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Original Article

Variability in Asthma Inflammatory Phenotype in Induced Sputum. Frequency and Causes☆

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ABSTRACT

Introduction: Recent studies have found variability in asthma inflammatory phenotypes determined by the inflammatory cells in induced sputum (IS). The aim of this study was to determine the frequency and factors affecting inflammatory phenotype variability in IS.
Methods: Retrospective observational study that included 61 asthmatic patients who underwent at least two IS tests over a period of 5 years. They were classified according to their baseline inflammatory phenotype and subsequently grouped according to phenotype variability (persistent eosinophilic, persistent non-eosinophilic and intermittent eosinophilic). Demographic, clinical and functional data and factors potentially influencing IS variability were collected in all cases.
Results: Of the 61 patients, 31 (50.8%) had a change with respect to baseline inflammatory phenotype. Of these, 16 (51.6%) were eosinophilic, 5 (16.1%) neutrophilic, 1 (3.2%) mixed and 9 (29.1%) paucigranulocytic. According to phenotype variability, 18 patients (29.5%) were classified as persistent eosinophilic, 17 (27.9%) non-persistent eosinophilic, and 26 (42.6%) intermittent eosinophilic. Smoking and recent asthma exacerbation were significantly associated with increased risk of variability of the IS inflammatory phenotype (OR=6.44; *P*=.013; 95% CI=1.49–27.80 and OR=5.84; *P*=.022; 95% CI=1.29–26.37, respectively).
Conclusion: Half of asthma patients, predominantly those with eosinophilic phenotype, present a change in IS inflammatory phenotype. This variability is associated with smoking and recent asthma exacerbation. Data suggest these factors can modify the classification of IS inflammatory phenotype in clinical practice.

Variabilidad del fenotipo inflamatorio del asma en el esputo inducido. Frecuencia y causas

Palabras clave:
Asma
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RESUMEN

Introducción: Estudios recientes han constatado variabilidad del fenotipo inflamatorio del asma en el recuento de las células inflamatorias del esputo inducido (EI). El objetivo del presente estudio fue determinar la frecuencia y los factores que condicionan la variabilidad del fenotipo inflamatorio del EI.
Métodos: Estudio observacional retrospectivo que incluyó 61 pacientes asmáticos a los que se les practicó un mínimo de dos EI en un período de 5 años. Los pacientes fueron clasificados según su fenotipo inflamatorio y posteriormente agrupados según la variabilidad del fenotipo (eosinofílicos persistentes, no eosinofílicos persistentes y eosinofílicos intermitentes). De todos los casos incluidos se recogieron datos demográficos y clínico-funcionales, así mismo se valoró los factores que pudiesen influir en la variabilidad del EI.
Resultados: De los 61 pacientes, 31 (50,8%) presentaron un cambio del fenotipo inflamatorio inicial. De estos, 16 (51,6%) eran eosinofílicos, 5 (16,1%) neutrofílicos; 1 (3,2%) mixto y 9 (29,1%) paucigranulocíticos. Según la variabilidad, 18 pacientes (29,5%) se clasificaron como eosinofílicos persistentes,

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17 (27,9%) no eosinofílicos persistentes y 26 (42,6%) eosinofílicos intermitentes. El tabaquismo y una exacerbación asmática reciente se asociaron significativamente con mayor riesgo de variabilidad del fenotipo inflamatorio del EI (OR = 6,44; p = 0,013; IC95% = 1,49–27,80 y OR = 5,84; p = 0,022; IC95% = 1,29–26,37, respectivamente).

Conclusión: La mitad de los pacientes asmáticos modifican el fenotipo inflamatorio del EI, predominando los de fenotipo eosinofílico. Esta variabilidad se asocia al tabaquismo y a una exacerbación asmática reciente. Los datos sugieren que estos factores podrían influir en la determinación del fenotipo inflamatorio del EI en la práctica clínica habitual.

Introduction

The study of bronchial inflammation has become a highly useful tool for characterizing and monitoring patients. Non-invasive techniques, such as inflammatory cell counts in induced sputum (IS), have been developed and applied to great benefit in determining inflammatory phenotypes in severe asthma and tailoring patient treatment.[1,2] Identification of the inflammatory phenotype from IS is more precise than determinations made from the fraction of exhaled nitric oxide (FE_{NO}).[3] Various clinical practice guidelines recommend the use of IS in the clinical evaluation of patients with uncontrolled severe asthma.[1,4]

