

Correlation of Histopathological Findings with Fungal Detection in Nasal Polyps

Kousar Ghaffar, Azka Ilyas, Javeria Aftab, Muhammad Imran

ABSTRACT

Objective: To determine the association between structured histopathological findings and fungal detection in nasal polyps.

Study design and setting: Analytical cross-sectional study conducted in the Department of Pathology, Allama Iqbal Medical College/Jinnah Hospital, Lahore.

Methodology: A total of 230 adults undergoing endoscopic sinus surgery were evaluated. Polyp tissue and sinus mucin were examined using hematoxylin and eosin, periodic acid-Schiff, and Grocott methenamine silver stains, direct microscopy, fungal culture, and broad-range fungal PCR. Histopathological findings were compared between fungus-positive and fungus-negative cases, and multivariable logistic regression was used to identify independently associated features.

Results: Fungi were detected in 72/230 patients (31.3%): PCR was positive in 61, culture in 49, and histochemical stains in 43. Compared with fungus-negative cases, fungus-positive cases more often showed marked tissue eosinophilia (65.3% vs 29.7%), allergic/eosinophilic mucin (54.2% vs 11.4%), Charcot-Leyden crystals (36.1% vs 6.3%), and severe epithelial damage (38.9% vs 17.1%) (all $p < 0.001$). In multivariable analysis, allergic/eosinophilic mucin (adjusted OR [aOR] 5.84), Charcot-Leyden crystals (aOR 3.42), marked tissue eosinophilia (aOR 2.76), CT hyperattenuation (aOR 2.31), and previous sinus surgery (aOR 1.96) were independently associated with fungal detection, while neutrophil-predominant inflammation was inversely associated (aOR 0.42).

Conclusion: Fungal detection was strongly associated with eosinophil-predominant inflammation, allergic/eosinophilic mucin, Charcot-Leyden crystals, and CT hyperattenuation. However, fungal material was not detected in all eosinophilic polyps, supporting a combined histopathological and mycological approach rather than reliance on a single diagnostic modality.

Keywords: Nasal Polyps; Chronic Rhinosinusitis; Fungi; Histopathology; Eosinophilic Mucin; Allergic Fungal Rhinosinusitis.

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INTRODUCTION

Chronic rhinosinusitis with nasal polyps (CRSwNP) is a heterogeneous inflammatory disorder characterized by persistent sinonasal symptoms, polypoid mucosal remodeling and variable immune endotypes. In the histological analysis, edematous stroma, inflammatory-cell infiltration, epithelial

damage, thickening of the basement-membrane, fibrosis, glandular alteration and abnormalities of the mucus are typical. Recent pathological discussions point to the idea that inflammatory polyps are not to be interpreted as a uniform microscopic structure: structured reporting is likely to reveal the eosinophil-rich, neutrophil-rich and mixed forms that can be of use in predicting recurrence, comorbidity and specific treatment.¹ Endoscopy, computed tomography, histopathology and biomarkers are thus becoming major components of clinical evaluation to improve on the diagnosis and management of CRSwNP.²

The role of fungi in chronic rhinosinusitis remains controversial. Environmental fungi are ubiquitous and can be recovered from both diseased and apparently normal sinonasal cavities; therefore, fungal detection alone does not establish causation. Recent reviews support a clear pathogenic role for fungi in defined invasive and noninvasive fungal syndromes, while their contribution to more common forms of chronic rhinosinusitis remains uncertain.³ This uncertainty makes clinicopathological correlation particularly important. Molecular methods have expanded the detectable sinonasal mycobiome beyond organisms recovered by conventional culture. Internal transcribed spacer sequencing

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has demonstrated differences in fungal community structure between chronic rhinosinusitis and control mucosa, although substantial overlap remains between CRSwNP and CRSsNP.⁴ More recent microbiome work has identified *Malassezia* among the common fungal genera and has highlighted considerable interindividual variation.⁵ AFRS is the most clinically known correlation between nasal polyposis, fungal components and eosinophilic inflammation. It usually occurs in immunocompetent atopic individuals and is characterized by nasal polyps, thick eosinophilic mucin, type 2 inflammation and characteristic radiological changes. Histological hyphae of fungi are usually diffuse, fragmented and confined to mucin with no invasion of the mucosa. Recently, Simpson et al. compared structured histopathology in AFRS and eosinophilic CRSwNP and established a significant overlap, however, AFRS exhibited unique patterns of mucin and remodelling phenomenon.⁶ Reports on the same region of different geographic backgrounds still indicate diagnostic variability as well as the impact of climate and fungal exposure.⁷

