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Clinico-pathological study of chronic rhinitis in adolescents

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Abstract

Through the increasing incidence and association of asthma, chronic rhinitis (CR) raises major problems in the pathology of children and adolescents. The evaluation of the inflammatory status together with the diagnosis and the tandem treatment of the two conditions can contribute to the improvement of the patients' quality of life. In this study, we analyzed the immunoexpression of cluster of differentiation (CD) 20, CD8, CD138 and eosinophil major basic protein (MBP) in 24 cases of CR in adolescents, in eight of them existing the association of asthma. Symptoms of CR and allergic status, as well as histopathological changes specific to a persistent inflammation, were identified in this study. The CD20/CD8 immunophenotype was more specific for CR, while the CD138/eosinophil MBP immunophenotype was specific for asthma-associated chronic rhinitis (AACR). The negative linear distribution of lymphocyte elements compared to plasmocytes and eosinophils specific for the allergic status can support the protective effect of CD8 T-lymphocytes and the presence of a semi-activated B-lymphocyte status in CR. The results may be useful for improving the stratification criteria of patients for therapy.

Keywords: chronic rhinitis, asthma, lymphocytes, eosinophils, plasmocytes.

Introduction

Considered in the past a banal condition, chronic rhinitis (CR) is a real health problem in children and adolescents today, especially by affecting the quality of life, but also by the relationship with the allergic status and inflammation of the lower airways [1, 2]. With an incidence of 10–40% in the general population, CR is defined as inflammation of the nasal mucosa that persists for more than 10 days and which in the population under 20 years can have non-infectious allergic etiology in about 75% of cases and non-allergic in 25%, the mixed aspects being also described [3, 4].

Due to the close relationship between respiratory inflammation with different locations, allergic rhinitis treatment guides with impact on asthma, indicates modulatory immunotherapy as a priority area of research [5]. Also, guidelines for the prevention and alleviation of asthma morbidity indicate the need to treat inflammation in the upper airway, including allergic or non-allergic rhinitis [5–7].

The molecular mechanisms by which rhinitis can initiate and maintain bronchial asthma are not fully understood, among them being described the disturbance of the barrier role of the nasal mucosa, systemic extension of inflammation, and disruption of nerve transmission [3, 8–10]. Quantifying inflammatory elements in CR can provide insights into the molecular mechanisms involved and therapeutic targets that can improve the quality of life of patients, in the context in which the immune system in children and adolescents has an adaptive capacity that can be exploited to influence the allergic respiratory status [10, 11].

Aim

In this study, we analyzed the immunoexpression of inflammatory elements present in the nasal mucosa in CR or associated with asthma-associated chronic rhinitis (AACR) in adolescents.

Patients, Materials and Methods

In this retrospective study, we included 24 cases of CR diagnosed in the Department of Pathology, Emergency County Hospital of Craiova, Romania. The patients were represented by adolescents hospitalized for persistent upper (16 cases) or upper/lower (eight cases) airways inflammation treated in the Departments of Pediatrics and Otorhinolaryngology from Emergency County Hospital and "Filantropia" Municipal Hospital (Craiova), over a period of seven years (2012–2018). The clinical diagnosis of CR was established following specific clinical and para-clinical investigations (laboratory tests, rhinomanometry, nasal provocation test, cytology from nasal secretions).

The epidemiological and clinical data (age, gender, history, symptoms) were taken from electronic Hospital's database and patient observation sheets. In this study, patients with upper airway structural defects, immuno-deficiency states, or who followed treatments for inflammation with locations other than respiratory were excluded. Also, were excluded patients with pathology associated with CR, as diabetes mellitus or cystic fibrosis.

The biological material was represented by nasal mucosa biopsy specimens, which were fixed in 10% buffered neutral formalin. The histopathological (HP) analysis was done by classic paraffin embedding and Hematoxylin–Eosin (HE) staining. In all studied cases, the HP results were reassessed using the last literature criteria for CR [12].

For all cases, serial sections of 4 μm were performed in order to use the immunohistochemical (IHC) technique for the assessment of some tissue inflammatory cells represented by lymphocytes, eosinophils and plasmocytes. After endogenous peroxidase blocking (hydrogen peroxide) and non-specific antigenic sites blocking (bovine serum albumin), the antigen enzymatic retrieval or Heat-Induced Epitope Retrieval (HIER) technique was performed. The antibodies used in this study were incubated overnight, at 4°C (Table 1).

Table 1 – ***Antibodies used in the study***

Antibody	Clone	Dilution	Antigen retrieval	External control
CD20	L26	1:200	Citrate buffer, pH 6	Tonsil
CD8	1A5	1:50	Tris-EDTA buffer, pH 9	Tonsil
CD138	MI15	1:150	Citrate buffer, pH 6	Plasmacytoma
Eosinophil MBP	BMK-13	1:100	Pepsin	Bronchial mucosa (asthma)

CD: Cluster of differentiation; MBP: Major basic protein; EDTA: Ethylene-diaminetetraacetic acid.

