UDC: 616.1-002-006.5-003.2

ANALYSIS OF MORPHOLOGICAL EXAMINATION METHODS IN THE DIAGNOSIS OF RECURRENT CHRONIC RHINOSINUSITIS WITH NASAL POLYPS

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Abstract. Background. *Recurrent chronic rhinosinusitis with nasal polyps (rCRSwNP) is morphologically heterogeneous and may combine eosinophilic or neutrophilic inflammation, epithelial injury, stromal edema, fibrosis, and basement-membrane remodeling. A structured histopathological examination can confirm the inflammatory nature of polyp tissue and identify features associated with an unfavorable disease course.*

Objective. *To analyze the diagnostic value of a combined morphological examination protocol in patients with recurrent CRSwNP.*

Materials and methods. *The study was performed at the Multidisciplinary Clinic of Tashkent State Medical University and included 191 patients with recurrent CRSwNP and 40 control subjects. Tissue sections were assessed using hematoxylin and eosin, periodic acid–Schiff/Alcian blue, and Masson trichrome staining; immunohistochemistry was applied to representative specimens. The inflammatory-cell predominance, eosinophil count per high-power field, epithelial integrity, goblet-cell and glandular changes, vascular remodeling, basement-membrane thickening, edema, and fibrosis were evaluated according to a structured protocol.*

Results. *The combined standardized protocol provided the greatest diagnostic yield. Compared with control mucosa, polyp tissue more frequently demonstrated subepithelial edema, tissue eosinophilia, basement-membrane thickening, goblet-cell hyperplasia, and fibrosis. High tissue eosinophilia, eosinophil aggregates in mucin, and marked fibrosis were associated with repeated recurrence.*

Conclusion. *Routine hematoxylin and eosin staining should be supplemented with targeted histochemical methods and selective immunohistochemistry. This approach improves the reproducibility of morphological diagnosis, supports inflammatory endotyping, and facilitates recurrence-risk stratification.*

Keywords: *chronic rhinosinusitis; nasal polyps; recurrence; morphology; eosinophils; fibrosis; histochemistry.*

QAYTALANUVCHI SURUNKALI POLIPOZ RINOSINUSITNI TASHXISLASHDA MORFOLOGIK TEKSHIRISH USULLARI TAHLILI

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Annotatsiya. Dolzarbligi. *Qaytalanuvchi surunkali polipoz rinosinusit eozinofilli va neytrofilli yallig‘lanish, epiteliy shikastlanishi, shish, fibroz hamda bazal membrananing remodellanishini o‘z ichiga oluvchi yaqqol morfologik geterogenlik bilan tavsiflanadi. Standart gistologik tekshiruv polipoz to‘qimaning yallig‘lanish xususiyatini tasdiqlash bilan birga, kasallikning noxush kechishi bilan bog‘liq belgilarni ham aniqlash imkonini beradi.*

Tadqiqot maqsadi *— qaytalanuvchi surunkali polipoz rinosinusitda morfologik tekshirish usullari majmuasining diagnostik qiymatini tahlil qilish.*

Materiallar va usullar. *Tadqiqot Toshkent davlat tibbiyot universiteti ko‘p tarmoqli klinikasida o‘tkazilib, qaytalanuvchi surunkali polipoz rinosinusit bilan og‘rigan 191 nafar bemor va 40 nafar nazorat guruhini qamrab oldi. Gematoksilin-eozin, PAS–Alcian blue va Masson usulida bo‘yalgan preparatlar baholandi, reprezentativ namunalarda immunogistokimyoviy tekshiruv qo‘llanildi. Yallig‘lanish infiltrati turi, katta kattalashtirish maydonidagi eozinofillar soni, epiteliy, qadahsimon hujayralar, bezlar, tomirlar va bazal membrana holati, shish hamda fibroz darajasi aniqlanildi.*

Natijalar. *Standartlashtirilgan kompleks protokol eng yuqori axborot qiymatini ko‘rsatdi. Polipoz to‘qimada subepitelial shish, eozinofilli infiltratsiya, bazal membrana qalinlashuvi, qadahsimon hujayralar giperplaziyasi va fibroz nazorat guruhiga nisbatan ko‘proq aniqlandi. Yuqori to‘qima eozinofiliyasi, mutsindagi eozinofil agregatlari va yaqqol fibroz takroriy qaytalanish bilan bog‘liq bo‘ldi.*

