THE EXPRESSION AND CLINICAL VALUE OF SP-A AND STAT3 IN CHRONIC SINUSITIS WITH NASAL POLYPS

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ABSTRACT

Objective*: To analyze the expression and clinical value of SP-A and STAT3 in chronic sinusitis with nasal polyps (CRSwNP).*

Methods: *Patients who underwent preoperative nasal endoscopy and sinus CT examination in our hospital from January 2019 to January 2020 were collected and divided into a chronic sinusitis group (n=52), a CRSwNP group (n=124), and the control group (n=40). According to the clinical classification and staging of chronic sinusitis and nasal polyps and the evaluation criteria for the efficacy of endoscopic sinus surgery, the CRSwNP components were divided into a type II stage 1 group (n=26), type II stage 2 group (n=35), type II stage 3 group (n=36), and type III group (n=27). The collected tissue pieces were made into paraffin sections, STAT3 was measured by immunohistochemistry PV6001 method, and SP-A level was measured by the conventional immunohistochemistry streptomyces antibioprotein-peroxidase staining method. A Kappa test was used to evaluate the consistency of SP-A and STAT3 positive rates with pathological results.*

Results: *The positive rates of SP-A and STAT3 in the CRSwNP group and chronic sinusitis group were significantly higher than those in the control group. The positive rates of SP-A and STAT3 in the CRSwNP group were significantly higher than those in the chronic sinusitis group, and the difference was statistically significant (P<0.05). There was no significant difference in the positive rates of SP-A and STAT3 among patients with different nasal polyps groups (P>0.05). The sensitivity of SP-A for diagnosing CRSwNP was 77.42%, and the specificity was 86.96%. The sensitivity of STAT3 for diagnosing CRSwNP was 74.19%, and the specificity was 85.87%. The sensitivity of the two combined diagnosis of CRSwNP was 88.26%, and the specificity was 92.38%.*

Conclusion: *SP-A and STAT3 have significantly high expression in CRSwNP patients. The extent of inflammation and the severity of lesions do not affect the expression of SP-A and STAT3. Both have certain value in the diagnosis of CRSwNP, and the combination of the two is of high value and can be widely used in clinical practice.*

Keywords: *SP-A, STAT3, chronic sinusitis, nasal polyps, expression, clinical, value, research.*

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Introduction

Nasal polyps are hyperplastic tissues protruding from the mucosa of the nasal cavity or sinuses. Chronic rhinosinusitis with nasal polyps (CRSwNP) is a subtype of nasal polyps, which seriously endangers the quality of life and health of patients[1]. Cytokines play a biological role in the occurrence and development of CRSwNP. Studies have shown that inflammatory factors affect the formation and development of CRSwNP[2]. The signal transducer and activator of transcription 3 (STAT3) are closely related to cell proliferation, apoptosis, inflammation, and neovascularization[3]. Studies have found that the abnormal expression of STAT3 in CRSwNP patients may be related to the occurrence and development of CRSwNP[4]. Pulmonary surfactant (PS) is a type of complex composed of lipids and surface-active proteins located on the surface of alveolar epithelial cells. There are four types of lung surfactant protein

(SP) discovered so far. Among them, SP-A plays an important role in maintaining the stability of the alveolar surface and maintaining the local immune function of the lung tissue. In recent years, studies at home and abroad have found that SP-A is also expressed on the surface of the nasal mucosa[5]. This experiment takes preoperative nasal endoscopy and sinus CT examination patients who were admitted to our hospital from January 2019 to January 2020 as the observation object and aims to analyze the expression and clinical value of SP-A and STAT3 in CRSwNP.

Materials and methods

A group of patients undergoing preoperative nasal endoscopy and sinus CT examinations who were admitted to our hospital from January 2019 to January 2020 were collected. According to the type of disease, they were divided into the chronic sinusitis group (n=52), CRSwNP group (n=124), and the control group (n=40).

Inclusion criteria for nasal polyps:

• The postoperative pathological diagnosis was CRSwNP;

• Patients and their families were informed and signed informed consent book.

Inclusion criteria for chronic sinusitis:

• Patients were diagnosed with deviation of nasal septum with chronic sinusitis;

• All the inferior turbinate mucosa tissues were resected when the nasal septum deviation was corrected, and the mucosa of inferior turbinate was partially excised;

• Patients and their families were informed and signed the informed consent book.