Four inflammatory phenotypes have been described in asthma patients, determined according to IS cellularity: eosinophilic, neutrophilic, mixed, and paucigranulocytic.[5] The identification of these phenotypes is not merely of academic interest; it can be used to guide treatment. For example, patients with non-eosinophilic phenotypes benefit less from treatment with inhaled corticosteroids (IC) than eosinophilics,[6,7] and it has been suggested that patients with neutrophilic phenotype respond favorably to long-term macrolide treatment,[8] and possibly to tiotropium.[9]

Nevertheless, the reliability of cell counts in IS has been questioned following recent reports of changes in the proportion of inflammatory cells in IS samples obtained from the same patient at different times. This variability has been reported to occur over the course of the day,[10] due to the effect of IC treatment,[11,12] or over the course of the disease, both in adults[13] and in children.[14] Other studies, however, have not reported any variability.[6,15] Be that as it may, the current recommendation is to perform more than one test in each patient in order to establish the correct inflammatory phenotype.[14,16,17]

Aside from the issues concerning the existence and incidence of this variability, little information is available on the factors associated with this phenomenon. An understanding of the causes of variability in inflammatory cell counts in IS would be of great importance in clinical practice. It would be useful, firstly, to identify the potential limitations of the IS technique for identifying asthma inflammatory patterns; and secondly, to anticipate or prevent such variability, if possible. The primary objectives of this study, then, were to determine the frequency of IS inflammatory phenotype variability in asthma and to identify factors that might cause phenotypes to change.

Methods

Design

This was a retrospective, observational study, analyzing the IS of 61 asthma patients on maintenance treatment, aged between 18 and 85 years. Asthma diagnosis was established if patients had consistent symptoms and confirmed variable airflow limitation (positive bronchodilator test, diurnal variability in peak expiratory flow >20% over 2 weeks or positive methacholine bronchial challenge test), according to the criteria of the Spanish Guideline on the Management of Asthma (GEMA 2009).[4] Study data were obtained from the clinical records of patients who attended asthma specialist clinics in our hospital and in whom IS testing had been performed either for clinical reasons (discrepancy between symptoms and FE_{NO} results, poor asthma control, and/or suspected concomitant gastroesophageal reflux) or due to their participation in another study (for determining inflammatory phenotype). All patients had undergone at least 2 IS tests between 2008 and 2013. The study design complied with the principles of the Declaration of Helsinki (1964) and was part of a subanalysis of another study approved by the Hospital Ethics Committee (ClinicalTrials.gov: NCT02028637).

Procedures

Demographic and clinical data were collected, including sex, age, smoking history, defined as active smokers or former smokers if they had stopped for at least 1 year. Asthma severity and stratification of IC doses were established according to GEMA 2009[4] and the Global Initiative for Asthma (GINA).[18] The Asthma Control Test (ACT), a self-administered questionnaire validated in Spanish, was used to evaluate asthma control.[19] Asthma exacerbation was any episode (infectious or otherwise) that changed the previous clinical status of the patient, irrespective of the severity of the event, according to ATS/ERS criteria.[20] Change in corticosteroid dose was defined as any modification in the patient's treatment involving the introduction, increase, discontinuation or reduction of inhaled or systemic corticosteroid treatment. Clinical and other data were collected within a period of 3 months prior to IS sampling.

Study procedures: skin prick testing was performed for common airborne allergens.[21] Spirometry was performed using a Datospir-600 (Sibelmed SA, Barcelona, Spain) device, according to SEPAR 2013 recommendations.[22,23] FE_{NO} was performed using an electrochemical device (NO Vario Analyzer. FILT Lungen and Thorax Diagnostic GmbH, Berlin, Germany) at a flow of 50 mL/s, according to ATS/ERS 2005 recommendations.[24] FE_{NO} levels of $\geq$50 ppb were considered significantly elevated.[25] Total serum IgE was determined using ImmunoCAP (Phadia 250). Sputum samples were induced and processed according to the standardized procedure published by Pizzichini et al.: all patients were premedicated with 200 μg of inhaled salbutamol. After 10 min of bronchodilation, IS was obtained after inhalation of a hypertonic solution aerosol for 7 min at each concentration (3%, 4% and 5%). Particles were generated in an ultrasonic nebulizer (Omron NE U07) with a mean diameter of 7 μm and an output of 3 ml/s. As a safety precaution, FEV1 was also measured after each inhalation period. Mucus plugs were treated with dithiothreitol (Sputolysin, Calbiochem Corp., San Diego, CA, USA) and phosphate-buffered saline solution and sputums were processed within 2 h. The cell suspension was filtered, and the total number of cells per gram of sputum, viability and total squamous cells indicating upper respiratory tract contamination were calculated using a blood cell counter and Trypan blue staining. Cell preparation and supernatant were obtained by cytocentrifugation. May–Grünwald–Giemsa staining was applied to this preparation for the differential cell count.[26]