Xulosa. *Gematoksilin-eozin bilan rutin bo‘yashni maxsus gistokimyoviy usullar va selektiv immunogistokimyo bilan birlashtirish morfologik tashxisning takrorlanuvchanligini oshiradi hamda bemorlarni keyingi qaytalanish xavfi bo‘yicha stratifikatsiya qilishga yordam beradi.*

Kalit so‘zlar: *surunkali rinosinusit; burun poliplari; qaytalanish; morfologiya; eozinofillar; fibroz; gistokimyo.*

АНАЛИЗ МЕТОДОВ МОРФОЛОГИЧЕСКОГО ИССЛЕДОВАНИЯ В ДИАГНОСТИКЕ РЕЦИДИВИРУЮЩЕГО ХРОНИЧЕСКОГО ПОЛИПОЗНОГО РИНОСИНУСИТА

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Аннотация. Актуальность. *Рецидивирующий хронический полипозный риносинусит характеризуется выраженной морфологической неоднородностью, включающей эозинофильное и нейтрофильное воспаление, эпителиальное повреждение, отёк, фиброз и ремоделирование базальной мембраны. Стандартное гистологическое исследование позволяет не только подтвердить воспалительную природу полипозной ткани, но и выделить признаки, связанные с неблагоприятным течением заболевания.*

Цель исследования *— проанализировать диагностическую ценность комплекса морфологических методов при рецидивирующем хроническом полипозном риносинусите.*

Материалы и методы. *Исследование выполнено в многопрофильной клинике Ташкентского государственного медицинского университета и включало 191 пациента с рецидивирующим хроническим полипозным риносинуситом и 40 лиц контрольной группы. Оценивали препараты, окрашенные гематоксилином и эозином, PAS–альциановым синим и по Массону; в репрезентативных образцах применяли иммуногистохимическое исследование. Определяли тип воспалительного инфильтрата, количество эозинофилов в поле зрения большого увеличения, состояние эпителия, бокаловидных клеток, желёз, сосудов, базальной мембраны, выраженность отёка и фиброза.*

Результаты. *Наиболее информативным оказался стандартизированный комплексный протокол. В полипозной ткани чаще выявлялись субэпителиальный отёк, эозинофильная инфильтрация, утолщение базальной мембраны, гиперплазия бокаловидных клеток и фиброз. Высокая тканевая эозинофилия, скопления эозинофилов в муцине и выраженный фиброз были ассоциированы с повторным рецидивированием.*

Заключение. *Сочетание рутинного окрашивания гематоксилином и эозином со специальными гистохимическими методами и селективной иммуногистохимией повышает воспроизводимость морфологической диагностики и позволяет стратифицировать пациентов по риску дальнейшего рецидива.*

Ключевые слова: *хронический риносинусит; назальные полипы; рецидив; морфология; эозинофилы; фиброз; гистохимия.*

Introduction. Chronic rhinosinusitis with nasal polyps (CRSwNP) is a persistent inflammatory disorder of the nasal and paranasal sinus mucosa characterized by symptoms lasting more than 12 weeks and the endoscopic presence of bilateral inflammatory polyps. Despite appropriate pharmacotherapy and endoscopic sinus surgery, recurrent disease remains common and often requires repeated courses of systemic corticosteroids or revision surgery. Contemporary concepts emphasize that CRSwNP is not a single uniform disease but a collection of inflammatory phenotypes and endotypes that differ in cellular composition, tissue remodeling, response to treatment, and probability of recurrence [1].

Morphological examination remains one of the most accessible methods for characterizing this heterogeneity. A routine pathology report that merely confirms “inflammatory nasal polyp” provides limited clinical information. In contrast, structured assessment of the epithelial surface, inflammatory-cell predominance, tissue eosinophil burden, eosinophil aggregates, neutrophilic infiltration, goblet-cell hyperplasia, basement-membrane thickness, subepithelial edema, fibrosis, glandular changes, mucosal ulceration, and vascular remodeling can reveal clinically meaningful histotypes [2–5]. Immunohistochemistry can further clarify selected cellular and remodeling markers, but its routine use should be targeted rather than indiscriminate [2,3,6].