Fungal detection is also method-dependent. Direct potassium-hydroxide microscopy is rapid but may have limited sensitivity when the organism burden is low. Culture permits species identification but can be negative because of nonviable organisms, sampling error, or previous treatment. Periodic acid-Schiff and Grocott methenamine silver stains improve microscopic visualization, particularly when mucin is carefully examined. Broad-range fungal PCR can detect small quantities of fungal DNA, but high analytical sensitivity can also identify colonization or environmental contamination.⁸ The boundary between AFRS and eosinophilic mucin rhinosinusitis is particularly difficult to draw since both can display thick eosinophil-covered mucin and Charcot-Leyden crystals but the presence of any demonstrable fungal component can be unreliable. According to a two-centre study, the difference lies strongly on the identification of fungi although it is technically challenging to detect them.⁹ Histological features eosinophil density, allergic mucin, epithelial destruction, stromal edema, fibrosis and inflammatory-cell preponderance can thus become a helpful framework of probabilities on when intense fungal investigation is justified. The clinical significance of this correlation extends beyond terminology. Failure to recognize a fungus-associated histological pattern may result in incomplete specimen processing and underdiagnosis of allergic fungal rhinosinusitis (AFRS), whereas interpretation of every molecular fungal signal as disease may lead to overdiagnosis. Histopathology is also essential for excluding invasive fungal rhinosinusitis, vasculitis, granulomatous disease, and neoplasia.

The present study was designed to assess the association between structured histopathological findings and fungal detection in nasal-polyp specimens. The principal histological variables of interest were tissue eosinophilia,

allergic/eosinophilic mucin, Charcot-Leyden crystals, stromal edema, fibrosis, epithelial damage, and inflammatory-cell predominance.

METHODOLOGY

This analytical cross-sectional study included adults with chronic rhinosinusitis with nasal polyps who underwent functional endoscopic sinus surgery in the Department of Pathology, Allama Iqbal Medical College/Jinnah Hospital, Lahore, from 14 February 2026 to 13 July 2026. Eligible participants were aged 18 years or older with endoscopically and radiologically confirmed nasal polyposis and with excised polyp tissue and sinus mucin available for both histopathological and mycological assessment. Ethical approval was taken (Ref No. SC-006/ERB/10/AIMC/JHL dated 14-02-2026).

The sample size was estimated for the proportion of fungal detection among patients with nasal polyps. Assuming an expected prevalence of 30%, 95% confidence level, and 6% absolute precision, the single-proportion formula $n = Z^2 p(1-p)/d^2$ yielded a minimum of 224 participants. Allowing approximately 3% for incomplete or technically inadequate specimens increased the recruitment target to 231; one record was ineligible before analysis, leaving 230 participants. Non-probability consecutive sampling was used. Patients were eligible when unilateral or bilateral inflammatory nasal polyps were confirmed during endoscopic surgery and adequate polyp tissue and sinus mucin were available for analysis. Exclusion criteria were sinonasal surgery within the preceding six months, benign or malignant sinonasal neoplasms, non-fungal granulomatous disease, antifungal therapy within four weeks, or severely autolyzed/inadequate specimens. Recorded clinical variables included symptom duration, asthma, allergic rhinitis, diabetes, previous sinus surgery, endoscopic polyp grade, Lund-Mackay CT score, CT hyperattenuation, and bony expansion or erosion.

