Ph.D Thesis

Summary

NEW CLINICAL, HISTOLOGICAL, IMMUNOHISTOCHEMICAL AND THERAPEUTIC INSIGHTS IN RHINOSINUSAL ALLERGY-INFECTED DISEASE

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Table of contents

1. Introduction  pg 3

2. Current state of knowledge  pg 3
   2.1. Anatomical generalities  pg 3
   2.2. Histological generalities  pg 3
   2.3. Epidemiology of nasal polyposis  pg 3
   2.4. Etiopathogenesis of nasal polyposis  pg 4
   2.5. Clinical and paraclinical diagnosis in allergy-infected nasal polyposis  pg 4
   2.6. Treatment of nasal polyposis  pg 5

3. Personal contribution  pg 5
   3.1. Working hypothesis and general aims  pg 5
   3.2. Research methodology  pg 6
      3.2.1. Theme approach  pg 6
      3.2.2. Material and method  pg 6
      3.2.3. Clinico-epidemiological study results  pg 6
      3.2.4. HP study results  pg 7
      3.2.5. IHC study results  pg 8

4. Discussions  pg 8

5. Conclusions  pg 9

References  pg 11

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1. Introduction

Nasal polyposis and chronic allergy-infected polypoid rhinosinusitis are the most common pathologies that the otolaryngologist faces daily. The motivation of the paper is that, although polypous disease had relatively stereotypical symptoms, over time there have been a number of associations of this pathology with various other entities: allergic rhinitis, bronchial asthma, chronic rhinosinusitis.

2. Current state of knowledge

2.1. Anatomical generalities

Paranasal sinuses are true "pneumatics" pairs in the bones of the same name, with variable shapes and sizes and are characterized by the fact that they can be present / absent, symmetrical / asymmetrical, hypo / hyperplastic. The lateral wall of the nostrils consists of the lateral nasal wall and the inferior nasal turbinates. In addition to the bony skeleton of the lateral region of the nasal fossae enter the medial pterygoid plaques. The posterior part is represented by the choanal region. The nasal vault is made up of the ethmoidal region, more precisely the ethmoidal roof and the cribriform blade.

2.2. Histological generalities

The nasal fossae are histologically characterized by the presence of respiratory mucosa. Its peculiarity consists in the fact that the vascularization is very well functionally differentiated. The anterior opening of the nasal fossae is made through the nostrils and later the communication with the nasopharynx is made through cones forming a true air duct. The respiratory mucosa has a diverse cellular and vascular-nervous equipment (1).

2.3. Epidemiology of nasal polyposis

Nasal polyposis occurs in approximately 0.6% of adults, but increases up to 15% in patients with bronchial asthma. Polyps are more common in nonasthmatic, allergic background male patients, while in asthmatic patients there is no difference in prevalence between men and women. Nasal polyposis
can cause serious complications if left untreated (2). Nasal polyposis is found in about 1 - 4% of the population and the incidence increases with age and in the presence of asthma. 6% of asthmatic adults have nasal polyposis. Men tend to be more affected by the disease than women.

2.4. Etiopathogenesis of nasal polyposis

The presence of polyps is motivated by inflammation of the mucosa, so there is a substrate of chronic rhinosinusitis. There are several theories: allergic, infectious, inflammatory, vasomotor, genetic. The mechanism by which polyps are formed starts from these theories, which means that several intricate etiological factors are present. It all starts from an aggression on the mucosa regardless of its nature, which will lead to the formation of edema and simultaneously with this stage there is an increase in the number of fibroblasts resulting in edematous hyperplasia.

2.5. Clinical and paraclinical diagnosis in allergy-infected nasal polyposis

The clinical background is dominated in order of frequency by nasal obstruction, hyposmia / anosmia associated or not with loss of taste, watery rhinorrhea in those with allergic background or mucopurulent in the chronic forms associated with bacterial infections, nasal pruritus, eye pruritus, fatigue, orbital pains, headache, epiphora, eye congestion, expiratory dyspnea, wheezing, chest constriction, cough. The feeling of shortness of breath, anxiety, facial pain, nasal sinuses or aural pressure are not exceptional either. Stating the clinical diagnosis is based on detailed anamnesis, quantification of symptoms, inspection, palpation, anterior and posterior rhinoscopy. Paraclinical diagnosis is based on a detailed hematological assessment, rigid and more modern flexible endoscopic exploration methods, CT and MRI imaging examinations that assess the real extent of the disease, supplemented by allergological and respiratory functional tests. Allergological and pneumological consultations and their conclusions are very useful.
2.6. Treatment of nasal polyposis

Medical treatment uses oral corticosteroids, topical steroids in the form of sprays and washes, oral antibiotics in cases complicated with infection, immunotherapy, leukotriene modifiers, antihistamines, monoclonal antibodies, nasal decongestants, nasal lavage with saline solutions. Surgical treatment supports medical treatment and aims to completely remove polyp formations, decommissioning all paranasal sinuses from which they originate, to prevent recurrences, reduce local inflammation, promote ventilation and facilitate correct application of topical drug treatments. At present, polypectomy associated with ethmoidectomy under endoscopic control is preferred.