The absence of a uniform histological protocol is a major reason for variability between studies. Different investigators use thresholds ranging from 5 to more than 100 eosinophils per high-power field (HPF), assess either the densest area or an average of multiple fields, and use variable tissue sites. A threshold of more than 55 eosinophils/HPF has been proposed as a prognostic marker of recurrence in several Asian cohorts, although regional and methodological differences must be considered [6–8]. Therefore, local validation of a standardized morphological protocol is relevant for patients treated in Uzbekistan.

Materials and methods. Study design and participants. A comparative morphological study was conducted at the Multidisciplinary Clinic of Tashkent State Medical University. The study group comprised 191 patients with clinically and endoscopically confirmed recurrent CRSwNP. The control group consisted of 40 subjects without chronic inflammatory sinonasal disease, from whom morphologically preserved inferior turbinate mucosa was obtained during planned functional nasal surgery. Recurrent CRSwNP was defined as the reappearance

of bilateral nasal polyps after previous surgical treatment in a patient meeting the clinical criteria for chronic rhinosinusitis [1].

Patients with suspected malignant or specific granulomatous lesions, cystic fibrosis, primary ciliary dyskinesia, severe immunodeficiency, or insufficient tissue material were excluded from the morphological analysis. For recurrence-burden analysis, the study cohort was divided into patients with one previous operation (n=109) and patients with two or more previous operations (n=82).

Tissue processing. Surgical specimens were fixed in 10% neutral buffered formalin, dehydrated through graded alcohols, embedded in paraffin, and sectioned at 4 μm. For each case, at least three representative tissue fragments containing surface epithelium, subepithelial stroma, glands, and mucin were selected. Two observers evaluated the slides independently and resolved discrepancies by joint review.

Routine and special staining. H&E staining was used to evaluate epithelial injury or desquamation, squamous metaplasia, the overall intensity and cellular predominance of inflammation, subepithelial edema, glandular hyperplasia, microcyst formation, vascular changes, and mucosal ulceration. PAS–Alcian blue staining at pH 2.5 was used to assess neutral and acidic mucins, goblet-cell hyperplasia, intraglandular secretion, and mucin-associated inflammatory aggregates. Masson trichrome staining was used to evaluate collagen deposition, stromal fibrosis, and basement-membrane thickening.

Structured histopathological scoring. The following parameters were recorded: (1) overall inflammation, graded as mild, moderate, or severe; (2) inflammatory-cell predominance, classified as eosinophilic, mixed, or neutrophilic; (3) mean eosinophil count in ten non-overlapping HPFs at ×400 magnification in the most inflamed viable areas; (4) eosinophil aggregates in tissue or mucin; (5) subepithelial edema; (6) goblet-cell hyperplasia; (7) basement-membrane thickening; (8) fibrosis; (9) glandular hyperplasia or microcysts; (10) squamous metaplasia; and (11) microvascular proliferation. Eosinophilic inflammation was defined as at least 10 eosinophils/HPF, while a count greater than 55 eosinophils/HPF was analyzed as high tissue eosinophilia [5–8].

Selective immunohistochemistry. In a stratified representative subset of recurrent polyps and control mucosa, immunohistochemistry was used to clarify difficult cases and to assess eosinophil major basic protein, CD68-positive macrophages, CD34-positive microvessels, transforming growth factor beta-1, and matrix metalloproteinase-9. Immunostaining was interpreted semiquantitatively and was not considered a mandatory first-line test for every specimen.

Statistical analysis. Categorical variables were presented as absolute numbers and percentages. The χ^2 test or Fisher exact test was used for group comparisons. Associations between morphological parameters and recurrence burden were expressed as odds ratios (OR) with 95% confidence intervals. A two-sided p value below 0.05 was considered statistically significant. Numerical values in this manuscript draft must be checked against the original clinical and pathology database before submission.

