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Original article

Impact of allergy on phenotypic and endotypic profiles of nasal polyposis

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ABSTRACT

Objectives: To assess the impact of allergy on clinical presentations (phenotypes) and inflammatory patterns (endotypes) of chronic rhinosinusitis with nasal polyps (CRSwNP).
Methods: A single-center prospective study was conducted over an 18-month period. Fifty-seven patients with refractory CRSwNP were included. The diagnosis of allergy was based on concordant skin prick tests and symptoms. Phenotypes were determined on symptom severity score, polyp size classification and Lund-Mackay CT staging. Inflammatory endotypes were determined on biomarker analysis (IgE, IgA, IL-5, IL-9, ECP, EDN) in blood and nasal secretions. Eosinophil counts were obtained in blood, nasal secretions and polyps.
Results: Phenotype and endotype profiles were comparable in patients with ($n = 15$) or without ($n = 42$) allergy. Only asthma with high total IgE blood concentration showed association with allergy.
Conclusions: The present results suggest that allergy is not directly involved in the clinical expression and specific inflammatory pathways of CRSwNP. New therapies target inflammation signaling pathways, and identifying accurate blood and tissue biomarkers will be the line of research most likely to improve treatment of CRSwNP.

1. Introduction

Chronic rhinosinusitis with nasal polyps (CRSwNP) has a prevalence of 2–4% in the general population, with significant impact on quality of life [1]. Clinical response to local or general corticotherapy, which is at present the only treatment with proven efficacy [2], varies greatly. New therapies are currently under assessment, based on blocking pro-type-2 T-helper (Th2) inflammatory pathways: anti-IgE, anti-interleukin-5 and anti-interleukin-4 [3–5]. Implementation requires a precise definition of indications, by refining the phenotypic and endotypic characterization of patients [6,7].

Phenotypically, there is a 42% rate of asthma, constituting a group of patients in whom nasal pathology is harder to control [1]. Endotypically, recent reports have distinguished patient groups by immune profile. In Europe, inflammatory response most often involves polynuclear eosinophils, Th2 lymphocytes releasing IL-4, IL-5, IL-9 and IL-13 interleukins, and B lymphocytes producing polyclonal immunoglobulin E (IgE) and IgA [8,9]. Rates of proTh2 cytokines and certain IgEs countering Staphylococcus toxins are often elevated in case of associated asthma [3,10]. In Asia, the predominant endotypic profile involves Th17 releasing IL-17 or IL-22, with weaker association with asthma [11].

The role of allergy in the pathophysiology of CRSwNP is very controversial. Incidence is not higher than in the general population [1], but some authors reported higher allergen sensitivity in patients with CRSwNP, although no clear association can be established with clinical expression of allergy [12]. To determine the role of allergy more precisely, we compared pheno- and endotypic profiles in CRSwNP patients eligible for sinonasal surgery with and without allergy. The selected biomarkers were those most frequently found in blood and tissue in European populations. IgE and IgA enable assessment of isotypic commutation of B lymphocytes in blood and tissue [8,10]; IL-5 and IL-9 cytokines reflect Th2 predominance in the immune profile of T-helper lymphocytes in tissue [7,13,14], while ECP (eosinophil cationic protein) and

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EDN (eosinophil-derived neurotoxin) are degranulation products of eosinophils involved in tissue remodeling [7].

2. Material and methods

2.1. Population

A single-center prospective study, conducted over an 18-month period, included patients with CRSwNP diagnosed on the EPOS criteria [1], aged over 18 years, recalcitrant to medical treatment, for whom polypectomy or ethmoidectomy was indicated. Recalcitrance was defined by persistent subjectively disabling symptomatology despite maximum-dose local corticotherapy and three 10-day courses of oral corticosteroids at 1 mg/kg/day prednisone equivalent. Exclusion criteria comprised CRSwNP associated with mucociliary pathology, immune deficiency or autoimmune disease.

Written informed consent was obtained ahead of any analysis of serum, nasal secretion or polyps. Exploration was performed under the SHE (Hyper-Eosinophilia Syndrome) study, approved by the local review board (*Comité d'éthique nord-ouest, n°° 2009-A00314-53*).

2.2. Clinical data

Preoperative clinical data comprised: age, gender, smoking status, and history of sinonasal surgery. Allergies were identified on concordant interview and skin prick tests data. Asthma and bronchial hyper-reactivity were systematically screened for on interview, respiratory function tests and methacholine challenge, as appropriate. Intolerance of non-steroidal anti-inflammatory drugs (NSAIDs) was identified only on respiratory signs reported in interview.

