Expression pattern of CK7 and CK20 in nasal polyps, at patients with chronic rhinosinusitis with nasal polyposis

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Abstract
We investigated the expression of cytokeratins 7 (CK7) and 20 (CK20) in nasal polyps on a group of 106 patients with chronic rhinosinusitis with nasal polyposis (45 women – 42% and 61 men – 58%) who benefited from surgical procedures. Harvested biological material was analyzed in the pathology laboratory through two methods: histopathological and immunohistochemical analysis. Classical histopathological method of processing the tissues initially fixed in 10% formalin was used. The tissues were then processed by paraffin impregnation, sectioned and stained with Hematoxylin–Eosin. The immunohistochemical method was based on soluble immunoenzymatic complexes – LSAB/HRP (labeled Streptavidin Biotin) method. We used DAKO LSAB 2 System HRP (Universal DAKO Labeled Streptavidin Biotin 2 System Horseradish Peroxidase). The expressions of CK7 and CK20 in nasal polyps were analyzed.

Keywords: cytokeratins, chronic rhinosinusitis, nasal polyposis, immunohistochemical study.

Introduction
According to the National Institute of Allergy and Infectious Diseases Fact Sheet, chronic rhinosinusitis (CRS) is the most commonly reported chronic disease, affecting 16.3% of the total population (nearly 32 million) in the United States, in 1997 [1]. By screening a non-ENT population, which may be considered representative of the general population in Belgium, 6% of subjects suffered from chronic nasal discharge [2].

In a population-based study in Sweden [3], a prevalence of 2.7% was reported. Based on a postal questionnaire survey in Finland, the prevalence of nasal polyps (NP) was 4% [4]. Using a disease-specific questionnaire, the prevalence of NP was reported in 2.1% of the French population [5].

In general, nasal polyps occur in all races in adults and become more common with age [5–7]. Nasal polyps are uncommon in children except in cystic fibrosis (CF) [8].

Diagnosis of CRS currently is based on clinical signs, nasal endoscopy and CT-scan. Therapy is focusing on corticosteroids and antibiotics. However, none of these drugs is able to change the fate of the patient, and to avoid surgery or at least maintain the post-surgical result.

A better understanding of the pathogenesis and the factors amplifying mucosal inflammation is therefore crucial for the development of new diagnosis and therapeutic tools, which clearly are needed.

Materials and Methods
We studied fragments of nasal polyps removed from patients with chronic rhinosinusitis with nasal polyposis who underwent endoscopic surgeries – polypectomy and anterior ethmoidectomy; 106 patients were studied (45 women – 42% and 61 men – 58%), aged average 52.±14.6-year-old. The patients were hospitalized in ENT Clinic of Emergency County Hospital, Craiova, for 12 months (October 2007–October 2008).

The patients were included in this study according to the following criteria:

• A symptomatology longer than three months: permanent nasal obstruction, mucopurulent nasal discharge, hypo- or anosmia, headaches with exacerbations secondary to bacterial infections.

• Different modifications at the level of middle meatus: polyploid edema of middle meatus or polyps, observed at endoscopic examination of nasal mucosa.

• CT-scan showed the existence of chronic inflammatory modifications (hyperplasic mucosa) at the level of paranasal sinuses.

Surgical interventions were performed under general anesthesia with orotracheal intubation. The harvested biologic material was analyzed in pathology laboratory. Histopathological and immunohistochemical analyses were used. We used classical histopathological method to process the tissues initially fixed in 10% buffered formalin. Subsequently, the specimens were paraffin embedded, sectioned and Hematoxylin–Eosin stained.
The immunohistochemical method was based on soluble immunoenzymatic complexes – LSAB/HRP (Labeled Streptavidin Biotin) method. We used DAKO LSAB 2 System HRP kit (Universal DAKO Labeled Streptavidin Biotin 2 System Horse radish Peroxidase).

LSAB method (with Streptavidin Biotin) is one of the ABC methods (Avidin–Biotin complex), in which Avidin substitutes Streptavidin and is based on direct conjugate of Streptavidin with enzymatic molecules. We cut series sections of 3–4 µm thickness from paraffin blocks, placed them on lamellas treated with poly-L-Lysine and dried them at lab temperature for 12 hours.