In this study, we used Labeled Streptavidin–Biotin (LSAB) 2 system (Dako, Redox, Romania, code K0675) for developing the reactions and 3,3'-Diaminobenzidine (DAB) tetrahydrochloride (Dako, Redox, Romania, code K3468) as chromogen. For the validation of reactions, the external positive and negative controls were used. From this study, were excluded the cases for which the harvested tissue did not revealed an optimum IHC reactivity for vimentin.

For the assessment of reactions, we used the positivity index (PI) resulted by reporting the average number of positive cells to the total number of tumor cells counted at 40× microscope objective, using 10 microscopic fields/ case.

The statistical analysis used mean values, standard deviations and comparison tests (Student's *t*-test, Pearson's test) within Statistical Package for the Social Sciences (SPSS) 10 software, the $p<0.05$ values being considered significant.

For the images acquisition, we used the Nikon Eclipse E600 microscope equipped with Lucia 5 software.

The informed consent was obtained for all cases and the local Ethics Committee approved the study.

Results

The 24 cases of CR were identified in adolescents aged between 13–18 years, with an average age of diagnosis of 16.1±1.7 years, the majority in male patients (16 cases, 66.7%).

Patient history indicated for eight cases the association of nasal symptomatology with asthma diagnosed in the last year. At the same time, patients with allergic family history (five cases) and exposure to smoking (seven cases) were identified. The symptoms of the patients were variable, for the patients with CR predominating nasal obstruction, rhinorrhea, sneezing, posterior nasal secretions, sinus pain, and in the case of the association of asthma (AACR), the episodic presence of wheezing, productive cough and thoracic constriction.

The HP analysis indicated changes specific to chronic inflammation with nasal location. At the level of the covering epithelium, we identified squamous metaplasia and hyperplasia of the basal layer, with focal or zonal extension that led to the increase in thickness. The mucus-secreting columnar cells (goblet) were increased in number, and sometimes the basement membrane was visibly thickened. Underlying the epithelium, at the stromal level we found areas of edema, collagenous sclerosis, capillary vessels with stasis and infiltration with inflammatory elements represented by macrophages, lymphocytes, plasmocytes, eosinophils and rare leukocytes. Sometimes inflammatory elements were present also at the intra-epithelial level. In the cases of CR, the hyperplasia of the epithelial cells and the squamous metaplasia prevailed (Figure 1A), whereas in the case of AACR, the goblet cell hyperplasia was evident (Figure 1B).

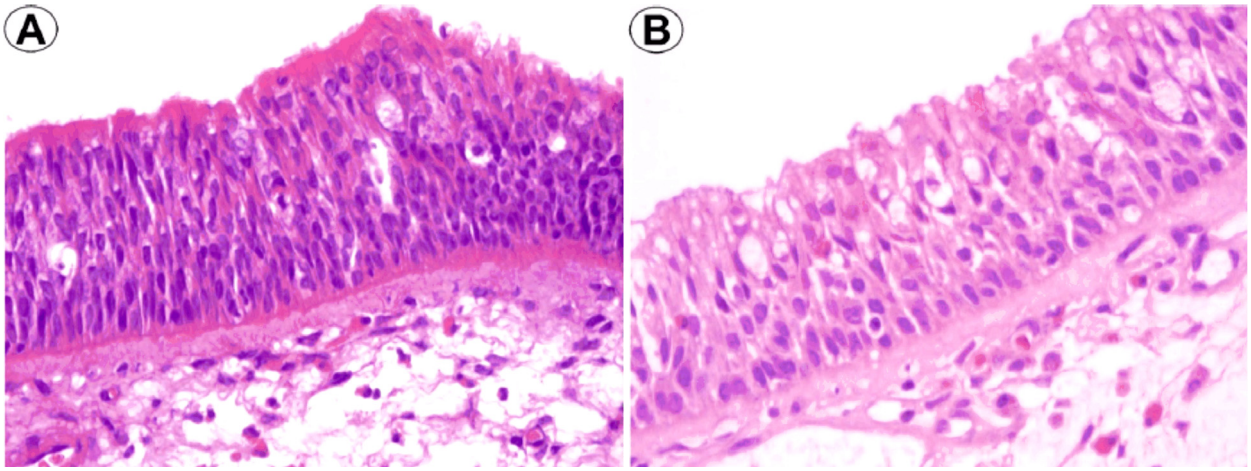

Figure 1 – ***(A) CR, basal cell hyperplasia, squamous metaplasia and basement membrane thickening (HE staining, ×200); (B) AACR, goblet cell hyperplasia (HE staining, ×200). CR: Chronic rhinitis; AACR: Asthma-associated chronic rhinitis; HE: Hematoxylin–Eosin.***

IHC analysis indicated the positivity of the analyzed markers in all cases of CR and AACR.