Inclusion criteria for the control group:

• Patients had simple septum deviation with turbinate hypertrophy;

• All patients underwent nasal septum deviation correction and partial inferior turbinectomy, and the excised turbinate tissue was retained;

• Patients and their families were informed and signed the informed consent book.

Three groups of exclusion criteria:

• Patients had a history of bronchial asthma, primary ciliary dyskinesia, aspirin-specific reactivity, cystic fibrosis, immune deficiencies, bronchiectasis, chronic obstructive pulmonary disease, or autoimmune diseases;

• Patients had a history of hormonal or antihistamine drug use;

• Respiratory tract infection occurred in the past two weeks;

• The patient refused the experiment or terminated the experiment for other reasons.

According to the "Clinical classification and staging of chronic sinusitis and nasal polyps and the evaluation criteria for the efficacy of endoscopic sinus surgery", formulated by the Otorhinolaryngology Branch of the Chinese Medical Association and the Editorial Committee of the Chinese Journal of Otorhinolaryngology[6], the CRSwNP components were then divided into a type II and phase 1 group (n=26), type II stage 2 group (n=35), type II stage 3 group (n=36), and type III group (n=27). There were 124 cases in the CRSwNP group, including 64 males and 60 females. The average age was (45.06±9.78) years old, and the average BMI value was (20.05±0.98) kg/m^2. There were 52 patients in the chronic sinusitis group, 28 males and 24 females, with an average age of (45.12±9.96) years and an average BMI value of (20.08±1.02) kg/m^2. There were 40 cases in the control group, 22 males and 18 females. The average age was (45.04±10.02) years, and the average BMI value was (20.04±1.03) kg/m^2. There was no significant difference in age, gender, or BMI value among the subjects in each group (P>0.05).

Observation indicators

Preparing the specimen

After processing the collected specimens, they were soaked in formalin fixative. After taking them out, the tissue masses were thoroughly washed with running water, and the tissue masses were soaked in 75%, 95%, and 95% alcohol for 60 minutes, respectively. They were soaked in anhydrous ethanol four times for 40 minutes each time, and then the tissue blocks were soaked in xylene for 15 minutes.

The tissue was placed in four waxed cups in turn. The tissues were embedded and marked at the same time. The cleaned glass slides were placed in acidic liquid for one day, dried and soaked in anhydrous ethanol for two hours, and dried and stored in a clean slide box. Polylysine was used to prevent it from falling off. The embedded wax block was trimmed, the section thickness was adjusted to 4 μm, and sectioning was undertaken. The slices were unfolded in a constant temperature water bath, the prepared glass slides were used to remove the slices, and they were placed in a 60 °C constant temperature box to bake the slices. The slices were unfolded in a thermostatic water bath box, the treated spare slide

dredges were used, and they were put into a 60 °C thermostatic oven for baking.

Determination of STAT3 by PV6001 immunohistochemistry method

The prepared paraffin sections complete the conventional dewaxing and hydration process. The slices were placed in hydrogen peroxide for 10 minutes at room temperature. First, they were rinsed with distilled water twice, and then rinsed with phosphate buffer three times for five minutes.

The slices were placed in a pressure cooker containing ethylenediaminetetraacetic acid diluted to the working concentration and boiled for two minutes after spraying. The section was taken out and let stand for a while, then rinsed with phosphate buffer three times for five minutes. The rabbit anti-human STAT3 antibody was diluted 1:100, and 50 μL rabbit anti-human STAT3 antibody was added dropwise. After dropping the primary antibody, the slices were placed in a refrigerator at 4 °C overnight. After slicing, they were rinsed with phosphate buffer three times for five minutes.

Biotin-labeled secondary antibody was added to the sliced tissue. Three five-minute washes with phosphate buffer were undertaken. Fifty μL of DAB chromogenic reagent was added to the sliced tissue. The color reaction was observed dynamically, and the reaction was finished by washing with running water; the hematoxylin was counter-stained for half a minute and washed with running water. Gradient alcohol was used for dehydration. The slices were put in xylene for transparency. They were fixed with neutral gum and covered with a cover glass.

Routine immunohistochemistry for detection of SP-A level by streptomyces avidin-peroxidase staining method

The prepared paraffin sections completed the conventional dewaxing and hydration process. They were repaired with ethylenediaminetetraacetic acid, then primary antibody and secondary antibody were added to incubate, color was developed with diaminobenzidine (DAB) and they were counterstained with hematoxylin, after dehydration with gradient alcohol, treatment with transparent xylene and being mounted with gum. The negative control used phosphate buffer instead of the primary antibody to make tablets.

