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Predicting the development of complications in patients with rhinosinusitis by cytokine profile indicators

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Abstract

Aim. To develop a method for predicting the development of complications in rhinosinusitis patients based on the cytokine profile study.

Methods. We examined 110 patients with rhinosinusitis and 30 healthy donors (control group). The patients were divided into the group without a complicated course of rhinosinusitis (first group, n=65) and the group with a complicated course of rhinosinusitis (second group, n=45). The blood serum levels of interleukin (IL)-1β, IL-4, IL-8, IL-10, IL-17, IL-18, interferon (IFN)-γ were determined by enzyme immunoassay. Statistical analysis of the results was performed by using Microsoft Excel 2013 and Statistica 12.0 software. Statistically significant differences between the compared groups were determined by using the Mann–Whitney U-test, and $p < 0.05$ was considered significant. Multivariate analysis included correlation analysis and stepwise regression analysis. The mathematical model consistency was determined by using Fisher's F-test, and $p < 0.05$ was considered significant.

Results. Changes in cytokine profile manifested by a decrease in the level of interleukin-1β (p=0.00001), interleukin-4 (p=0.045) and an increase in the level of interleukin-18 (p=0.00001) were revealed in patients with uncomplicated course of rhinosinusitis. The complicated course of rhinosinusitis was characterized by a decrease in the level of interleukin-1β (p=0.00002), interleukin-4 (p=0.049) and an increase in the level of interleukin-8 (p=0.023), interleukin-17 (p=0.00015) and interleukin-18 (p=0.0002). Comparative analysis of the first and the second groups of patients showed an increased level of interleukin-8 (p=0.00001), interleukin-17 (p=0.0001) and reduced levels of interleukin-18 (p=0.00045) in the group of patients with complicated course of rhinosinusitis. Based on interleukin-17, interleukin-8 and interleukin-18 levels, the mathematical model for predicting the development of complications in patients with rhinosinusitis was developed.

Conclusion. The complicated course of rhinosinusitis was characterized by decreased levels of interleukin-1β, interleukin-4 and increased levels of interleukin-17, interleukin-8 and interleukin-18, indicating a more pronounced inflammatory process; for personalized therapy, an approach based on interleukin-17, interleukin-8 and interleukin-18 levels was developed on which the development of a complicated course in patients with rhinosinusitis can be predicted.

Keywords: rhinosinusitis, cytokines, immune system, prognosis.

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Background. The prevalence of rhinosinusitis (RS) in Russia and the world is increasing annually from 5% to 15% of the population [1,2]. Altered immunological reactivity plays a major role in the pathogenesis of RS [3]. As a result, not only the clinical course but also the duration of the disease, as well as the possibility of a complicated course of RS, in particular development of otitis media (OM), depends on the nature of immune disorders.

In the structure of otorhinolaryngological pathology, acute inflammatory diseases of the middle ear accounts for up to 20% of cases [4]. The pathogenesis of OM is based on the classical response of the immune system to damage by foreign agents (such as bacteria and viruses), which upon entry to the mucous membrane of the nasal cavity, pharynx, and auditory tube orifice trigger a cascade of sequential reactions [5].

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Recently, studies have confirmed the main role of cytokines in determining the course of the pathological process and the development of immunological defense reactions in response to the introduction of pathogens and their participation in the inflammatory process in RS and OM [6, 7].

In RS with a prolonged sluggish course, other paranasal sinuses are involved in the inflammatory process. Thus, in 15%–25% of cases, patients with RS had various complications, including acute OM and tubootitis. In the acute inflammatory process and frequent exacerbations of a chronic disease, a rapid generalization of the process occurs following a decrease in the barrier function of tissues and impairment of the immune system [8].

However, despite the existing contemporary methods of examination and treatment, various modern antibacterial drugs, incidence of RS and OM, and frequency of associated complications not only decrease, but steadily grow [9].

This work aimed to develop a method for predicting the development of complications in patients with RS based on their cytokine profile.