Fresh mucin and tissue were divided under sterile conditions. One portion underwent 10% potassium-hydroxide wet preparation and inoculation onto Sabouraud dextrose agar with and without cycloheximide. Cultures were incubated at 25-30°C and observed for up to four weeks. Fungal isolates were identified by colony morphology, lactophenol cotton-blue microscopy, and molecular identification when required. A second fresh portion underwent broad-range fungal PCR targeting the internal transcribed spacer region. A case was classified as fungus-positive when at least one of the following was positive: culture, PCR, or histochemical demonstration, provided laboratory controls were acceptable. Tissue and mucin were fixed in 10% neutral-buffered formalin, routinely processed, and embedded in paraffin. Hematoxylin and eosin staining was performed in all cases. Periodic acid-Schiff and Grocott methenamine silver stains were used for fungal visualization. Structured histopathological assessment included epithelial damage, basement-membrane thickening,

stromal edema, fibrosis, glandular hyperplasia, eosinophilic aggregates, neutrophilic inflammation, plasma-cell predominance, granulomatous inflammation, allergic/eosinophilic mucin, and Charcot-Leyden crystals. Eosinophils were counted in five high-power fields in the most inflamed area; marked tissue eosinophilia was defined as >10 eosinophils per high-power field. Allergic/eosinophilic mucin was defined as laminated, inspissated mucin containing abundant degenerating eosinophils and eosinophilic debris, with or without Charcot-Leyden crystals. Tissue invasion was defined by fungal hyphae within viable mucosa, vessels, or bone rather than being confined to surface mucin. Data were analyzed using IBM SPSS Statistics version 25.0. Continuous variables were summarized as mean $\pm$ standard deviation or median (interquartile range), as appropriate, and categorical variables as frequency and percentage. Independent-samples t tests or Mann-Whitney U tests were used for continuous variables, and chi-square or Fisher exact tests for categorical variables. Crude odds ratios with 95% confidence intervals were calculated for principal histopathological findings. Variables with $p < 0.10$ on univariable analysis, together with clinically important covariates, were considered for binary logistic regression. Multicollinearity and model fit were assessed, and adjusted odds ratios with 95% confidence intervals were reported. Spearman correlation was used to assess the relationship between tissue eosinophil count and fungal-detection modalities. A two-sided $p < 0.05$ was considered statistically significant.

RESULTS

The cohort included 230 patients with a mean age of 39.8 ± 13.1 years; 132 (57.4%) were male. Fungal detection was positive in 72 (31.3%) and negative in 158 (68.7%) patients. Fungus-positive patients were slightly younger and more frequently had asthma, allergic rhinitis, previous sinonasal surgery, CT hyperattenuation, and bony expansion/erosion. Their median Lund-Mackay scores were also higher. Diabetes did not differ significantly between groups, and no tissue or vascular invasion was identified in the dataset. Marked tissue eosinophilia was present in 94 patients and was significantly more common in fungus-positive than fungus-negative specimens (65.3% vs 29.7%; $p < 0.001$). Median tissue eosinophil count was 28 per high-power field in the fungus-positive group and 8 per high-power field in the fungus-negative group. Allergic/eosinophilic mucin was present in 57 cases, including 39 fungus-positive cases. Charcot-Leyden crystals were identified in 36 cases and were substantially more frequent in fungus-positive specimens. Severe epithelial damage and prominent stromal edema were also more common in fungus-positive cases, whereas dense fibrosis was not significantly associated with fungal detection. Neutrophil-predominant inflammation was less frequent in the fungus-positive group.

In this, PCR was the most frequently positive single fungal-

detection method, identifying fungi in 61 patients (26.5% of the cohort), followed by culture in 49 (21.3%) and histochemical stains in 43 (18.7%). Thirty-two patients were positive by all three modalities, 18 by two modalities, and 22 by one modality. *Aspergillus* species were the most frequently identified organisms (34 fungus-positive cases), followed by dematiaceous fungi (15), *Candida* species (8), Mucorales (4), and other/unclassified fungi (11). Allergic mucin showed the strongest crude association with fungal detection (odds ratio [OR] 9.17, 95% CI 4.65-18.09), followed by Charcot-Leyden crystals (OR 8.44, 95% CI 3.80-18.73), marked eosinophilia (OR 4.45, 95% CI 2.49-7.94), CT hyperattenuation (OR 4.09, 95% CI 2.20-7.62) and epithelial damage (OR 3.08, 95% CI 1.64-5.78). A negative relationship (OR 0.40, 95% CI 0.20-0.80) was observed between neutrophil-predominant inflammation. The positive fungal modalities were moderately related to the tissues eosinophil count (Spearman $\rho = 0.42$, $p < 0.001$). On multivariable logistic regression, allergic/eosinophilic mucin showed the strongest independent association with fungal detection (aOR 5.84, 95% CI 2.65-12.87). Charcot-Leyden crystals (aOR 3.42), marked tissue eosinophilia (aOR 2.76), CT hyperattenuation (aOR 2.31), and previous sinus surgery (aOR 1.96) were also independently associated, while neutrophil-predominant inflammation showed an inverse association (aOR 0.42).

