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Cortactin expression in nasal polyps of Aspirin-Exacerbated Respiratory Disease (AERD) patients

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ABSTRACT

Purpose: The term aspirin-exacerbated respiratory disease (AERD) refers to a combination of asthma, chronic rhinosinusitis with nasal polyposis (CRSwNP), and acute respiratory tract reactions to nonsteroidal anti-inflammatory drugs. AERD has now been included among the CRSwNP endotypes, and is considered one of the most aggressive in terms of disease recurrence.

Cortactin is a multi-domain protein with a part in several cellular mechanisms involving actin assembly and cytoskeleton arrangement. Cortactin seems to have a role in inflammatory responses and to be implicated in human airway secretion and contraction mechanisms.

The novel aim of the present study was to examine cortactin expression in nasal polyps of a consecutive cohort of AERD patients and in nasal mucosa of a control group of patients.

Materials and methods: Cortactin expression was assessed immunohistochemically in nasal polyps from 18 consecutive AERD patients who underwent endoscopic sinus surgery and in nasal mucosa of 19 patients without chronic rhinosinusitis.

Results: Concomitant allergy was found in 11 AERD patients, most of them male (8 cases; $p = 0.02$). Cortactin expression in nasal polyps was definitely high (+3) in 17 out of 18 cases, in both epithelial cells (cytoplasmic and membranous immunoreactivity) and activated fibroblasts. A higher cortactin expression was seen in female than in male AERD patients ($p = 0.05$).

Conclusions: Given this preliminary evidence of cortactin upregulation in the polyps of AERD patients, prospective studies could further investigate the role of cortactin in the biology of AERD, and the potential role of cortactin-targeted approaches in integrated AERD treatments.

1. Introduction

The term aspirin-exacerbated respiratory disease (AERD) refers to a combination of asthma, chronic rhinosinusitis with nasal polyposis (CRSwNP), especially the eosinophilic histotype, and acute upper and/or lower respiratory tract reactions to the ingestion of nonsteroidal anti-inflammatory drugs (NSAIDs) [1]. Additional features can include blood hypereosinophilia, abdominal and dermatological disorders, and intolerance of alcoholic beverages [2]. AERD affects from 0.3% to 0.9% of the general population, usually developing in the third and fourth decades of life [3]. It has now been included among the CRSwNP endotypes, and is one of the most aggressive in terms of disease recurrence [1].

Cortactin is a multi-domain protein that takes part in several cellular mechanisms involving actin assembly and cytoskeleton arrangement [4,5]. The locus of cortactin CTTN is in the 11q13 chromosomal region. It is a gene frequently amplified in several types of human cancer (adenocarcinoma of the breast and colon, squamous cell carcinoma of the esophagus or head and neck) [6,7]. Cortactin overexpression has consequently been associated with a tumor's aggressiveness, invasiveness, and metastatic potential, and with a poor prognosis [8–10].

Several studies [11–15] have recently investigated the role of cortactin in non-neoplastic diseases, and particularly in inflammatory disorders involving smooth muscle contraction and mucus secretion in the human airways.

Given the emerging role of cortactin in regulating inflammatory responses, and its implication in human airway secretion and contraction mechanisms, the novel, main aim of the present study was to examine cortactin expression in nasal polyps from a consecutive cohort of

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AERD patients, who were stratified by their demographic, clinical, laboratory, and prognostic features; cortactin immunostaining was determined also in normal nasal mucosa from a control group of patients without chronic rhinosinusitis. A second endpoint of the study was to assay blood basophils, eosinophils, and calculate the neutrophils-to-lymphocytes ratio (NLR) in the same cohort of patients.

2. Methods

2.1. Patients

This retrospective study was approved by our Otolaryngology Section's in-house committee and conducted according to the principles of the Helsinki Declaration. All patients and controls signed a detailed informed consent form regarding the processing and publication of their data and images.

The study was performed on a consecutive cohort of Caucasian adult patients (≥18 years) with a diagnosis of AERD. Asthma was diagnosed clinically and confirmed by spirometry and methacholine challenge tests. Hypersensitivity to aspirin and other cyclooxygenase-1 (COX-1) inhibiting NSAIDs was documented on the grounds of patients' clinical history. Rigid 0° and 30° (Ø 4 mm) endoscopes, and CT scans of the paranasal sinuses were used to detect and classify CRSwNP, as well as for follow-up purposes. Patients with endoscopic evidence of polyps grades 2 or 3 according to Mackay and Lund [16] and failing to respond to topical and oral steroid therapy given for three consecutive months as recommended in the European Position Paper on Rhinosinusitis and Nasal Polyps (EPOS) guidelines [17] underwent endoscopic sinus surgery (ESS).

The following were reasons for exclusion: pregnancy, autoimmune disease, acute or chronic infectious diseases other than sinusitis, malignancies, hematological disorders, or chronic renal insufficiency.