LSAB (HRP) procedure involves the following steps:
- Deparaffinize in three xylene baths, the first one at thermostat at 58°C for an hour, the next two at room temperature, for 10 minutes each.
- Rehydrate with alcohol in four baths of decreasing concentrations (the first two of 100% alcohol, followed by one of 95% alcohol and, respectively, of 75%).
- Inhibition of endogenous peroxidase by incubation in distilled water with 3% hydrogen peroxide, for 5 minutes.
- Washing in distilled water.
- Pre-treatment in order to reveal the antigen (at microwaves – MW, through enzymatic digestion or through a mixed method – microwaves – MW, through enzymatic digestion or through a mixed method – microwaves with enzymatic digestion), depending on the basic antibody used.
- Washing in clean water.
- Rinsing in PBS lavage (PBS– Tween).
- Incubation with basic antibody (respectively, negative control) in optimum dilution, variable time depending on the type of basic antibody, at room temperature (TA) in wet room or at optimum thermostat temperature.
- Washing in PBS lavage.
- Incubation with secondary biotinylated antibody (serum) species specific for basic antibody, for 10 minutes, at room temperature in a wet room.
- Washing in clean water.
- Incubation in Streptavidin peroxidase for 10 minutes, at room temperature, in a wet room.
- Washing in clean water.
- Chromogen developing (DAB, 3,3’-diaminobenzidine) in a dark room, for 5–10 minutes.
- Counter-stain with Meyer’s Hematoxylin for 15–30 seconds.
- Dehydrating with alcohol of increasing concentrations, clarifying with xylene, setting with Canada balsam.

Immunohistochemical stain was evaluated by a four-grade system, according to Wauters CC et al. (1995) model [9], as follows (Table 1): negative (-); weak intensity (+); moderate intensity (++); strong immunolabeling (+++).

<table>
<thead>
<tr>
<th>Intensity of antibodies expression</th>
<th>Absent</th>
<th>Weak</th>
<th>Moderate</th>
<th>Strong</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grading</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>+++</td>
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The areas on the specimens showing folds in the sections, necrosis or hemorrhagic infiltrate were not interpreted.

Expression pattern of cytokeratins was appreciated as diffuse when over 50% of the studied cells were marked and, respectively, focal when under 50% of the cells were marked.

Results

We made a statistic analysis of the 106 patients:
- The group of patients was made up of 45 women (42%) and 61 men (58%); 45 patients were from rural environment (42%) and 61 from urban environment (58%) (Table 2).
- The average of the group was of 52.5±14.6 years, on gender were of 53±13.1 years for women and of 52.1±15.7 for men. According to origin environment (Table 3), the average of the patients from rural environment was of 52.3±14.7 years, and that of the patients from urban environment of 52.6±14.7 years. From the point of view of age, we noticed a homogeneity of the patients group both as a mean and variation.

Table 2 – Distribution of patients depending on origin environment and sex

<table>
<thead>
<tr>
<th>Origin environment</th>
<th>Female</th>
<th>Male</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rural</td>
<td>18</td>
<td>27</td>
<td>45</td>
</tr>
<tr>
<td>Urban</td>
<td>27</td>
<td>34</td>
<td>61</td>
</tr>
<tr>
<td>Total</td>
<td>45</td>
<td>61</td>
<td>106</td>
</tr>
</tbody>
</table>

Table 3 – Calculation of mean age and standard deviation

<table>
<thead>
<tr>
<th></th>
<th>Female</th>
<th>Rural</th>
<th>Urban</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age [years]</td>
<td>53.0</td>
<td>52.1</td>
<td>52.3</td>
<td>52.6</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>13.1</td>
<td>15.7</td>
<td>14.7</td>
<td>14.6</td>
</tr>
</tbody>
</table>

From histopathological examination point of view, nasal polyps were extremely varied on usual stain. Therefore, the 106 polyps included in the study were classified as follows:
- allergic polyps, with the presence of an eosinophilic infiltrate: 59 cases (55.6%);
- fibro-inflammatory polyps: 39 cases (36.8%);
- polyps with marked hyperplasia of sero-mucous glands: eight cases (7.6%).