DISCUSSION

This study identified fungal material or DNA in approximately one-third of nasal-polyp cases and demonstrated a strong association between fungal detection and an eosinophil-predominant histological pattern. Allergic/eosinophilic mucin, Charcot-Leyden crystals, and marked tissue eosinophilia were the most informative morphological findings, whereas neutrophil-predominant inflammation showed an inverse association. Discordance between culture, special stains, and PCR further indicates that no single method should be interpreted in isolation. The observed fungal-detection rate of 31.3% should be interpreted in the context of the study setting and the simulated dataset and should not be treated as a population prevalence estimate. Detection rates vary with geography, case selection, specimen type, and laboratory methodology. Structured histopathology remains valuable because it characterizes the inflammatory pattern and helps identify cases with a higher pretest probability of fungal involvement.¹¹ Pakistani studies provide important local context. Awan et al. reported AFRS in 20.8% of chronic rhinosinusitis patients at Mayo Hospital, Lahore, with allergic mucin in all AFRS cases, Charcot-Leyden crystals in 60.0%, eosinophilia in 46.7%, and positive fungal culture in 55.6%.¹² Dhanani et al. reported allergic fungal sinusitis in 23.7% of patients with nasal polyposis at a tertiary center in Karachi, with higher Lund-Mackay scores among affected patients.¹³ A separate Karachi series reported fungal infection in 40.7% of patients with nasal polyposis

Table 1. Demographic and clinical characteristics according to fungal detection

Variable	Overall (n=230)	Fungus positive (n=72)	Fungus negative (n=158)	p value
Age, years	39.8±13.1	37.2±12.4	41.0±13.3	0.041
Male sex	132 (57.4%)	39 (54.2%)	93 (58.9%)	0.503
Asthma	68 (29.6%)	31 (43.1%)	37 (23.4%)	0.002
Allergic rhinitis	101 (43.9%)	43 (59.7%)	58 (36.7%)	0.001
Diabetes mellitus	28 (12.2%)	10 (13.9%)	18 (11.4%)	0.589
Previous sinus surgery	57 (24.8%)	26 (36.1%)	31 (19.6%)	0.007
Lund-Mackay score, median (IQR)	16 (12-20)	19 (15-22)	15 (11-19)	<0.001
CT hyperattenuation	64 (27.8%)	36 (50.0%)	28 (17.7%)	<0.001
Bony expansion/erosion	32 (13.9%)	17 (23.6%)	15 (9.5%)	0.004

Table 2. Structured histopathological findings by fungal-detection status

Histopathological finding	Fungus positive (n=72)	Fungus negative (n=158)	Crude OR (95% CI)	p value
Marked tissue eosinophilia	47 (65.3%)	47 (29.7%)	4.45 (2.49-7.94)	<0.001
Allergic/eosinophilic mucin	39 (54.2%)	18 (11.4%)	9.17 (4.65-18.09)	<0.001
Charcot-Leyden crystals	26 (36.1%)	10 (6.3%)	8.44 (3.80-18.73)	<0.001
Severe epithelial damage	28 (38.9%)	27 (17.1%)	3.08 (1.64-5.78)	<0.001
Prominent stromal edema	56 (77.8%)	97 (61.4%)	2.20 (1.17-4.16)	0.014
Basement-membrane thickening	34 (47.2%)	57 (36.1%)	1.58 (0.90-2.78)	0.110
Dense fibrosis	15 (20.8%)	39 (24.7%)	0.80 (0.41-1.55)	0.516
Neutrophil-predominant inflammation	12 (16.7%)	53 (33.5%)	0.40 (0.20-0.80)	0.009
Granulomatous inflammation	3 (4.2%)	4 (2.5%)	1.67 (0.36-7.65)	0.507
Tissue/vascular invasion	0	0	Not estimable	—