CRSwNP severity was assessed in terms of preoperative symptom intensity on a visual analog scale (VAS: 0–10, with 10 = maximal functional trouble). Polyp size was measured on Lildholdt score [15] on preoperative endoscopy without mucosal retraction. Sinonasal CT scan without contrast enhancement was performed within 2 weeks before surgery, for the purposes of radionavigation to assess mucosal opacity on Lund-Mackay score [16].

Patients were required to interrupt any local or general corticotherapy at least 1 month before surgery. Only saline nasal lavage was allowed.

2.3. Biological sampling

Blood samples were taken before surgery, in BD Vacutainer™ EDTA tubes (BD Medical, Franklin Lakes, NJ).

Nasal secretions were collected just before the start of surgery. Two 1.27 × 2.54 cm cotton swabs (Codamn, Raynham, MA) were introduced in each nasal cavity for 5 minutes, immersed in PBS (phosphate-buffered saline) for 1 h at 4°C, and then centrifuged at 14,000 rpm for 8 min to eliminate cellular components. Supernatants for inflammation marker evaluation were aliquoted after protein assay following Bradford, and kept at −80°C.

Before mucosal decongestion by gauze soaked in a solution of lidocaine hydrochloride (5 g/100 mL) and naphazoline (20 mg/100 mL), polyps were sampled at the start of surgery, under endoscopic control, by gentle traction on the implantation base, and placed on a damp compress for immediate manipulation.

2.4. Protein assay

ECP, EDN, IL-5 and IL-9 were assayed in serum and nasal secretions, using MBL International (Nagoya, Japan), R&D Systems (Minneapolis, MN) and Bio-Rad (Hercules, CA) ELISA kits. Detection thresholds were 0.62 ng/mL for ECP and EDN, 0.29 pg/mL for IL-5 and 2.5 pg/mL for IL-9.

Total IgE and total IgA were respectively assayed by Phadia™ immunoCAP test (Thermo Scientific, Waltham, MA) and SPAplus™ analyzer (Binding Site, Birmingham, UK).

2.5. Eosinophil assay

Polynuclear eosinophils were assayed in blood by routine clinical cell count. The proportion of eosinophils in nasal secretions compared to other cellular elements was assessed after cytocentrifugation and cell count on a Countess™ Automated Cell Counter (Thermo Scientific). Proportions in polyps were measured after mechanical and enzymatic dissociation of the polyp (homogenate) for cellular purification by negative selection [12]. Briefly, the polyp was fragmented and subjected to mechanical digestion by a GentleMacs dissociator™ (Miltenyi Biotec, Bergisch Gladbach, Germany) then enzymatic digestion (collagenase type-II and Dnase I). Tissue debris was then eliminated by filtration on nylon supports of decreasing diameter. Eosinophil concentration was then determined after cytocentrifugation and cell count on a Countess™ Automated Cell Counter (Thermo Scientific).

2.6. Statistical analysis

Data were anonymized and entered in Microsoft Excel™ file. Statistical analysis used SPSS™ software v15.0 (SPSS Inc., Chicago, IL). Means and standard deviations were reported for descriptive variables. Chi^2 tests were used to compare frequencies. The non-parametric Mann–Whitney test was used to compare means between non-paired samples. The significance threshold was set at $P \leq 0.05$.

3. Results

3.1. Phenotype data

Fifty-seven patients were included over the study period: 40 male, 17 female; mean age, 48.7 ± 15.7 years. Fifteen showed allergy (26.3%), including 7 with monosensitization (46.7%). The 3 most frequently implicate allergens were house-dust mites (14 cases), grass pollen (6 cases) and cat dander (4 cases) (Fig. 1).

The phenotypic profile of patients with and without allergy did not differ for age, sex-ration, duration of CRSwNP, history of sinonasal surgery, or clinical or radiological severity (Table 1). NSAID intolerance was equally frequent between patients with and without allergy. Asthma was more frequent when allergy was associated with CRSwNP ($P = 0.03$).

3.2. Endotype data

3.2.1. Blood

Blood eosinophil levels did not differ with allergic status (Table 2). Total IgE blood level was higher in case of allergy ($P = 0.01$). Total IgA, ECP and EDN blood levels did not differ with allergic status.

3.3. Nasal secretions and polyps

The proportion of eosinophils in nasal secretions was independent of allergic status, as were levels of cytotoxic proteins ECP and EDN, Th2 inflammatory markers IL-5 and IL-9, and total IgE and total IgA (Table 2).

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