CD20 immunoexpression was identified in the cytoplasm and membrane of B-lymphocytes, with predominantly subepithelial distribution and presence in the stroma of small lymphoid follicles. The average PI CD20 value for the whole group was 19.5±10.3, with values between 3–40 elements/×400. The number of CD20-positive lymphocytes was higher in the case of CR, where the value was between 8–40 elements/×400 (mean 10.2±5.4), compared to AACR for which we found 3–15 elements/×400 (mean 24.5±8.8), aspects that were statistically significant (p=0.001, Student's t-test) (Table 2, Figure 2, A and B).

CD8 immunoexpression was present in the membrane of the subset of cytotoxic T-lymphocytes, the inflammatory elements being present intraepithelially or stromal predominantly perivascular. The average PI CD8 total value was 13.1±5.1, with values between 5–22 elements/×400. The number of CD8-positive lymphocytes was higher in the case of CR compared to AACR, respectively 10–22 elements/×400 (mean 15.8±4.1) compared to 5–12 elements/×400 (mean 8.2±2.4), which was statistically significant (p<0.001, Student's t-test) (Table 2, Figure 2, C and D).

Table 2 – *PI average values and statistical significance depending on inflammation type*

PI value / Inflammation	CD20	CD8	CD138	Eosinophil MBP
CR	24.5±8.8	15.8±4.1	22.9±7	21.8±5.9
AACR	10.2±5.4	8.2±2.4	37.5±11.3	53.1±12.5
p-value (Student's t-test)	0.001	<0.001	<0.001	<0.001

PI: Positivity index; CR: Chronic rhinitis; AACR: Asthma-associated chronic rhinitis; CD: Cluster of differentiation; MBP: Major basic protein.