All patients underwent preoperative laboratory tests at least 3 months after withdrawing oral steroids, and at least 1 month after stopping nasal steroid treatments. In particular, a blood sample was taken to obtain neutrophil, lymphocyte, eosinophil, and basophil counts, and the NLR was calculated [18]. All assays were performed at the same laboratory (EIA Unit, Laboratory Medicine Service, Padova General Hospital; certified in accordance with ISO 15189).

For the histopathological examination, surgical specimens were stained with hematoxylin and eosin, and 3 high-power fields (HPF) (magnification 400×) from each specimen were examined by a pathologist (FM) to quantify the eosinophil component. As previously reported [19], CRSwNP was classified as eosinophilic (≥10 eosinophils/HPF) or non-eosinophilic (< 10 eosinophils/HPF).

After surgery, all patients performed nasal irrigations with isotonic saline solution twice daily and applied the same topical nasal steroids as they had used preoperatively, in accordance with the EPOS guidelines [17]. During periods of pollination, patients with a known pollen allergy were treated with antihistamines.

Follow-up endoscopies were scheduled 3, 6, and 12 months after ESS, and yearly thereafter. Patients with endoscopic evidence of at least grade 1 polyposis [16] were classified as cases of recurrence.

2.2. Immunohistochemistry

Immunohistochemical reactions were conducted on sections 4–5 μm thick obtained from formalin-fixed and paraffin-embedded samples from 18 patients with AERD and 19 patients without chronic rhinosinusitis that underwent nasal biopsy and resulted completely normal at histological examination. All immunohistochemical stains were performed with a fully automated system (BondmaX Leica Microsystems, Wetzlar, Germany), using the Bond Polymer Refine Detection kit (Leica Microsystems, Wetzlar, Germany), and a rabbit anti-cortactin antibody (monoclonal EP1922Y; Abcam, Cambridge, UK; working dilution 1:200, 30 min, citrate buffer), according to the manufacturer's instructions. The prepared sections were lightly counterstained with hematoxylin. Appropriate positive controls, and sections incubated without any primary antibody as negative controls were run concurrently according to the manufacturer's recommendations. For each slide, cortactin immunostaining was scored by a pathologist (LN). In AERD patients' specimens, cytoplasmic cortactin expression was measured as the percentage of positive cells and scored as: 0 = < 1%; +1: 1–25%; +2: 26–65% and +3: > 66%. Staining intensity was graded as: 0 = none; +1: weak; +2: moderate; +3 = strong. An overall H-score was calculated as: (1 + intensity) / (3 × expression score) [20].

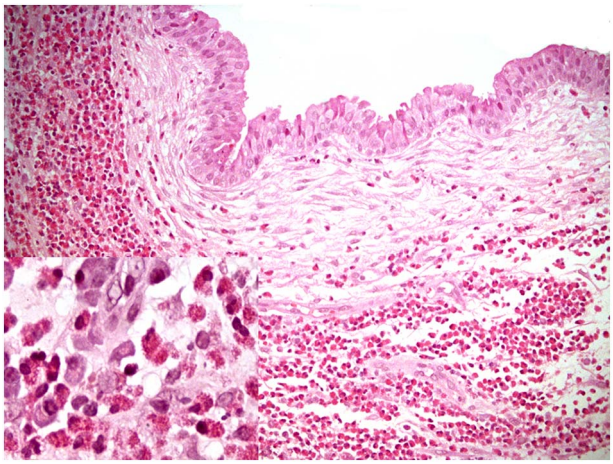

Fig. 1. Representative case of eosinophilic polyp mucosa showing high eosinophil density (box on left; hematoxylin and eosin, magnification 200×).

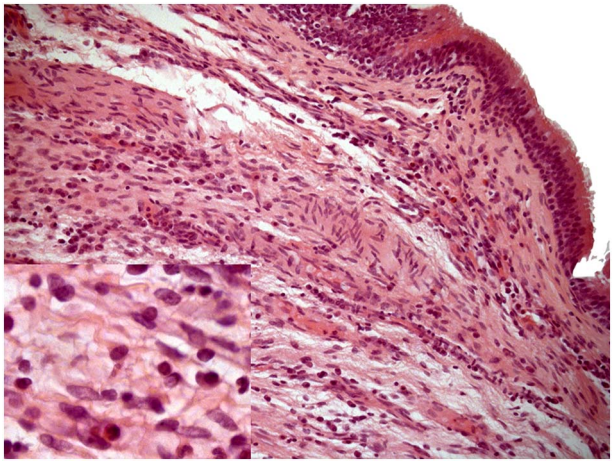

Fig. 2. Representative case of non-eosinophilic polyp mucosa (box on left; hematoxylin and eosin, magnification 200×).

2.3. Statistical analysis

Because of the small sizes of the sub-cohorts considered, the statistical significance of any differences between means was ascertained using a non-parametric test (the Mann–Whitney test) to compare between-group findings. Fisher's exact test and Spearman's rank correlation test were also used, as appropriate. A p value < 0.05 was considered significant, while values in the range of $0.08 \geq p \geq 0.05$ were considered as indicating a statistical trend. The STATA 8 statistical package (Stata Corp LP, College Station, Texas) was used for all analyses.