Result analysis

Two pathologists used a double-blind method to score the expression of SP-A and STAT3 in each group of tissues. The positive rate of cells: If no positive cells were seen under the microscope magnification 400 times, and the ratio of the total number of cells was less than 5%, zero points were scored. If the positive rate of cells was between 5%–35%, one point was scored.If the positive rate of cells was between 35%–65%, two points were scored.

If the cell positive rate was greater than 65%, three points were scored. Degree of color development: Zero points were given for no obvious color development or no color development; one point was given for earthy yellow color development; two points were given for brownish yellow color development; and three points were given for dark brown color development.

The scores obtained by the two criteria mentioned above were added and divided by 2; that is, the score of a certain 400x field of view. Using the principle of randomness, a total of five different 400-fold visual fields were taken to calculate their average score and set as the score of the experimental specimen. A score of 0 was considered negative (-), and a score of 0.5-3 was considered positive (+).

Statistical methods

The data in this study were analyzed by the SPSS20.0 software package. All measurement data were compared with ($\bar{x}\pm s$), and the comparison between groups was by t test; the count data were all represented by percentages, and the comparison between groups was by χ^2 test.

A Kappa test was used to evaluate the consistency between the positive rates of SP-A and STAT3 and the pathological results. Kappa <0.40 indicated poor consistency between the two; Kappa between 0.40–0.75 indicated general consistency; Kappa >0.75 indicated better consistency. The statistical result was statistically significant at $P<0.05$.

Results

Comparison of SP-A and STAT3 positive rates in each group of patients

The positive rates of SP-A and STAT3 in the CRSwNP group and chronic sinusitis group were significantly higher than those of the control group. The positive rates of SP-A and STAT3 in the CRSwNP group were significantly higher than those in the chronic sinusitis group, and the difference was statistically significant ($P<0.05$). See Table 1, 2 and Figure 1, 2.

Group	Case	SP-A		
		-	+	Positive rates
Control	40	38	2	5.00%
Chronic sinusitis	52	42	10	19.23%
CRSwNP	124	38	96	77.42%
χ^2				76.905
P				<0.001

Table 1: Comparison of SP-A positive rate of patients in each group (cases, %).

Group	Case	STAT3		
		-	+	Positive rates
Control	40	39	1	2.50%
Chronic sinusitis	52	40	12	23.08%
CRSwNP	124	32	92	74.19%
χ^2				80.104
P				<0.001

Table 2: Comparison of STAT3 positive rate of patients in each group (cases, %).

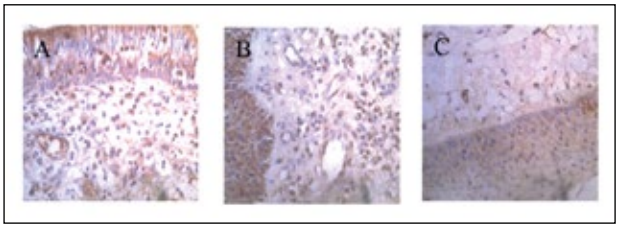

Figure 1: SP-A expression of patients in each group.
A: The expression of SP-A in the CRSwNP group; B: The expression of SP-A in the chronic sinusitis group; C: The expression of SP-A in the control group.

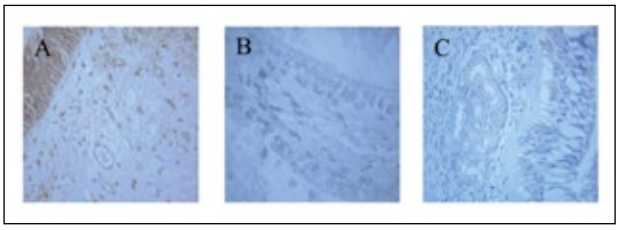

Figure 2: TAT3 expression of patients in each group.
A: The expression of STAT3 in the CRSwNP group; B: The expression of STAT3 in the chronic sinusitis group; C: The expression of STAT3 in the control group.

Comparison of the positive rates of SP-A and STAT3 in patients with different nasal polyps

There was no significant difference in the positive rates of SP-A and STAT3 among patients with different nasal polyps groups (P>0.05). See Tables 3 and 4.

