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Nasal lavage is better than blood count in predicting sputum eosinophilia

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To the Editor:

The ERS/ATS guidelines for severe asthma evaluation recommends the use of sputum eosinophil count as biomarker to guide therapy in adults, due to it places a higher value on possible clinical benefits from adjusting the treatment. However, it is useful only for patients who can produce sputum with persistent or, at least, intermittent eosinophilia and have severe asthma with frequent exacerbations¹. Surrogate biomarkers of airways inflammation have been long investigated. The importance of monitoring and managing asthma based on biomarkers is especially important for patients with discordance between symptoms and eosinophilic airway inflammation².³. Although the sputum eosinophil count has been proposed for phenotyping severe and uncontrolled asthma, it is crucial to identify a biomarker to evaluate the future risk in controlled asthma patients.

The sputum samples are always difficult to be obtained and managed in routine clinical practice. Several studies have been done to determine whether eosinophilia in easier and more accessible biological samples can be used as a biomarker of asthma phenotyping. Firstly, peripheral blood eosinophil count has been also proposed as a practical alternative to predict eosinophilia inflammatory profile improving asthma prognosis^{4,5}. Secondly, nasal lavage cytology can be used as a surrogate for sputum cytology in determining eosinophilic airway inflammation and our group have already shown it previously².

In this report, we aimed to determine if nasal lavage or blood eosinophil count were related to sputum eosinophil count in assessing inflammatory pattern in asthma patients under controlled status.

Specific details of our study methods have been published previously². In brief, this study included 140 patients over 18 years old, never smokers or ex-smokers, with moderate-severe asthma, who were undergoing combination therapy for at least three months. Patients with allergic nasal symptoms were allowed to use nasal corticoids. Each patient was evaluated to determine the current level of asthma control according to Asthma Control Questionnaire (ACQ controlled score < 1.5)⁶ and was included only if they were under control. They underwent clinical evaluation, spirometry, skin prick test, blood sample collection and nasal lavage followed by induction of sputum. All exams were performed in the morning at same time of day. All patients provided written informed consent and the study protocol were approved by the local ethic committee.

The sputum, nasal lavage and blood samples were considered eosinophilic when the relative eosinophil counts were $\geq 3\%$ ⁷, $\geq 5\%$ ⁸ and $\geq 3\%$ ⁵, respectively. Blood samples were also considered eosinophilic when the absolute eosinophil count was $\geq 220/\text{mm}^3$ ⁵. The sputum and nasal lavage were considered neutrophilic when the relative cells counts were $\geq 65\%$ ³ and $\geq 50\%$ ⁸ respectively.

Nasal lavage was performed before induced sputum to avoid the hypertonic saline nasal epithelium challenge or the induction of chemotactic factor release. As an advantage of nasal lavage samples over induction sputum was the time to obtain a

sputum cell count (50 minutes vs. 120 minutes). Moreover, nasal lavage collection is an easier process and more accessible than induced sputum for any special population who have difficulties in the sputum collection, as children and elderly ⁹.

The accuracy of nasal and blood eosinophil counts in measuring lower airway inflammation was determined using induced sputum cell counts as a gold standard in a ROC curve. Spearman's coefficient was used to assess correlation between nasal or blood cell count and sputum count. A multivariate logistic regression model was applied to characterize sputum eosinophilia. A p value < 0.05 was regarded as significant. All analyses were conducted by using IBM SPSS Statistics, Version 19.0. Armonk, NY: IBM Corp.

The mean age was 46.8 ± 13.1 years old, the majority of the subjects were female (73%) and had a positive skin prick test (88%) at the visit. The subjects presented mild to moderate obstruction with positive response to bronchodilators in the spirometry.

Although all patients were under control according to ACQ, 54 patients (38.5%) presented eosinophilic inflammatory activity characterized by sputum eosinophil $\geq 3\%$ (35 sputum eosinophil $\geq 3\%$ + 19 eosinophil $>3\%$ + neutrophil $\geq 65\%$)

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In the ROC curve analysis to assess the discriminative power of nasal and blood eosinophil counts to predict eosinophilic sputum, the nasal lavage showed the best value of AUC compared to blood samples, with the highest sensitivity and specificity predicting sputum eosinophilia (Fig. 1A).

Additionally, we found a strong correlation between relative nasal lavage eosinophil count and relative sputum eosinophil count using Spearman's correlation ($r = 0.72$, $p < 0.0001$) (Fig 1B), but only a weak correlation between absolute blood eosinophil count ($r = 0.32$, $p < 0.001$) (Fig. 1C).

In the multivariate logistic regression model the nasal lavage count was an independent predictor to characterize the sputum eosinophilia more than 3%, adjusted for absolute blood eosinophil count of $\geq 220/\text{mm}^3$, age, male gender and pre bronchodilator FEV_1 % of predicted value. (relative nasal eosinophils OR 61.0; 95%CI: 14.6-254.4; $p < 0.001$; and absolute blood eosinophil OR 0.1; 95%CI: 0.29-2.99; $p = 0.91$).

Although blood eosinophil count does correlate with sputum eosinophilia, we found that nasal eosinophil count exhibits a stronger Spearman's correlation, and thus it is a better predictor for sputum eosinophilia. In logistic regression model applied in our casuistic, the eosinophilic nasal lavage showed greater increasing in odds of eosinophilic sputum ($p < 0.001$), blinding a significant effect of eosinophilic blood

count²_ENREF_1_ENREF_1 confirming both co-linearity between blood and nasal eosinophilia and weak correlation between blood and sputum eosinophilia.

We further investigated the factors associated to sputum eosinophilia by using a graph to show the factor analysis variables associated to sputum eosinophilia and non-eosinophilia, and its 95% confidence intervals. While clinical symptoms and spirometric values were insensitive to recognize sputum eosinophilia, blood sample and nasal lavage cytology were able. Then, those factors could be considered a tool for enhance treatment or to recognize corticoid resistance or even a poor adhesion to treatment (Fig. 1D).

One limitation of this study was that airway expiratory NO and other expiratory condensate biomarkers were not investigated. Since that our objective is to identify asthma biomarkers that can be measured and evaluated in routine clinical practice, we decided not to include those measurements because they are not easily be performed in that scenario. In addition, some concerns have been raised regarding the specificity and clinical utility of the association between FENO and airway eosinophil count⁴.

Our results showed that it is possible to detect the existence of lower airway inflammation using nasal lavage cytology, even among asymptomatic patients who appear to be under good control by ACQ. Nasal cytology could be considered as an alternative marker for sputum cytology in the identification and assessment of eosinophilic asthma phenotype.

Since nasal lavage samples are relatively easy to be obtained in clinical practice, it should be included in routine evaluation of asthmatic patients.

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