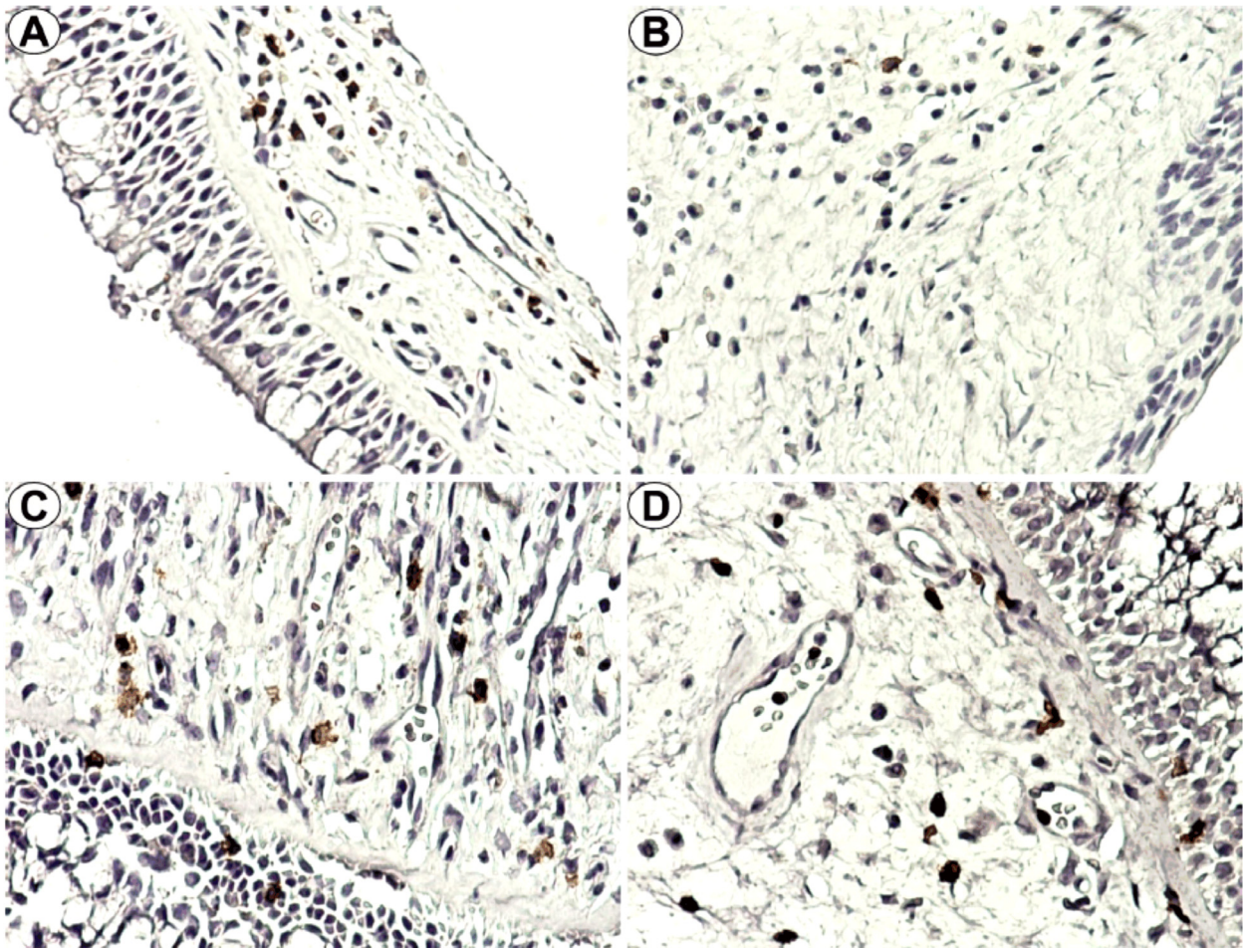

Figure 2 – ***(A and C) CR; (B and D) AACR. Anti-CD20 antibody immunostaining: (A and B) ×200. Anti-CD8 antibody immunostaining: (C and D) ×200. CR: Chronic rhinitis; AACR: Asthma-associated chronic rhinitis; CD: Cluster of differentiation.***

CD138 immunoreactions were identified at the level of the apical cytoplasm and membrane of the plasmocyte elements and of the basal layer of the epithelium. The mean value of PI CD138 for the analyzed group was 28±11, with values between 10–55 elements/×400. The number of plasmocytes was higher in the case of AACR for which 20–45 elements/×400 were observed (mean 37.5±11.3), while for CR the values were between 10–35 elements/×400 (mean 22.9±7). The aspect was statistically significant (p<0.001, Student's t-test) (Table 2, Figure 3, A and B).

Eosinophil MBP immunoexpression was present in the cytoplasm and membrane of eosinophils, both intraepithelially and at the level of the underlying stroma. The mean PI eosinophil MBP value for the whole group was 32.7±17.4, the values being between 15–70 elements/×400. The number of positive eosinophils was higher in the case of AACR compared to CR, respectively 30–70 elements/×400 (mean 53.1±12.5) compared to 15–32 elements/×400

(mean 21.8±5.9), which was statistically significant (p<0.001, Student's t-test) (Table 2, Figure 3, C and D).

Our study did not identify statistically significant associations of PI values for the markers analyzed with age, gender groups or symptomatology of patients.

In this study, for the entire analyzed group, plasmocytes and eosinophils were more frequent compared to CD20 and CD8 lymphocytes. However, CD20- and CD8-positive lymphocytes predominated in CR, whereas plasmocytes and eosinophils were more frequent in AACR. Statistical analysis indicated non-significant positive linear relation between CD20 and CD8 values (p=0.103, Pearson's test) and significant relation between CD138 and eosinophil MBP values (p=0.005, Pearson's test). We also found negative linear relationships between the two groups of markers, statistically significant only for eosinophil MBP values with CD20 (p=0.005, Pearson's test) and CD8 (p=0.004, Pearson's test) (Figure 4).