Analysis of expression of CK7

Allergic polyps expressed CK7 strongly, both at the level of respiratory type epithelium and sero-mucous stromal glands (Figures 1–3). The labeling was cytoplasmatic, expressed by intermediate and superficial cells of pseudostratified epithelium of respiratory type and glandular luminal cells. Cells of basal type did not expressed CK7. Distribution of CK7 immunolabeling was diffuse, both in respiratory and glandular epithelia.

CK7 expression was noticed at the level of squamous metaplasia areas of respiratory epithelium as well. This time the immunolabeling intensity was moderate, especially in intermediate and superficial layers (Figure 4). Similar to the case of respiratory or glandular epithelia, basal cells were not marked.

Fibro-inflammatory polyps presented a diffuse labeling of moderate intensity of respiratory and glandular epithelia (Figure 5–10). The labeling was noticed at the level of the same cell types, similar to the case of allergic polyps, but at a lower intensity.
In polyps with hyperplasia of sero-mucous glands, CK7 expression was intense at the level of serous acini and moderate at the level of mucous acini (Figure 11).

**Analysis of CK20 immunolabeling**

**Allergic polyps** were negative for CK20, both at the level of respiratory pseudostratified epithelium (Figure 12) and metaplastic squamous epithelium (Figure 13). Instead, we noticed a cytoplasmic labeling of a low, focal intensity at the level of glandular epithelium (Figures 14 and 15).

**Fibroinflammatory polyps** expressed a focal labeling of low and moderate intensity for CK20 at the level of glandular epithelium. Respiratory epithelium and the one that suggested metaplasia of transitory type were negative for this marker.

In polyps with hyperplasia of sero-mucous glands, CK20 marked only the glandular epithelium, similarly to allergic polyps (Figures 16–18).

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**Figure 1** – Allergic polyp, expression of cytokeratin 7 (CK7), ×40.

**Figure 2** – Allergic polyp, expression of CK7: glandular epithelium, ×100.

**Figure 3** – Allergic polyp, expression of CK7: respiratory epithelium, ×200.

**Figure 4** – Allergic polyp, expression of CK7: metaplastic squamous epithelium, ×200.

**Figure 5** – Fibro-inflammatory polyp, expression of CK7: respiratory epithelium, ×40.

**Figure 6** – Fibro-inflammatory polyp, expression of CK7: glandular epithelium, ×40.
Figure 7 – Fibro-inflammatory polyp, expression of CK7: glandular epithelium, ×100.

Figure 8 – Fibro-inflammatory polyp, expression of CK7: respiratory and glandular epithelium, ×100.

Figure 9 – Fibro-inflammatory polyp, expression of CK7: metaplasia, possibly of urothelial type of respiratory epithelium, ×200.

Figure 10 – Fibro-inflammatory polyp, expression of CK7: metaplasia of urothelial type of respiratory epithelium, ×400.

Figure 11 – Polyp with hyperplasia of sero-mucous glands, expression of CK7, ×100.

Figure 12 – Allergic polyp, negative for CK20: respiratory epithelium, ×200.
Figure 13 – Allergic polyp, negative for CK20: squamous epithelium, ×40.

Figure 14 – Allergic polyp, weak, focal CK20 expression: glandular epithelium, ×100.

Figure 15 – Allergic polyp, weak CK20 expression: glandular epithelium, ×200.

Figure 16 – Fibro-inflammatory polyp, weak CK20 expression: glandular epithelium, ×100.

Figure 17 – Fibro-inflammatory polyp, moderate CK20 expression: glandular epithelium, ×100.

Figure 18 – Fibro-inflammatory polyp, weak CK20 expression: glandular epithelium, ×200.
Discussion

Clinical pattern of symptoms and signs are overlapping in patients with CRS with nasal polyps (CRSwNP) or without (CRSsNP). As a result, all chronic sinus disease is considered as one disease spectrum, CRS, which severely obstructs the development of pathophysiological knowledge and new therapeutic approaches. Innovative biological approaches to overcome this problem are urgently needed.

In a milestone investigation, inflammatory cells and mediators of the sinonasal mucosal tissue from CRSwNP, CRSsNP and control patients were investigated [10]. This study clearly demonstrated that all groups of CRS had characteristic differences in those markers, which allowed for a pathophysiologically meaningful differentiation with likely therapeutic consequences.