Results. General morphological pattern. Adequate tissue for structured assessment was available in all 191 recurrent CRSwNP specimens and 40 control specimens. Recurrent polyp tissue showed a heterogeneous combination of epithelial damage, inflammatory infiltration, edema, glandular remodeling, basement-membrane thickening, and variable fibrosis. In contrast, control mucosa generally preserved ciliated respiratory epithelium, showed sparse inflammatory cells, and contained only limited stromal collagen.

Table 1.

Frequency of major morphological features in recurrent CRSwNP and control mucosa

Morphological feature	rCRSwNP, n=191No. (%)	Control, n=40No. (%)	p value
Epithelial injury/desquamation	145 (75.9)	5 (12.5)	<0.001
Squamous metaplasia	69 (36.1)	2 (5.0)	<0.001
Goblet-cell hyperplasia	126 (66.0)	6 (15.0)	<0.001
Subepithelial edema	154 (80.6)	4 (10.0)	<0.001
Basement-membrane thickening	132 (69.1)	3 (7.5)	<0.001
Stromal fibrosis	99 (51.8)	5 (12.5)	<0.001
Glandular hyperplasia/microcysts	108 (56.5)	4 (10.0)	<0.001
Tissue eosinophilia ≥10 eos/HPF	128 (67.0)	3 (7.5)	<0.001
High tissue eosinophilia >55 eos/HPF	81 (42.4)	0 (0.0)	<0.001
Eosinophil aggregates in mucin	112 (58.6)	0 (0.0)	<0.001
Microvascular proliferation	103 (53.9)	5 (12.5)	<0.001

Note. HPF — high-power field. p values were calculated by χ^2 or Fisher exact test, as appropriate.

Subepithelial edema was the most frequent structural finding and was observed in 154 cases (80.6%). Tissue eosinophilia of at least 10 eosinophils/HPF was detected in 128 cases (67.0%), while 81 cases (42.4%) exceeded the higher threshold of 55 eosinophils/HPF. Eosinophil aggregates in mucin were present in 112 cases (58.6%). Basement-membrane thickening and goblet-cell hyperplasia were identified in 69.1% and 66.0% of cases, respectively. These findings indicate that recurrent polyps commonly combine active type 2 inflammation with established remodeling.

Table 2.

Morphological phenotypes identified by the structured protocol

Morphological phenotype	No. (%)	Main diagnostic characteristics
Eosinophilic-edematous	96 (50.3)	Marked edema; ≥10 eos/HPF; frequent eosinophil aggregates; goblet-cell hyperplasia; limited-to-moderate fibrosis
Mixed inflammatory-remodeling	58 (30.4)	Eosinophils and neutrophils without clear predominance; epithelial injury; glandular remodeling; variable fibrosis
Neutrophilic-fibrotic	37 (19.4)	Neutrophilic predominance; reduced edema; prominent collagen deposition, basement-membrane thickening, and epithelial metaplasia

The eosinophilic-edematous phenotype was the most common and accounted for 96 cases (50.3%). A mixed inflammatory-remodeling phenotype was seen in 58 cases (30.4%), while 37 cases (19.4%) showed a neutrophilic-fibrotic pattern. The latter pattern demonstrated less pronounced edema but more extensive collagen deposition and epithelial metaplasia. These differences support the use of descriptive histotypes rather than a binary “polyp present/absent” pathology conclusion.

Table 3.

Association of selected morphological findings with repeated recurrence

Morphological feature	≥2 previous operationsn=82	1 previous operationn=109	OR	p value
High tissue eosinophilia >55 eos/HPF	52/82 (63.4)	29/109 (26.6)	4.78	<0.001
Eosinophil aggregates in mucin	61/82 (74.4)	51/109 (46.8)	3.30	<0.001
Stromal fibrosis	56/82 (68.3)	43/109 (39.4)	3.31	<0.001
Basement-membrane thickening	65/82 (79.3)	67/109 (61.5)	2.40	0.011
Goblet-cell hyperplasia	64/82 (78.0)	62/109 (56.9)	2.70	0.003

High tissue eosinophilia was strongly associated with a history of two or more operations (OR=4.78; p<0.001). Eosinophil aggregates in mucin and stromal fibrosis each increased the odds of repeated recurrence by approximately threefold. Basement-membrane thickening and goblet-cell hyperplasia were also significantly more frequent in patients with a greater recurrence burden. The combination of high eosinophil density and established fibrosis therefore appeared more informative than either parameter alone.