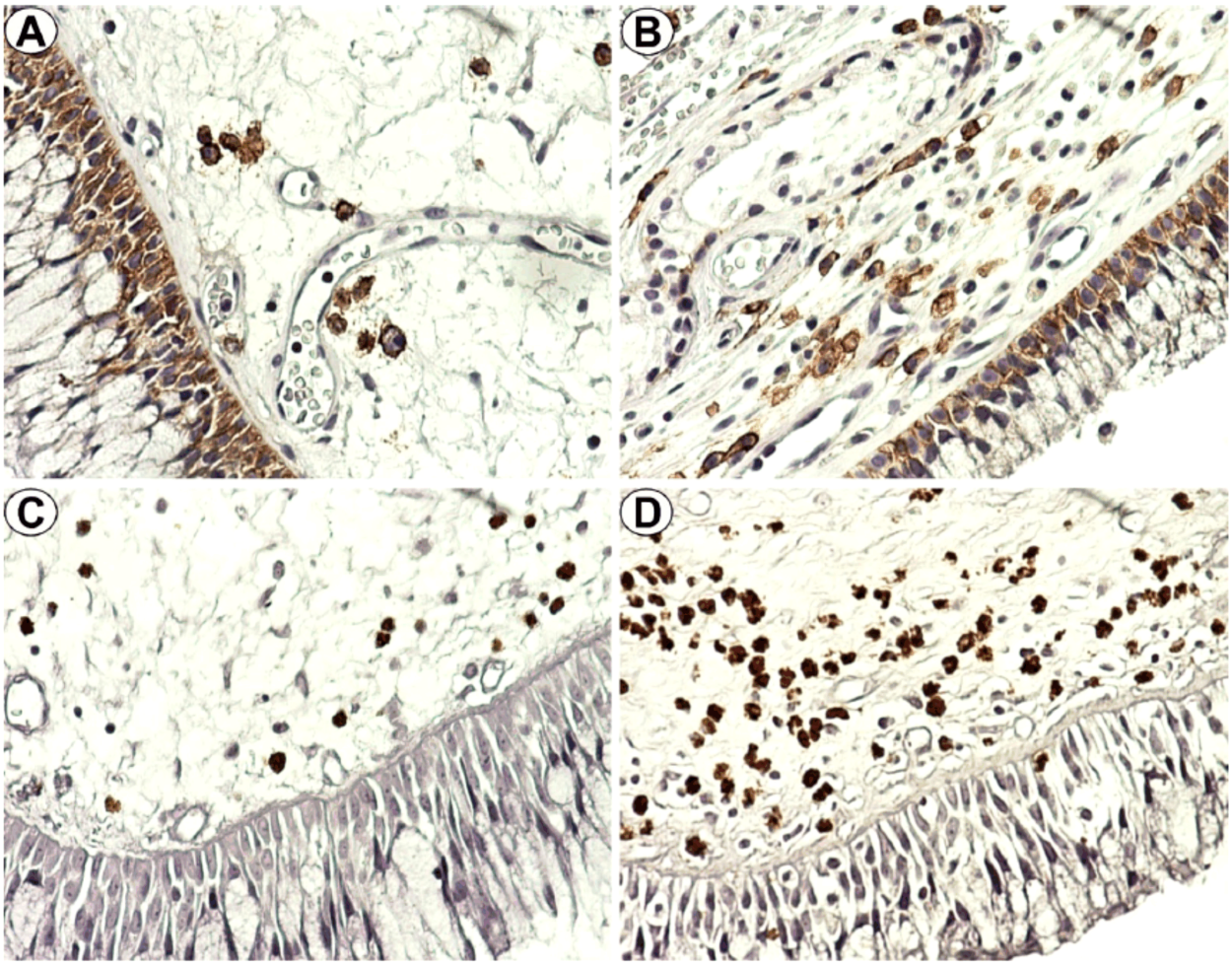

Figure 3 – ***(A and C) CR; (B and D) AACR. Anti-CD138 antibody immunostaining: (A and B) ×200. Anti-eosinophil MBP antibody immunostaining: (C and D) ×200. CR: Chronic rhinitis; AACR: Asthma-associated chronic rhinitis; CD: Cluster of differentiation; MBP: Major basic protein.***

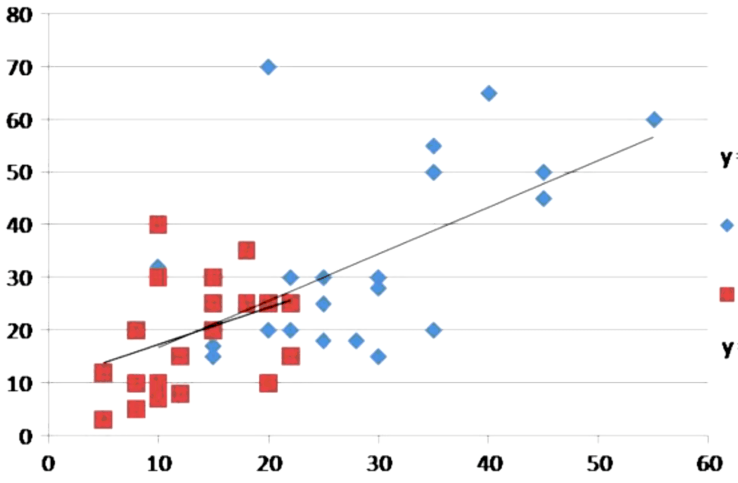

Figure 4 – ***Distribution of PI values for CD20, CD8, CD138 and eosinophil MBP. PI: Positivity index; CD: Cluster of differentiation; MBP: Major basic protein.***

Discussions

Common in children and adolescents, CRs have an increasing incidence and may be associated with asthma [9]. Depending on the region, CR can affect up to 45% of the pediatric population [13]. The etiology is variable and can be allergic, vasomotor, hormonal, infectious, iatrogenic, idiopathic or in complex disorders, such as: Churg–Strauss disease, Kartagener syndrome, diabetes mellitus, mucoviscidosis [12, 14]. Of the CRs, allergic ones are the most frequent and most intensively studied, including in pediatric pathology [3]. In this study, the diagnosis was of CR in 66.7% of cases and AACR in 33.3% of cases.