Analysis of the value of SP-A and STAT3 in the diagnosis of nasal polyps

The sensitivity of SP-A for diagnosing CRSwNP was 77.42% and the specificity was 86.96%; the sensitivity of STAT3 for diagnosing CRSwNP was 74.19%, and the specificity was 85.87%; the sensitivity of the two combined diagnosis of CRSwNP was 88.26%, and the specificity was 92.38 %. See Table 5.

Group	Case	SP-A		
		-	+	Positive rates
Type II Phase 1	26	10	16	61.54%
Type II Phase 2	35	13	22	62.86%
Type II Phase 3	36	12	24	66.67%
Type III	27	8	19	70.37%
χ^2				0.591
P				0.898

Table 3: Comparison of SP-A positive rate of patients with different nasal polyps.

Group	Case	SP-A		
		-	+	Positive rates
Type II Phase 1	26	8	18	69.23%
Type II Phase 2	35	10	21	60.00%
Type II Phase 3	36	11	24	66.67%
Type III	27	10	22	81.48%
χ^2				0.016
P				0.999

Table 4: Comparison of STAT3 positive rate among different groups of patients with nasal polyps.

Index	Sensitivity	Specificity
SP-A	77.42%	86.96%
STAT3	74.19%	85.87%
Combined	88.26%	92.38%

Table 5: Analysis of the value of SP-A and STAT3 in the diagnosis of nasal polyps.

Discussion

CRSwNP is most common in the maxillary sinus, ethmoid sinus, middle nasal passage, and middle turbinate. The common manifestations are nasal obstruction, increased nasal discharge, headache, loss of smell, and runny nose. It can also cause complications such as otitis media, rhinopharyngitis, and snoring. It has an obvious tendency to relapse, is closely related to a variety of respiratory inflammatory diseases, and is one of the important diseases that seriously affect quality of life and physical health[7-9].

At present, surgical treatment and drug treatment are mainly used in clinical practice, but the recurrence rate after treatment is still at a high level[10]. In recent years, with the development of nasal endoscopic surgery technology and the

progress of postoperative treatment, the recurrence rate of CRSwNP has been reduced to 15%. However, there are still patients who relapse after treatment, which seriously affects the health of patients, reduces their quality of life, and brings them a heavy psychological pressure and spiritual burden[11]. Therefore, analyzing the etiology and pathogenesis of CRSwNP is of great significance for early diagnosis and early treatment.

SP-A is secreted by the respiratory epithelium and exists in the liquid on the surface of the respiratory tract. It has the function of regulating natural immune cells and is the first line of defense against pathogenic microorganisms[12]. Recent studies have found that SP-A exists in tissues and organs other than the human lungs. Sinus mucosal epithelium belongs to the upper respiratory tract epithelium, and SP-A is involved in natural immune regulation and may be related to the pathogenesis of chronic sinusitis[13]. STAT3 is a nuclear transcription factor discovered in recent years.

It can enter the nucleus when stimulated by extracellular signals such as growth factors, regulate cell functions, and is closely related to tumor occurrence, development, and apoptosis. In recent years, studies have found that in a variety of human malignant tumors, STAT3 has abnormal expression and enhanced activity, and is an oncogene[14]. Studies have found that STAT3 is abnormally expressed in patients with sinus squamous cell carcinoma[15]. This experiment speculated that STAT3 also plays an important role in the occurrence and development of CRSwNP. In this experiment, the positive rates of SP-A and STAT3 in the CRSwNP group and the chronic sinusitis group were significantly higher than those in the control group, and the positive rates of SP-A and STAT3 in the CRSwNP group were significantly higher than those in the chronic sinusitis group, and the difference was statistically significant ($P<0.05$). There was no significant difference in the positive rates of SP-A and STAT3 among patients with different nasal polyps groups ($P>0.05$).

It was suggested that SP-A and STAT3 and CRSwNP play a role in the pathogenesis of CRSwNP, and the extent of inflammation and the severity of the lesion do not affect the expression of SP-A and STAT3, which may provide strong test support for the diagnosis of CRSwNP.

In order to further analyze the relationship between SP-A and STAT3 and CRSwNP, this experiment used a Kappa test to evaluate that the sensitivity of SP-A for diagnosing CRSwNP was 77.42% and the specificity was 86.96%. The sensitivity of STAT3 for diagnosing CRSwNP was 74.19%, and the specificity was 85.87%. The sensitivity of the two combined diagnoses of CRSwNP was 88.26%, and the specificity was 92.38%. It was suggested that SP-A and STAT3 have better predictive value in diagnosing CRSwNP, and the combined detection has better predictive value in diagnosing CRSwNP than single index detection, which helps doctors diagnose CRSwNP early and take corresponding measures is extremely important for the effective treatment of patients.