3. Personal contribution

3.1. Working hypothesis and general aims

During the doctoral study, I researched about nasal polyposis in detail, identifying in a large number of cases the simultaneous existence of an allergic component, or an infectious one. The topic of interest on which the thesis itself was built, is the clarification of the interrelationship between allergic and infectious phenomena associated with nasal polyposis. For the analysis of clinical, paraclinical, endoscopic, imaging and therapeutic parameters I used classical and modern means leading to valuable results and conclusions and treating this subject in detail in all its complexity. All the results obtained were subjected to a thorough statistical analysis, which led me to state the essential conclusions of the multiplanar study.

Study aims were:

1. Establishing a causal relationship between nasal polyposis and the most common allergic and infectious pathological entities associated: asthma, allergic rhinitis, rhinosinusitis; 2. Case study from an epidemiological, clinical and paraclinical point of view; 3. Histopathological findings of polypous formations with the calculation of scores given to the investigated parameters; 4.
Immunohistochemistry evaluation in order to identify molecular targets for the application of personalized therapies.

3.2. Research methodology

3.2.1. Theme approach

The clinical study was performed after the informed consent of a number of 373 patients who presented as main clinical diagnosis – nasal polyposis from the total number of patients admitted to the Otorhinolaryngology Clinic (ENT) of the Craiova County Emergency Clinical Hospital over a period of 5 years, more precisely 1st January 2015 – 31st December 2019. After diagnosis, patients underwent a combined medical and surgical treatment, some of the tissue specimens obtained during surgery being subjected to HP (150 cases out of a total of 373) and IHC (50 cases out of a total of 150 cases analyzed by HP) exams.

3.2.2. Material and method

The clinical-epidemiological, paraclinical, imaging and therapeutic study aimed to establish simultaneous and overlapping relationships of nasal polyposis with other pathologies (asthma, allergic rhinitis, chronic allergin-infected rhinosinusitis), epidemiological, imaging and therapeutic characteristics objectified by using and calculating clinical (VAS, SNOT 22), endoscopic (Lund-Kennedy, Lildholdt) and imaging (Lund-Mackey) scales and scores.

The HP study involved a detailed analysis of some histological features of classic stained resected fragments - hematoxylin-eosin, obtaining specific scores and comparing my own data with those published in the literature.

The IHC study involved the use of specific antibodies that targeted and highlighted the presence of certain inflammatory cells in different histological areas of resected fragments (polyp formations).

3.2.3. Clinico-epidemiological study results

The clinical-epidemiological study found an incidence of 2.83% in the total number of patients hospitalized in the ENT Clinic of SCJU Craiova during Jan. 1st, 2015 – 31st Dec. 2019, of which 61.4% men (229 patients) and 38.6% women (144 patients), 54.95% (205 cases) from urban areas, 72 cases identified in 2015,
72 in 2016, 68 in 2017, 81 in 2018 and 80 cases in 2019. This pathology most frequently affected subjects belonging to the decades 40-49 years (23.05% - 86 cases), 60-69 years (22.52% - 84 cases) and 50-59 years (19.83% - 74 cases). The main associated comorbidities were asthma (106 cases - 28.41%), chronic allergy-infected rhinoinusitis (98 cases - 26.27%) and allergic rhinitis (87 cases - 23.32%) and there were also some overlaps. Depending on the symptoms, nasal polyposis was dominated in order of frequency by nasal obstruction, followed by hypo/anosmia, nasal voice and oral respiration.

The average VAS score was 6.2 for point b) and 37.4 for c). Applying the preoperative SNOT 22 score resulted in an average score of 51.42 and postoperatively 4.82 at one month, 4.37 at 3 months and 3.56 at 6 months.

The Lildholdt grading system quantified by endoscopy identified grade 1 polyps in 17.42% (65 cases), grade 2 - 23.86% (89 cases) and grade 3 - 58.71% (219 cases).

The preoperative Lund-Kennedy score ranged from 3 to 14, most commonly with 8 to 69 cases (18.49%). Postoperatively it varied between 0 and 6, the most common being 2 - 99 cases (26.54%).

The Lund-Mackey score obtained by tomographic evaluation was the most common 7 on the right and 8 on the left, in a total of 98 cases analyzed.

The standard treatment applied consisted of polypectomy associated with endoscopically assisted ethmoidectomy, supplemented with intraoperative antibiotic followed postoperatively with cortisone and antihistamine anti-inflammatory treatment.

3.2.4. HP study results

The present study included a number of 150 allergic nasal polyps, for which we aimed to evaluate HP parameters that were given scores, both for the epithelial and stromal components, which were the basis for calculating the composite histological score (CHS). Changes in the epithelial component were in order of frequency: goblet cell hyperplasia in 121 cases (80.6%), basement membrane thickening in 118 cases (78.6%), basal layer hyperplasia in 87 cases (58%),
epithelial alteration in 44 cases (29.3%), eosinophilic infiltration in 16 cases (10.6%) and squamous metaplasia in 15 cases (10%). Changes in the stromal component were, in order of frequency: infiltration with eosinophils in all 150 cases (100%), edema in 132 cases (88%), infiltration with plasma cells in 89 cases (59.3%), infiltration with lymphocytes in 74 cases (49.3%) and macrophage infiltration in 11 cases (7.3%). All these changes laid the basis for calculating the CHS which had high values in 41 cases (27.4%) and low in 109 cases (72.6%).