The prevalence of allergic rhinitis and comorbidities/ complications in the pediatric population is constantly increasing, almost double compared to the 90s [15]. In adolescence, is indicated the higher frequency of allergic rhinitis in males and non-allergic rhinitis females [2]. In our study, the mean age of diagnosis was 16.1±1.7, the cases predominating in males.

Allergic rhinitis is allergen-induced nasal mucosal inflammation and accompanied by immunoglobulin E (IgE)-mediated nasal hypersensitivity symptoms including rhinorrhea, nasopharyngeal secretions, nasal obstruction of varying degrees, nasal pruritus and sneezing [5]. It is considered intermittent or persistent if the symptomatology is less than four days and at least four days per week for one month respectively. Allergic rhinitis can be perennial, seasonal or occupational, being frequently underdiagnosed [5, 7]. In our study, the symptomatology of allergic rhinitis was present in all cases, including the presence of risk factors in some cases (smoking, family history).

The diagnosis of non-allergic rhinitis is one of exclusion, without signs of allergic hypersensitivity, without infection, relatively difficult to identify especially in pediatric pathology [1, 9]. The prevalence of non-allergic rhinitis in different studies ranges between 17–50% [3].

Asthma is an inflammatory condition of the lower airways characterized by reversible obstruction, hyper-reactivity and wheezing episodes, productive coughing and choking sensation [6, 16], aspects that were present in this study in the case of AACR.

Allergic rhinitis and asthma are common chronic conditions in children and adolescents, over 60% of asthma cases presenting inflammation of the nasal mucosa [7, 17]. The role of chronic allergic rhinitis in the severity of asthma in pediatric pathology is controversial and although some studies indicate a clear relationship, others do not support this hypothesis [18].

Allergic rhinitis and asthma have the same pattern of inflammation, and there are numerous studies that support comorbidity and the role of nasal inflammation in the initiation and persistence of asthma symptoms, some authors considering the two entities different faces of so-called chronic allergic respiratory syndrome [6, 19]. Usually, allergic rhinitis precedes asthma or it appears concomitantly, while in preschoolers asthma may be the first, but exist also two-way pathogenic hypotheses between the two entities [10]. A study on children and adolescents indicated that about 20% of patients with asthma also had allergic rhinitis [20]. At the same time, *The Royal College of Paediatrics and Child Health* (RCPCH) *Science and Research Department* believes that the two entities have immunopathogenic similarities and multidisciplinary therapeutic management should be addressed to the upper and lower airways [21].

In our study, the characteristic changes of a chronic nasal inflammation were present. In the case of CR, CD20 B-lymphocytes and CD8 T-lymphocytes predominated, and in the case of AACR, there were more plasmocytes and eosinophils.

From the HP point of view, the CR with allergic component has a duration of more than six weeks and the diagnostic elements after this period are nonspecific, except for the increased number of eosinophils, including on the cytological smears, respectively values between 4–25 elements, which appear in over 60% of allergic rhinitis [12, 22, 23]. However, the rhinocytogram is only indicative for allergic rhinitis, which indicates different inflammatory patterns depending on the allergen [3].

IgE-producing plasmocytes, dendritic cells, macrophages, mast cells, lymphocytes, eosinophils and basophils are the main inflammatory cells present in CR, and the pathogenic allergic mechanism seems to be maintained by molecular pathways of mutual activation through inflammation mediators [6]. There are studies that have indicated massive epithelial and subepithelial infiltration with eosinophils in the case of allergic mechanisms, and that by prolonged tissue remodeling can contribute to mucosal thickening [24].

The CR analyzed did not have aspects specific to a certain type of chronic inflammation being classified as nonspecific CR. The high number of eosinophils and plasmocytes compared to lymphocytes indicates the presence of an allergic component of hypersensitivity not only in the AACR group but also in the CR group. However, data from the literature indicate the existence of so-called nonallergic rhinitis with eosinophils, mast cells or mixed [25].

CD20 is a membrane antigen expressed at the level of the lymphocyte line B being involved in the process of differentiation of the B-lymphocytes into plasmocytes and precursors. Besides the role of antigen presentation, antibody formation after differentiation also has the role of activating and differentiating T-lymphocytes and regulating the immune response [26]. In our study, the expression of CD20-positive elements was higher in CR compared to AACR, which may suggest a semi-activated status of these elements on the B-plasmocyte–IgE release lineage.