Materials and methods of research. The study included 110 patients with RS, who were distributed into two groups. The first group consisted of 65 patients with uncomplicated RS, and the second group included 45 patients with complicated RS. The uncomplicated group included patients aged 30–65 years, 43 women, and 22 men. The average age of the patients was 42.75 ± 2.25 years. The complicated group included patients aged 29–62 years, 27 women and 18 men. The average age of the patients was 39.7 ± 2.3 years. The control group which included 30 healthy people (19 women and 11 men) aged 27–65 years (mean age, 40.7 ± 1.3 years), comparable in gender and age with the patient groups, were examined.

To confirm and validate the results, 75 patients with RS were additionally examined.

The study was conducted in the Otorhinolaryngology Department of the G.A. Zakharyin Clinical Hospital No. 6 (Penza) in the period from 2019 to 2020. Immunological studies were performed in the central research laboratory of the Penza Institute for Advanced Medical Education. RS was diagnosed in all patients in accordance with the new European EPOS 2020 guidelines. In patients with complicated RS, OM developed which was diagnosed based on the All-Russian Clinical Recommendations of 2016. Informed consent was obtained from the patients, and the study was approved by the local ethics committee of the Penza Institute for Advanced Medical Education (Protocol No. 85 dated 02/20/2013).

In this study, 5 ml of blood was sampled from the cubital vein of patients and healthy individuals; the sample was placed into Vacuette vacuum tubes with a blood coagulation activator. In the patient groups and control group, the levels of interleukins (IL), namely, IL-1β, IL-8, IL-17, IL-18, IL-4, and IL-10, as well as interferon γ (Vector-Best, Novosibirsk), were determined in the blood serum by the enzyme immunoassay method. An enzyme immunoassay analyzer Stat Fax 3200 was used to measure the concentration of cytokines, and the values obtained were expressed in picograms per 1 ml (pg/ml).

Statistical analysis was performed using the Statistica 12.0 and Microsoft Excel 2013 software (USA). Values were expressed as a median and interquartile range ($Q_{0.25}$–$Q_{0.75}$). Statistical differences between the groups were assessed using the Mann–Whitney U-test, and their significance was set at $p < 0.05$. Multivariate analysis included correlation analysis and stepwise regression analysis. The significance of the mathematical model was established using Fisher's F-test and was set at $p < 0.05$.

Results and discussion. Table 1 presents the data obtained on the indicators of the cytokine profile of patients with complicated and uncomplicated RS and of healthy individuals.

The analysis of indicators of cytokines in the studied groups revealed significant differences. The uncomplicated and complicated groups presented a decrease in the IL-1β level compared with the control group ($p = 0.00001$ and $p = 0.00002$, respectively; Mann–Whitney U-test). The immunological role of IL-1β consists in triggering the first stages of the immune response, with the involvement of immunocompetent cells in the process. However, low concentrations of IL-1β disrupt the processes of antigen presentation and do not trigger an immune response. As a result, insufficient synthesis of IL-1β contributes to the formation of a protracted and recurrent course of purulent RS [10, 11].

In addition, both patient groups showed a lower level of IL-4 than the control group ($p = 0.045$ and $p = 0.049$, respectively; Mann–Whitney U-test). The most important function of IL-4 is stimulation of differentiation of antigen-activated T-helper lymphocytes $CD4^+$ toward Th2 [12]. In addition, IL-4 has the ability to suppress Th1 activation and the synthesis of tumor necrosis factor and interferon γ and thereby reduce their immunostimulating effect [13].

The level of IL-8 was increased in the complicated group in comparison with the control group ($p = 0.023$; Mann–Whitney U-test) and uncomplicated group ($p = 0.00001$; Mann–Whitney U-test). IL-8 regulates the migration of neutrophilic granulocytes into the tissue, which are the first to eliminate pathogenic microorganisms [13]. There is evidence of an increased level of IL-8 in the focus of inflammation in OM [14]. In addition, increased

Table 1. Analysis of cytokine values in groups of patients with complicated and uncomplicated rhinosinusitis and in healthy individuals