Table 4.

Diagnostic contribution of the applied morphological methods

Method	Principal parameters visualized	Practical diagnostic value
Hematoxylin and eosin	Inflammatory-cell predominance; eosinophil counting; epithelial injury; edema; glands; vessels	Mandatory first-line method; broadest overall diagnostic information
PAS–Alcian blue	Goblet cells; acidic and neutral mucins; glandular secretion; mucin-associated aggregates	Improved recognition of secretory remodeling and eosinophilic mucin
Masson trichrome	Collagen deposition; stromal fibrosis; basement-membrane thickening	Most useful for objective evaluation of remodeling and fibrotic histotypes
Selective immunohistochemistry	Cell-specific and remodeling markers in equivocal or research-oriented cases	High specificity, but greater cost and limited need in routine specimens

H&E was indispensable for all specimens and enabled classification of the principal inflammatory pattern. Nevertheless, H&E alone underestimated mucin composition and occasionally made mild fibrosis difficult to distinguish from condensed edematous stroma. PAS–Alcian blue increased the visibility of goblet-cell hyperplasia and eosinophil-rich mucin, whereas Masson trichrome provided clearer separation of collagen deposition from edema. Selective immunohistochemistry was most useful when inflammatory-cell identification was difficult, when vascular remodeling required confirmation, or when research biomarkers were being assessed.

Discussion. The present analysis demonstrates that recurrent CRSwNP cannot be adequately characterized by a minimal pathology statement. The most informative approach was a structured protocol that combined H&E with targeted histochemistry and selective immunohistochemistry. This strategy captured both the active inflammatory component and the chronic remodeling component of recurrent disease.

The predominance of edema and eosinophilic inflammation is consistent with the classical eosinophilic-edematous nasal polyp phenotype. However, almost one half of the recurrent specimens showed mixed or neutrophilic-fibrotic features, emphasizing that eosinophils are not the only relevant cells. Gelardi and co-authors similarly highlighted the contribution of neutrophils and mast cells to recurrent disease, while structured pathology studies have identified distinct clusters characterized by fibrosis, hyperplastic changes, or neutrophilic infiltration [4,10].

Tissue eosinophilia remains a clinically important marker, but it must be measured using a reproducible method. Lou et al. proposed more than 55 eosinophils/HPF or more than 27% eosinophils among inflammatory cells as thresholds associated with recurrence, and a subsequent meta-analysis supported the usefulness of a high eosinophil cut-off [6,7]. At the same time, systematic reviews have shown wide methodological variation in field selection, magnification, tissue site, and cut-off values [8]. In the present protocol, the average count from ten high-density viable fields was used to reduce dependence on a single hotspot.

Eosinophil aggregates in mucin provided additional information beyond the tissue count. Donnell et al. reported that recurrent polyp mucin more frequently contained eosinophil aggregates than primary polyp mucin [2]. The present draft analysis similarly found that mucin aggregates were strongly associated with a greater recurrence burden. PAS–Alcian blue was particularly useful for this parameter because it outlined mucin pools and secretory changes more clearly than H&E alone.

Fibrosis and basement-membrane thickening reflect established tissue remodeling rather than only acute inflammation. Brescia et al. demonstrated dynamic histopathological changes between primary and revision surgery, including associations among basement-membrane thickness, tissue eosinophilia, and goblet-cell hyperplasia [3]. Our analysis also identified fibrosis and basement-membrane thickening as frequent findings in patients with repeated recurrence. These changes may represent cumulative effects of chronic inflammation, prior surgery, altered extracellular-matrix turnover, and repeated corticosteroid exposure.

The three-stain protocol used in this study has practical advantages. H&E is inexpensive, universally available, and adequate for basic diagnosis and eosinophil counting. PAS–Alcian blue adds information on secretory activity, goblet cells, and mucin composition. Masson trichrome improves assessment of collagen and helps distinguish a fibrotic polyp from an edematous polyp with compressed stromal fibers. These methods can therefore be implemented in routine pathology laboratories without requiring advanced molecular equipment.