In summary, SP-A and STAT3 have significantly high expression in CRSwNP patients. The extent of inflammation and the severity of lesions do not affect the expression of SP-A and STAT3. Both have certain value in the diagnosis of CRSwNP. The combination of the two has high value and can be widely used in clinical practice.

References

1) Liu JX, Liao B, Yu QH, Wang H, Liu YB, et al. The IL-37-Mex3B-Toll-like receptor 3 axis in epithelial cells in patients with eosinophilic chronic rhinosinusitis with nasal polyps. J Allergy Clin Immunol 2020; 145: 160-172.
2) Dadgarnia M, Rahmani A, Baradaranfar M, Atighechi S, Zand V, et al. The relationship between endoscopic and radiologic findings and olfactory status of patients with chronic rhinosinusitis with nasal polyps before and after the endoscopic sinus surgery. Eur Arch Otorhinolaryngol 2019; 276: 397-400.
3) Veloso-Teles R, Cerejeira R. Endoscopic sinus surgery for chronic rhinosinusitis with nasal polyps: Clinical outcome and predictive factors of recurrence. Am J Rhinol Allergy 2017; 31: 56-62.
4) Wang J, Xiang B, Dolinsky VW, Kardami E, Cattini PA. Cardiac Fgf-16 Expression Supports Cardiomyocyte Survival and Increases Resistance to Doxorubicin Cytotoxicity. DNA Cell Biol 2018; 37: 866-877.
5) Hong JH, Lu YC, Huang CY, Pu YS. Androgen deprivation therapy increased the risk of pulmomary embolism in patients with prostate cancer - from 24,464 patients, Taiwan National Health Insurance Research Database. Eur Urol 2018; 17: 1085-1088.
6) Zhou H, Chen ZB, Chen X, Chen RX, Gong XY, et al. Guidance and Progress Tour of the Journal of Chinese Medical Association (Otorhinolaryngology Head and Neck Surgery) Suqian Station Meeting Minutes. J Otolaryngol Ophthalmol Shandong Univ 2018; 32: 119-120.

7) Li QC, Cheng KJ, Wang F, Zhou SH. Role of atopy in chronic rhinosinusitis with nasal polyps: does an atopic condition affect the severity and recurrence of disease? J Laryngol Otol 2016; 130: 640-644.
8) Won HK, Kim YC, Kang MG, Park HK, Lee SE, et al. Age-related prevalence of chronic rhinosinusitis and nasal polyps and their relationships with asthma onset. Ann Allergy Asthma Immunol 2018; 120: 389-394.
9) Du J, Ba L, Zhou J, Yu L, Liu R, et al. The role of cysteinyl leukotrienes and their receptors in refractory nasal polyps. Prostaglandins Leukot Essent Fatty Acids 2017; 126: 39-48.
10) Frendø M, Håkansson K, Schwer S, Rix I, Ravn AT, et al. Asthma in ear, nose, and throat primary care patients with chronic rhinosinusitis with nasal polyps. Am J Rhinol Allergy 2016; 30: 67-71.
11) Gelardi M, Iannuzzi L, De Giosa M, Taliente S, De Candia N, et al. Non-surgical management of chronic rhinosinusitis with nasal polyps based on clinical-cytological grading: A precision medicine-based approach. Acta Otorhinolaryngol Ital 2017; 37: 38-45.
12) Jacintha M, Neeraja P, Sivakumar M, Chinnaraj K. Comparative Study of MnFe2 O 4 Nanoparticles Synthesized by Sol-gel Method with Two Different Surfactants. J Supercond Nov Magn 2018; 30: 1-6.
13) Weiss-Errico MJ, O'Shea KE. Detailed NMR investigation of cyclodextrin-perfluorinated surfactant interactions in aqueous media. J Hazard Mater 2017; 329: 57-65.
14) Huynh J, Chand A, Gough D, Ernst M. Therapeutically exploiting STAT3 activity in cancer - using tissue repair as a road map. Nat Rev Cancer 2019; 19: 82-96.
15) Shinde A, Paez JS, Libring S, Hopkins K, Solorio L, et al. Transglutaminase-2 facilitates extracellular vesicle-mediated establishment of the metastatic niche. Oncogenesis 2020; 9: 16.

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