3.2.5. IHC study results

From the IHC point of view, we analyzed a number of 50 synonasal allergic polyps, of which 12 cases with high CHS and 38 cases with low CHS. BMK-13 immunoassay analysis indicated cytoplasmic positivity at the level of stromal eosinophilic infiltrate in all cases analyzed (100%), while at the epithelial level the immunolabeling was identified in only 16 cases (32%). Analysis of CLA immunoexpression (CD45R0) in the 50 cases analyzed indicated cytoplasmic positivity at the epithelial level in 14 of them (28%), while the label was identified at the stromal level in 45 cases (90%). Analysis of CD4 immunoexpression indicated cytoplasmic positivity in 26 of the analyzed cases (52%), and for CD8 in 45 of them (90%). Analysis of CD20 immunoexpression indicated cytoplasmic positivity in a small number of cases, respectively 15 of the analyzed cases (30%), both in the epithelial cell infiltrate (7 cases) and stromal (15 cases). Analysis of CD138 immunoexpression indicated cytoplasmic positivity in 41 of the analyzed cases (82%), only at the stromal level. Analysis of CD68 immunoexpression indicated cytoplasmic positivity at the epithelial level in 9 cases (18%) and at the stromal level in 22 cases (44%).

4. Discussions

The incidence of nasal polyposis was 2.8% in my study, a value consistent with the 1-4% recorded in the total American population (3). The patients in my study were 61.39% male, similar to the 60.3% result of a 2005 study (4). As in another study (5), we found that asthma is the most common comorbidity
associated with nasal polyposis, in 28.41%. It was demonstrated in my study, as well as in literature, that a high Lund-Mackey CT (LMCTS) score correlates with a high preoperative SNOT 22 score and very good postoperative results, with significant improvement in quality of life (QOL) (6), is a significant decrease in postoperative symptoms.

In the present study we found quite frequently aspects of hyperplasia of the basal layer, sometimes over large areas (58%), but only rarely did we identify aspects of squamous metaplasia (10%) and only focal type. In no case did I notice any dysplastic changes. Goblet cell hyperplasia is next to squamous metaplasia among the most common epithelial changes in edematous nasal polyps (7). In the study, we found the alteration of the epithelium in 44 cases (29.3%), with an aspect that varied from superficial and partial denudation of the lining epithelium to its complete denudation. Stromal edema is quite commonly associated with varying degrees of severity. Inflammatory infiltrate is different in allergic and non-allergic polyposis. Polyp stroma regardless of subtype generally contains mixed inflammatory cells, but the most common histopathologic form of nasal polyposis is the eosinophilic type (90%), compared to the non-eosinophilic form, in which the dominant stromal cells are lymphocytes and plasma cells (8).

The immune cells infiltration identified in nasal polyps is mixed, and includes almost constantly eosinophils, which represent over 10% of inflammatory cells of PN (9). In the study we observed cytoplasmic immunoexpression BMK-13 at the stromal level in all analyzed cases (100%) and epithelial in only 32% of analyzed cases, being identified statistically significant associations of increased BMK-13 scores with high CHS values. Lymphocytes are a major component of cellular infiltrate in PN.

5. Conclusions

1. Nasal polyposis is becoming more frequent, with a real social and economical impact by limiting the work capacity of patients and by the days of
leave necessary for postoperative recovery, in some cases repeatedly due to the recurrent nature of this pathology.

2. Although the scores of Lund Kennedy, Lund Mackay, Lildholdt grading system are validated in international literature, none of them provides the possibility to predict the evolution of polyp disease or the frequency of recurrences, being useful only in the primary diagnosis and in follow-up of cases from a subjective and objective point of view.

3. Stromal infiltration with eosinophils, present in all studied cases, certifies the hypothesis that nasal polyposis development requires as a mandatory condition the presence of allergic background.

4. The composite histological score allowed the division of the group of 150 cases into two categories, one with low CHS present in 109 cases (72.6%) and one with high CHS - 41 cases (27.4%).

5. The most common inflammatory elements in the epithelial compartment identified were BMK-13+ eosinophils, followed by CD68+ macrophages, CLA+ elements, CD8+ lymphocytes and CD20+ lymphocytes. CD4+ lymphocytes and CD138+ plasma cells were not identified in the epithelial compartment.

6. The presence of all the inflammatory elements analyzed was highlighted in the stromal compartment, the most numerous being the eosinophils BMK-13+, followed in order of frequency by CLA+ elements, CD138+ plasma cells, CD8+ lymphocytes, CD4+ lymphocytes, CD20+ lymphocytes and CD68+ macrophages.

7. The results obtained in this study can be used to characterize the pathogenic mechanisms involved in the initiation, progression and persistence of allergic nasal polyps, as well as to improve the criteria for streamlining the treatment of these lesions.
References