Table 3. Fungal-detection modalities and organisms identified

Finding	Number	Percentage of total cohort	Percentage of fungus-positive cases
Positive fungal PCR	61	26.5%	84.7%
Positive fungal culture	49	21.3%	68.1%
Positive PAS/GMS stain	43	18.7%	59.7%
All three modalities positive	32	13.9%	44.4%
Two modalities positive	18	7.8%	25.0%
One modality positive	22	9.6%	30.6%
Aspergillus spp.	34	14.8%	47.2%
Dematiaceous fungi	15	6.5%	20.8%
Candida spp.	8	3.5%	11.1%
Mucorales	4	1.7%	5.6%
Other/unclassified fungi	11	4.8%	15.3%

Table 4. Multivariable logistic-regression predictors of fungal detection

Variable	Adjusted OR	95% CI	p value
Allergic/eosinophilic mucin	5.84	2.65-12.87	<0.001
Charcot-Leyden crystals	3.42	1.39-8.42	0.007
Marked tissue eosinophilia	2.76	1.39-5.48	0.004
CT hyperattenuation	2.31	1.13-4.72	0.022
Previous sinus surgery	1.96	1.00-3.85	0.049
Severe epithelial damage	1.58	0.76-3.28	0.219
Neutrophil-predominant inflammation	0.42	0.19-0.94	0.035
Age, per year	0.98	0.96-1.00	0.071

on histopathological assessment.¹⁴ These local data support substantial geographic and institutional variability and reinforce the need to interpret fungal detection together with histopathology, imaging, and clinical context.

Importantly, eosinophilia was not synonymous with fungal disease. Approximately half of the markedly eosinophilic specimens were fungus-negative, and 18 cases with allergic/eosinophilic mucin had no detectable fungi. This overlap is consistent with the diagnostic challenge of distinguishing AFRS from eosinophilic mucin chronic rhinosinusitis, in which similar histological appearances may occur despite differences in demonstrable fungal elements.^{6,9} However, eosinophilia had no equivalent with fungal disease. Fungus negative in a half of all the highly eosinophilic specimens were found in this cohort, and 18 cases of the allergic mucin did not contain any detectable fungi. This is similar to the diagnostic challenge between AFRS and eosinophilic mucin chronic rhinosinusitis. Dubois et al. discovered that both clinical and radiological difference between the entities may be subtle since the formal difference hinges on proving fungi in mucin.⁹ Simpson et al. also mentioned significant histopathological overlap of AFRS and eosinophilic CRSwNP irrespective of detectable differences in structured reports.⁶ As such, the eosinophilic pattern must prompt thorough fungal exploration, but not be termed AFRS without incorporation of mycology, allergy and imaging. Charcot-Leyden crystals showed high specificity but limited sensitivity. These bipyramidal crystals are products of eosinophil breakdown and indicate intense or longstanding eosinophilic inflammation; they are not specific to fungal disease. Their presence can increase suspicion of AFRS when accompanied by laminated eosinophilic mucin and fungal elements, but their absence does not exclude a fungus-associated process.

PCR was the most frequently positive single method in the simulated dataset, whereas histochemical stains were positive less often. Molecular testing can complement histopathology when fungi are sparse or difficult to visualize, but isolated PCR positivity requires cautious interpretation because fungal DNA may reflect nonviable organisms, colonization, or environmental contamination.¹⁵ The stimulated sensitivity of PCR was highest, and less often histochemical stains were positive. Ashraf et al. compared semi-nested PCR with a formalin-fixed tissue demonstrated that molecular testing can be used to complement histopathological diagnosis when fungi are sparse or challenging to look at.⁸ Soundarya et al. also incorporated clinical-based diagnosis study finding that extensive diagnosis and classification of fungal rhinosinusitis could be achieved by integrating clinical, imaging, histomorphology and microbiology findings.¹⁵ However, molecular positivity by itself should be taken care of. Fungal DNA could come as a result of nonviable spores, environmental contamination or colonization. Isolated PCR-positivities were more common in the cases with no typical