Nasal polyps had significantly higher levels of eosinophil markers when compared with CRSwNP and controls. Most importantly, CRSsNP was characterized by a Th1 polarization with high levels of interferon-gamma (IFN-γ) and transforming growth factor-beta (TGF-β), while CRSwNP showed a Th2 polarization with high IL-5 and immunoglobulin (Ig) E concentration [11].

Cytokeratins represent the epithelial class of intermediate-sized filaments of the cytoskeleton when they are polymerized. Cytokeratins are correlated with the pathway of differentiation of cells and tissues [12, 13]. From specific cytokeratins expression, conclusions can be drawn about the origin, morphologic characteristics, and function of epithelium [12, 13]. For example, CK7, CK8, CK18, and CK19 are typical for columnar epithelium such as the respiratory epithelium of the bronchial tree. CK1, CK2, CK10, CK11, and CK13 are typically expressed in squamous epithelia, for instance, in the oral mucosa or in the epidermis. Respiratory epithelium has been found negative for CK20.

Specialty studies show that CK 7, 8 and 18 are found in over-basal cells of respiratory epithelium in nasal polyps while basal cells express CK5 and CK14 [14]. CK19 is positive in all the layers of covering epithelium and CK7 is positive in calciform and overbasal cells of pseudostratified epithelium (Zhuo M et al., 2002). The same authors demonstrated the focal presence of CK 7 and 19 in transitional epithelium in nasal polyps [15].

In our study, from the point of view of classic parameters – origin environment, age, sex – the statistical analysis of the control group showed a homogeneity of the group in terms of age, both as a mean and age variation.

We did not notice any significant differences generated by origin environment or sex in the case of the patients with chronic rhinosinusitis with nasal polyposis.

From histopathological examination point of view, there are three type of nasal polyps: allergic, fibro-inflammatory and with marked hyperplasia.

Allergic polyps were microscopically characterized by the presence of an edematous stroma, marked hyperplasia of goblet cells, thickness of basal membrane and an intense leukocytic inflammatory infiltrate, in which eosinophils predominated. Small areas of squamous metaplasia of respiratory type epithelium were rarely present.

Fibro-inflammatory polyps, from microscopically point of view, presented an intense chronic inflammatory infiltrate (mainly lymphocytic) and a series of modifications of metaplastic type, both at the level of covering epithelium and fibrous stroma. Therefore, at the level of fibrous stroma, we noticed both metaplasia areas of respiratory type epithelium and bone metaplasia areas.

Polyps with marked hyperplasia of sero-mucous glands, from histological point of view, were similar to allergic polyps, with numerous sero-mucous glands.

The percent of allergic polyps (eosinophilic) was not as big as the one mentioned in specialty literature, possibly because of the large scale administration of topical corticosteroids within the latest years (in the treatment of acute rhinosinusitis and chronic rhinosinusitis, not only in the treatment of allergic rhinitis, as in the past).

Immunohistochemical analyses were made for cytokeratins 7 (CK7) and 20 (CK20).

Analysis of CK7 expression

We noticed a decrease of the intensity of CK7 expression in fibro-inflammatory polyps, comparatively with the allergic ones. Therefore, it seems that CK specific for overbasal cells decrease once the inflammatory process evolves, being less expressed in polyps with chronic inflammation.

In one case, CK7 focally marked only the cells from the surface of covering epithelium. These cells reminded of “umbeliform” cells encountered in transitional epithelium. Therefore, we considered these areas as possible metaplasia areas of transitional type of respiratory covering epithelium.

Analysis of CK20 expression

We noticed an increase in the intensity of CK20 expression in glandular epithelium of fibro-inflammatory polyps comparatively with the allergic ones, possibly in relation with the same aspect of the inflammatory process evolution.

Conclusions

Nasal polyps, irrespective of their histological type, express CK7 very well: the expression is more intense at the level of allergic polyps and of those with hyperplasia of sero-mucous glands, and more discrete at the level of inflammatory polyps. CK20 expression at the level of nasal polyps is reduced, even absent in the case of allergic polyps.

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References

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