Values	Uncomplicated group (n = 65), Me [$Q_{0.25}$–$Q_{0.75}$]	Complicated group (n = 45), Me [$Q_{0.25}$–$Q_{0.75}$]	Healthy individuals (n = 30), Me [$Q_{0.25}$–$Q_{0.75}$]
IL-1β, pg/ml	2.4 [0–3.2]*	2.6 [0–3.2]*	5.9 [3.1–8.3]
IL-4, pg/ml	0.73 [0.2–1.7]*	1.2[0–1.8]*	2.5 [1.5–3.7]
IL-8, pg/ml	2.6 [0.7–5.9]**	7.6 [7.0–8.2]*	4.5 [3.0–5.9]
IL-10, pg/ml	1.4 [0–4.5]	2.7 [1.0–3.8]	2.2 [1.5–2.8]
IL-17, pg/ml	0 [0–0.8]**	2.1 [1.8–2.3]*	0.6 [0–1.0]
IL-18, pg/ml	309.2 [219.0–399.8]*.**	143.6 [121.0–171.2]*	98.1 [75.0–112.8]
Interferon γ, pg/ml	0 [0–6.1]	2.3 [0–7.3]	4.3 [2.7–5.8]

Note: *statistically significant differences between the patient groups and the control group ($p < 0.05$); **statistically significant differences between the uncomplicated and complicated groups ($p < 0.05$); IL, interleukin.

synthesis of IL-8 leads to the formation of a chronic focus of aseptic inflammation in RS [15].

The level of IL-17 was significantly increased in the complicated group in comparison with the control group ($p = 0.00015$; Mann–Whitney U-test) and complicated group ($p = 0.0001$; Mann–Whitney U-test). IL-17 is a product of a subpopulation of T-helpers-17 (Th17), having a pro-inflammatory activity, and its increased level indicates and inflammatory activity in patients with otorhinolaryngological pathology [12, 16].

The level of IL-18 significantly increased in the uncomplicated group ($p = 0.00001$; Mann–Whitney U-test) and complicated group ($p = 0.0002$; Mann–Whitney U-test) in comparison with the control group. Moreover, the level of IL-18 significantly increased in the uncomplicated group ($p = 0.00045$; Mann–Whitney U-test). IL-18 functions as one of the key pro-inflammatory cytokines in the formation of an innate and adaptive immune response. As IL-18 stimulates the synthesis of interferon γ, this cytokine is significant in purulent otorhinolaryngological pathology [17, 18].

Thus, a comparative analysis of the uncomplicated and complicated groups showed increased levels of IL-8 and IL-17 and a reduced level of IL-18 in the complicated group, which indicates a more severe and pronounced inflammatory process in these patients.

In addition, the correlation analysis revealed a strong direct significant relationship between the complicated and uncomplicated groups in terms of IL-17 ($R = 0.7231$, $p = 0.0000$) and IL-8 ($R = 0.7947$, $p = 0.0000$) levels, as well as the presence of medium-strength feedback with the IL-18 index ($R = –0.6070$, $p = 0.0002$). Therefore, levels of IL-8, IL-17, and IL-18 were taken as immunological criteria for a complicated course of RS, which were used in this study to predict the development of a complicated course of RS.

For accurate and convenient prediction using stepwise regression analysis, the following mathematical model was created, which included the immunological criteria identified:

$$y=0{,}000391\times x_1+0{,}389649\times x_2+0{,}000827\times \times x_3+0{,}358001,$$

where y is the development of a complicated course in patients with RS, 0 without development, and 1 with development and x_1, x_2, and x_3 represents IL-8, IL-17, and IL-18, respectively.

The quality of the obtained mathematical model for predicting the development of a complicated course of RS was assessed according to the coefficient of determination, which revealed its informational significance, value of the F-criterion, and level of the model significance. As a result, the model has a high informational significance (determination coefficient R2 = 0.71800228) and statistical value [$F(3.26) = 22.0664$; $p < 0.0000$].

By substituting the values of IL-8, IL-17, and IL-18 into the indicated mathematical equation, the criteria for referring patients with RS to having a complicated course or to having an uncomplicated course were obtained. Thus, y value of 0–0.49 indicates absence of complicated course of RS and y value of 0.5 and higher indicates the development of a complicated course of RS.