CD8 is a transmembrane glycoprotein identified on the surface of cytotoxic T-cells. In contrast to CD4 T-helper (Th) 2 lymphocytes whose role is known in the release of chemical mediators with an effect on the initiation and maintenance of an allergic proinflammatory status, the role of CD8 lymphocytes is less investigated [27]. Proinflammatory, regulatory and protective effects against inflammation are described, an aspect suggested by some studies and with possible therapeutic impact

[27, 28]. In our study, the number of CD8-positive lymphocytes was higher in CR, inversely proportional to the population of eosinophils and plasmocytes, which supports the protective hypothesis.

In this study, the expression of plasmocytes and eosinophils was higher in the case of AACR, inflammatory elements that are described in the literature as being associated with the status and allergic forms [6, 24]. We found the presence of six cases not associated with asthma that had a number of eosinophils and plasmocytes of at least 25/×400 microscopic field. For such cases, the aspect may indicate the HP diagnosis of allergic rhinitis only in the clinical, paraclinical and imaging context.

The presence of CD8 and CD20 lymphocytes in higher numbers in CR and of plasmocytes and eosinophils in the case of asthma can support the hypothesis that in the case of CR the evolution may be towards the consolidation of an allergic nasal field that becomes trigger for the initiation/extension of inflammation at the bronchial level.

The limitations of this experimental study can be considered the size group and inclusion of patients after initiation of treatment that could have an impact on the inflammatory population of the nasal mucosa. However, nasal biopsy is justified and accepted by patients most often after the appearance of mucosal remodeling phenomena, and the persistence of the inflammatory elements identified in this study may suggest the directions of regulation within immunomodulatory therapy.

In the same context, the current classifications of the clinical and HP forms of CR are unclear. Future studies on human tissue at different pediatric ages are needed both for the clear definition of the clinico-pathological entities and the efficiency of the therapy.

Conclusions

The study indicated different inflammatory patterns for CR and AACR. The CD20 and CD8 lymphocyte immunophenotype were associated with CR, while the CD138 and eosinophil MBP immunophenotype were AACR specific. The expression of lymphocytic elements revealed a negative linear relation with the plasmocytes and eosinophils, which may suggest the existence of an evolutionary process of the allergic status initiation. The results obtained can be used to classify patients in order to optimize immunomodulatory therapy.

Conflict of interests

The authors declare that they have no conflict of interests.

Authors' contribution

Rebecca Cristiana Şerban and Elena Loredana Şelaru equally contributed to this article.

References

[1] Erwin EA, Faust RA, Platts-Mills TA, Borish L. Epidemiological analysis of chronic rhinitis in pediatric patients. Am J Rhinol Allergy, 2011, 25(5):327–332.

[2] Roberts G, Xatzipsalti M, Borrego LM, Custovic A, Halken S, Hellings PW, Papadopoulos NG, Rotiroti G, Scadding G, Timmermans F, Valovirta E. Paediatric rhinitis: position paper of the European Academy of Allergy and Clinical Immunology. Allergy, 2013, 68(9):1102–1116.

[3] Poddighe D, Gelardi M, Licari A, Del Giudice MM, Marseglia GL. Non-allergic rhinitis in children: epidemiological aspects, pathological features, diagnostic methodology and clinical management. World J Methodol, 2016, 6(4):200–213.

[4] Chiang WC, Chen YM, Tan HK, Balakrishnan A, Liew WK, Lim HH, Goh SH, Loh WY, Wong P, Teoh OH, Goh A, Chay OM. Allergic rhinitis and non-allergic rhinitis in children in the tropics: prevalence and risk associations. Pediatr Pulmonol, 2012, 47(10):1026–1033.

[5] Brozek JL, Bousquet J, Baena-Cagnani CE, Bonini S, Canonica GW, Casale TB, van Wijk RG, Ohta K, Zuberbier T, Schünemann HJ; Global Allergy and Asthma European Network; Grading of Recommendations Assessment, Development and Evaluation Working Group. Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines: 2010 revision. J Allergy Clin Immunol, 2010, 126(3):466–476.

[6] Jeffery PK, Haahtela T. Allergic rhinitis and asthma: inflammation in a one-airway condition. BMC Pulm Med, 2006, 6(Suppl 1):S5.

[7] de Groot EP, Nijkamp A, Duiverman EJ, Brand PL. Allergic rhinitis is associated with poor asthma control in children with asthma. Thorax, 2012, 67(7):582–587.

[8] Bonay M, Neukirch C, Grandsaigne M, Leçon-Malas V, Ravaud P, Dehoux M, Aubier M. Changes in airway inflammation following nasal allergic challenge in patients with seasonal rhinitis. Allergy, 2006, 61(1):111–118.

[9] Chawes BL, Bønnelykke K, Kreiner-Møller E, Bisgaard H. Children with allergic and nonallergic rhinitis have a similar risk of asthma. J Allergy Clin Immunol, 2010, 126(3):567–573.e1–573.e8.