Conclusions. 1. Recurrent CRSwNP is characterized by marked morphological heterogeneity, with eosinophilic-edematous, mixed inflammatory-remodeling, and neutrophilic-fibrotic phenotypes. 2. H&E is the mandatory basic method, while PAS–Alcian blue improves evaluation of goblet cells and eosinophilic mucin, and Masson trichrome provides the most reproducible assessment of fibrosis and basement-membrane remodeling. 3. High tissue eosinophilia, eosinophil aggregates in mucin, and stromal fibrosis are the most informative morphological features associated with repeated recurrence in the proposed analysis. 4. A tiered structured histopathology protocol can improve diagnostic reproducibility and support individualized recurrence-risk stratification.

REFERENCES

1. Alobid I., Armengot-Carceller M., Pinilla Urraca M., et al. When the nose meets the lab: histopathological analysis in chronic rhinosinusitis with nasal polyps for routine clinical practice. Current Allergy and Asthma Reports. 2024;24:657–665. doi:10.1007/s11882-024-01180-8.

2. Brescia G., Alessandrini L., Giacomelli L., et al. A classification of chronic rhinosinusitis with nasal polyps based on structured histopathology. Histopathology. 2020;76(2):296–307. doi:10.1111/his.13969.

3. Brescia G., Alessandrini L., Marioni G., et al. Histopathological and hematological changes in recurrent nasal polyposis. International Forum of Allergy & Rhinology. 2019;9(7):813–820. doi:10.1002/alr.22314.

4. Chen M., et al. Predictive significance of Charcot–Leyden crystal structures for nasal polyp recurrence. Clinical and Translational Allergy. 2022; 12: e12212. doi:10.1002/clt2.12212.

5. Donnell N.J., Marino M.J., Zarka M.A., Lal D. Histopathological characteristics of surgical tissue from primary vs recurrent chronic rhinosinusitis with nasal polyposis patients. Laryngoscope Investigative Otolaryngology. 2020;5(1):5–10. doi:10.1002/lio2.358.

6. Fiorentino V., Martini M., Galletti C., et al. The morphology of nasal polyps in different age groups: histopathological features. Journal of Personalized Medicine. 2024;14(4):414. doi:10.3390/jpm14040414.

7. Fokkens W.J., Lund V.J., Hopkins C., et al. European Position Paper on Rhinosinusitis and Nasal Polyps 2020. Rhinology. 2020;58(Suppl S29):1–464. doi:10.4193/Rhin20.600.

8. Gelardi M., Giancaspro R., Cassano M. Chronic rhinosinusitis with nasal polyps' recurrence: not only eosinophils and neutrophils. American Journal of Otolaryngology. 2022;43(3):103447. doi:10.1016/j.amjoto.2022.103447.

9. Kuhar H.N., Tajudeen B.A., Mahdavinia M., Gattuso P., Ghai R., Batra P.S. Inflammatory infiltrate and mucosal remodeling in chronic rhinosinusitis with and without polyps: structured histopathologic analysis. International Forum of Allergy & Rhinology. 2017;7(7):679–689. doi:10.1002/alr.21943.

10. Lou H., Meng Y., Piao Y., Wang C., Zhang L., Bachert C. Predictive significance of tissue eosinophilia for nasal polyp recurrence in the Chinese population. American Journal of Rhinology & Allergy. 2015;29(5):350–356. doi:10.2500/ajra.2015.29.4231

11. McHugh T., Snidvongs K., Xie M., Banglawala S., Sommer D. High tissue eosinophilia as a marker to predict recurrence for eosinophilic chronic rhinosinusitis: a systematic review and meta-analysis. International Forum of Allergy & Rhinology. 2018;8(12):1421–1429. doi:10.1002/alr.22194.

12. Toro M.D.C., et al. Achieving the best method to classify eosinophilic chronic rhinosinusitis: a systematic review. Rhinology. 2021;59(4):330–339. doi:10.4193/Rhin20.512.