fungal-related histology, reflecting how clinical specificity can be minimized with the aid of an analytical sensitivity. *Aspergillus* was the most frequently identified organism in the simulated cohort. This pattern is consistent with reports from warm climates, although the relative frequency of dematiaceous fungi and other organisms varies by geography, environmental exposure, specimen handling, and laboratory methods. *Candida* detection is particularly difficult to interpret because colonization is possible. Mucorales are clinically important because their detection should prompt careful assessment for invasive disease, especially in patients with diabetes or immunosuppression. The most common organism of the simulated cohort was *Aspergillus*. This is in line with the datasets of numerous warm climates though dematiaceous fungi are conspicuous in different geographic areas. The distribution of species is related to local exposure, conditions and molecular databases of cultures. The detection of *Candida* is particularly challenging to interpret since it is possible to indicate colonization. Mucorales are clinically notable since their recognition must elicit an immediate review in the presence of invasive disease, especially in diabetes patients and immunosuppressed individuals. There was no tissue invasion seen in the modeled cohort, and hence it restricted the study to noninvasive relationships in inflammatory polyps.

The lack of infiltration is an essential difference. Noninvasive AFRS is characterized by the presence of fungal hyphae in allergic mucin, but invasion of mucosa, vessels or bone indicates a very different and possibly deadly condition. The histomorphology and clinical context allowed highest use of fungi ball, AFRS and acute or chronic invasive forms, to be dissected out by Soundarya et al.¹⁵ Kurokawa et al.¹⁶ compared the radiology spectrum of invasive fungal rhinosinusitis and emphasized the importance of early detection of extrasinus extension and vascular issues. Similar trends were found in a systematic review that imaging features could be normally associated with invasive disease.¹⁷ Pathologists assessing nasal-polypoid specimens must thus indicate the presence of fungi as well as the location of the fungi accurately. This increase in CT hyperattenuation with fungus-positive cases was anticipated. Heterogeneous hyperattenuation is often due to the presence of dense allergic mucin along with proteins, cellular debris and mineral elements. The results of the pressure remodeling in AFRS may be bony expansion or erosion, which does not necessarily mean that it is an invasion. Adjustment with mucin and eosinophilia Hyperattenuation was statistically significant in the multivariable model as an independent variable, thus showing that radiological and histological information added value to diagnosing. Zhu et al. illustrated that the radiomic features derived with the help of imaging are able to find eosinophilic CRSwNP¹⁸, which promotes a greater idea that the information about the inflammatory endotype is contained in the imaging. It is also worth noting the negative correlation

with neutrophil-dominated inflammation. Neutrophilic CRSwNP is another type of inflammatory situation which could be associated with bacterial dysbiosis, epithelial cytokine and resistance to treatment. A No known interactions with other bacteria were observed by Jiang and Liang in both eosinophilic and noneosinophilic CRSwNP.¹⁹ The inverse correlation modeled does not mean that fungi are not able to interact with neutrophils, but indicates that the typical fungal-associated polyp type is more type 2 and eosinophilic. Mixed inflammation is to be identified since microbial interactions and previous exposure to corticosteroids/antibiotics can change cellular composition.²⁰

Age showed only a weak association in the adjusted analysis. Fungus-positive patients were slightly younger, but CRSwNP and fungal rhinosinusitis occur across a broad age range. Age should therefore be interpreted as a contextual variable rather than a defining diagnostic feature. Methodological strengths of the study model included a relatively large sample, paired tissue and mucin assessment, use of multiple fungal-detection methods, structured histopathological evaluation, and multivariable analysis. Limitations: The definition modeled only counted positivity by any means, maximizing sensitivity but at the cost of analogizing colonization with disease. The special stains were presumed to be used everywhere, which is not likely to be a useful strategy. Transport, previous therapy and laboratory experience may influence the culture and PCR findings. The cross-sectional design would be able to measure but not to determine causality. Lastly, total IgE, fungal-specific IgE, postoperative recurrence and skin testing was not observed, which restricted the formal classification of AFRS. In this cohort, fungal detection was associated with allergic/eosinophilic mucin, Charcot-Leyden crystals, marked tissue eosinophilia, epithelial damage, and CT hyperattenuation, while neutrophil-predominant inflammation showed an inverse association. Discordance among culture, histochemical staining, and PCR supports a combined diagnostic approach integrating histopathology, mycology, imaging, and clinical findings.

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Authors Contribution:

Kousar Ghaffar: Conception and design, acquisition of data analysis and interpretation of data, drafting and critical revision, final approval of the version to be published.

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