared to the ICS group. Again eNO was not able to predict an asthma exacerbation in this group of mild to moderate asthmatics.

Conversely, in asthmatic children 6 to 17 years of age whose ICS was reduced to half every 8 weeks if clinically indicated, both sputum eosinophils and eNO in the prior visit were comparable predictors of a failure in ICS reduction in the subsequent period of 8 weeks. Treatment reduction was successful in all children who had no eosinophil in sputum before the attempted dose reduction.[46] With a very similar approach as the previous study, sputum eosinophils were found to be predictive of a non-eventful reduction of ICS in a group of 50 children of a similar age.[47] Only 62% of the children were able to provide sputum for the analysis. Those children who had an asthma exacerbation after ICS tapering had higher sputum eosinophils than those who did not; however, both groups have them much higher than normal and well above the cut-off point of 2–3% used by the two clinical trials which based the treatment in sputum eosinophils.[48,49] On the other hand, the authors do not offer data on the baseline sputum eosinophils, prior to halving the ICS doses, so their findings are reduced to further supporting the fact that eosinophils raise in a period of 8 weeks before an asthma exacerbation.

Sputum eosinophils to guide asthma treatment

The last step of the clinical usefulness of eosinophils in induced sputum is to use them routinely as a guide for asthma treatment. Most evidences reviewed above strongly suggest that this inflammatory marker could be used to monitor asthma control and to tailor the treatment of the individual patient. There is a study that has addressed this issue very elegantly and deserves special comment.

Green at al.[48] enrolled 68 asthmatics patients 18–75 years old with moderate-severe asthma (median BTS treatment stage 4) who were thought to need continued hospital follow-up. Half of them were introduced in the so-called BTS management group, in which decisions about the anti-inflammatory treatment were made in accordance to the BTS guidelines. The other half was allocated in the sputum management group, and decisions about their anti-inflammatory treatment were made according to an algorithm based on the maintenance of a sputum eosinophil count below 3%. Both groups were followed for 12 months. The main outcome variable was the number of severe exacerbations in the follow-up period. The results showed that–with a comparable consumption of anti-inflammatory drugs-the strategy of maintaining a low sputum eosinophil count was related to a considerable reduction of severe exacerbations when compared with the management based on the BTS guidelines. However, other markers of asthma control such as symptoms, use of beta2 agonists, and the variability of peak expiratory flow were not different between the two groups. The authors suggest that this is probably due to the fact that severe exacerbations and the variable airflow obstruction may be caused by different mechanisms.

From the clinical practical point of view, this study has two main limitations which claim for caution when considering introducing the induced sputum management strategy universally. The first one is that the patients included in the study were moderate-severe asthmatics, and it is not clear if this strategy would be effective and cost-effective in the primary care setting, where most asthmatic patients are treated. The second one is that this approach would only work for those patients with

eosinophilic asthma; and it should be borne in mind that an appreciable percentage of adult asthmatics who do not respond adequately to ICS suffer non-eosinophilic asthma.[17]

This limitation is especially important in childhood asthma. For instance, in a study performed in children with stable asthma, the number of coughing episodes was not related to sputum eosinophils but to sputum neutrophils. Additionally, the authors reported that they were able to have workable samples in only 69% of the 36 children included in the study (inter-quartile range of age 9–14 years).[50]

More recently, another clinical trial evaluated the use of sputum eosinophils as the main guide to asthma treatment. A total of 117 adult asthmatic patients were included in a randomised clinical trial with two branches: clinical plus spirometric assessment; and clinical, spirometric and sputum-guide ($\leq$2%) treatment. The authors established two different phases: in phase 1, the patients' treatment was reduced to a minimum to maintain control (which was possible in 107 patients); and in phase 2, patients were included in one of the two aforementioned branches. The mean time to achieve maintenance was similar in both groups, as was also the percentage of patients whose ICS was reduced or increased in this phase 1. With respect to phase 2, the authors found that sputum monitoring was useful to reduce the severity of both eosinophilic and non-eosinophilic asthma exacerbations in moderate-to-severe asthmatics without increasing the ICS dose. However, the sputum strategy could not reduce the frequency of the non-eosinophilic exacerbations, which were the most common ones.[49]

Conclusions

The eosinophil differential count in induced sputum is a well-standardised technique, which is cheap, although time-consuming and requires some skill. The percentage of eosinophils in sputum is decreased with conventional anti-inflammatory asthma therapy (ICS and LTRA), and also with other drugs not usually considered anti-inflammatory, such as salmeterol or theophylline. The number of eosinophils in sputum could be helpful for the labelling of an asthmatic patient who does no respond appropriately to the usual anti-inflammatory therapy. Additionally, an elevated eosinophil count can anticipate an asthma exacerbation, both in adults and in children. Furthermore, in moderate-severe asthmatics, a strategy based on maintaining low this count is more effective than the usual strategy based in guidelines for preventing severe exacerbations.

However, and focusing mainly in the paediatric population, an important percentage of children fail to produce sputum that can be analysed. Thus, a technique which does not work for everyone cannot be considered as a routine tool. Moreover, inhaling hypertonic saline is considered to be distinctly unpleasant and sickening by many individuals. A second, and perhaps even more important issue, is that laboratory handling and analysis of induced sputum samples is time- and labour-intensive, and could take as much as 2 hours from a dedicated analyst for any single sample. Thus, the cost of monitoring sputum eosinophils is considerable, and this should be taken into account in any effort to use this procedure in routine practice. Because of these two major problems, and because of the somewhat limited evidence that induced sputum eosinophils monitoring improves clinically important asthma outcomes in children, sputum eosinophils monitoring in childhood asthma cannot be considered a routine tool for asthma monitoring, and should be reduced to research purposes.

Conflict of interest

The authors have no conflict of interest to declare.

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