[10] Tsilochristou OA, Douladiris N, Makris M, Papadopoulos NG. Pediatric allergic rhinitis and asthma: can the march be halted? Paediatr Drugs, 2013, 15(6):431–440.

[11] Calderon MA, Gerth van Wijk R, Eichler I, Matricardi PM, Varga EM, Kopp MV, Eng P, Niggemann B, Nieto A, Valovirta E, Eigenmann PA, Pajno G, Bufe A, Halken S, Beyer K, Wahn U; European Academy of Allergy and Clinical Immunology. Perspectives on allergen-specific immunotherapy in childhood: an EAACI position statement. Pediatr Allergy Immunol, 2012, 23(4):300–306.

[12] Brandwein-Gensler M. Head and neck. Series: "Cambridge Illustrated Surgical Pathology", Cambridge University Press, 2010.

[13] Aït-Khaled N, Pearce N, Anderson HR, Ellwood P, Montefort S, Shah J; ISAAC Phase Three Study Group. Global map of the prevalence of symptoms of rhinoconjunctivitis in children: The International Study of Asthma and Allergies in Childhood (ISAAC) Phase Three. Allergy, 2009, 64(1):123–148.

[14] Settipane RA, Kaliner MA. Chapter 14: Nonallergic rhinitis. Am J Rhinol Allergy, 2013, 27(Suppl 1):S48–S51.

[15] Gentile D, Bartholow A, Valovirta E, Scadding G, Skoner D. Current and future directions in pediatric allergic rhinitis. J Allergy Clin Immunol Pract, 2013, 1(3):214–226; quiz 227.

[16] Bousquet J, Jeffery PK, Busse WW, Johnson M, Vignola AM. Asthma. From bronchoconstriction to airways inflammation and remodeling. Am J Respir Crit Care Med, 2000, 161(5): 1720–1745.

[17] Masuda S, Fujisawa T, Katsumata H, Atsuta J, Iguchi K. High prevalence and young onset of allergic rhinitis in children with bronchial asthma. Pediatr Allergy Immunol, 2008, 19(6):517–522.

[18] Hamouda S, Karila C, Connault T, Scheinmann P, de Blic J. Allergic rhinitis in children with asthma: a questionnaire-based study. Clin Exp Allergy, 2008, 38(5):761–766.

[19] Togias A. Rhinitis and asthma: evidence for respiratory system integration. J Allergy Clin Immunol, 2003, 111(6):1171–1183; quiz 1184.

[20] Thomas M, Kocevar VS, Zhang Q, Yin DD, Price D. Asthma-related health care resource use among asthmatic children with and without concomitant allergic rhinitis. Pediatrics, 2005, 115(1):129–234.

[21] Vance G, Lloyd K, Scadding G, Walker S, Jewkes F, Williams L, Dixon L, O'Beirne C, Fletcher P, Brown T, Hughes J, Sohi D, Piddock C, Shields M, McKean M, Warner J; Science and Research Department, Royal College of Paediatrics and Child Health. The 'unified airway': the RCPCH care pathway for children with asthma and/or rhinitis. Arch Dis Child, 2011, 96(Suppl 2):*i*10–*i*14.

[22] Chinnakkannan SK, Singh M, Das RR, Mathew JL, Saxena AK. Association of allergic rhinitis and sinusitis with childhood asthma. Indian Pediatr, 2017, 54(1):21–24.
[23] Kaur J, Chugh K, Sachdeva A, Satyanarayana L. Under diagnosis of asthma in school children and its related factors. Indian Pediatr, 2007, 44(6):425–428.
[24] Howarth PH, Salagean M, Dokic D. Allergic rhinitis: not purely a histamine-related disease. Allergy, 2000, 55(Suppl 64):7–16.
[25] Gelardi M, Maselli del Giudice A, Fiorella ML, Fiorella R, Russo C, Soleti P, Di Gioacchino M, Ciprandi G. Non-allergic rhinitis with eosinophils and mast cells constitutes a new severe nasal disorder. Int J Immunopathol Pharmacol, 2008, 21(2):325–331.
[26] Sarvaria A, Madrigal JA, Saudemont A. B cell regulation in cancer and anti-tumor immunity. Cell Mol Immunol, 2017, 14(8):662–674.
[27] Lourenço O, Fonseca AM, Taborda-Barata L. Human CD8+ T cells in asthma: possible pathways and roles for NK-like subtypes. Front Immunol, 2016, 7:638.
[28] Baraldo S, Turato G, Cosio MG, Saetta M. Which CD8+ T-cells in asthma? Attacking or defending? Eur Respir J, 2016, 48(2):287–290.

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