To confirm and test the obtained mathematical equation for predicting complications in the form of OM in patients with RS, we analyzed IL-8, IL-17, and IL-18 indicators in 75 patients with RS who visited the Otorhinolaryngology Department. The mathematical calculation predicted the development of OM in 16 of 75 patients with RS. Further monitoring of these patients revealed that 15 of them developed a complication in the form of OM, despite the standard therapy received. Thus, a correct prognosis was confirmed in 96% of cases.

This mathematical model can be recommended as an additional laboratory method for predicting the development of complications in the form of OM in patients with RS, which provides a personalized approach to patient therapy. Prescribing immunotherapy to patients in addition to the standard therapy based on the levels of IL-17, IL-8, and IL-18 will increase the efficiency of treatment, as well as minimize the risk of complications in patients with RS.

CONCLUSIONS

1. Compared with the control group, the complicated group had decreased levels of IL-1β and IL-4 and an increased level of IL-8, IL-17, and IL-18, which indicates a shift in the balance of cytokines toward pro-inflammatory cytokines and pronounced inflammatory process.

2. A complicated course of RS is determined using levels of IL-8, IL-17, and IL-18.

3. For personalized therapy, an approach was developed that used IL-8, IL-17, and IL-18, which can be used to predict the development of complications in patients with RS.

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REFERENCES

1. Volkov A.G., Stagnieva I.V., Eroshenko A.Iu. Significance of local pain syndrome in patients with frontitis. *Vestnik otorinolaringologii*. 2010; (4): 38–40. (In Russ.)

2. Lopatin A.S., Gamov V.P. *Ostryy i khronicheskiy rinosinusit: etiologiya, patogenez, klinika, diagnostika i printsipy lecheniya.* (Acute and chronic rhinosinusitis: etiology, pathogenesis, clinical picture, diagnosis and treatment principles.) M.: Pervyy MGMU im. I.M. Sechenova, 2013; 91 p. (In Russ.)

3. Svistushkin V.M, Shevchik E.A. Topical antibacterial therapy in the practice of otolaryngologist. *Meditsinskiy sovet*. 2019; (8): 10–17. (In Russ.) DOI: 10.21518/2079-701X-2019-8-10-17.

4. Eremeyeva K.V., Budeikina L.S. Specifics of the local treatment of acute otitis media. *Meditsinskiy sovet*. 2017; (8): 84–86. (In Russ.) DOI: 10.21518/2079-701X-2017-8-84-86.

5. Kryukov A.I., Kunelskaya N.L., Ivoylov A.Yu., Garov E.V., Garova E.E., Yanovskiy V.V. New trends in the treatment of uncomplicated acute otitis media in adults: evidence-based approach. *Meditsinskiy sovet*. 2013; (7): 36–40. (In Russ.) DOI: 10.21518/2079-701X-2013-7-36-40.

6. Bayke E.V., Urazova O.I. The cytokine profile of blood in dependence on polymorphism of cytokine genes in patients with chronic purulent otitis media. *Bulletin of Siberian Medicine.* 2018; 17 (1): 24–35. (In Russ.) DOI: 10.20538/1682-0363-2018-1-24-35.

7. Baranova N.I., Shkurova N.A., Fedin A.V. Features of the microbial picture, the activity of the phagocytosis system and the profile of key cytokines in patients with rhinosinusites and otitis media caused by the type 2 diabetes. *Izvestiya vysshikh uchebnykh zavedeniy. Povolzhskiy region*. 2020; (2): 5–14. (In Russ.) DOI: 10.21685/2072-3032-2020-2-1.

8. Popov N.N., Gariuk G.I., Filatova I.V., Ogniven-ko E.V. Clinical immunological characteristics of the course of purulent maxillary sinusitis in patients with diabetes mellitus. *Mezhdunarodnyy meditsinskiy zhurnal*. 2007; (1): 103–107. (In Russ.)

9. Gurov A.V. Features of antibacterial therapy of acute purulent sinusitis and acute purulent otitis media. *Meditsinskiy sovet*. 2018; (6): 78–82. (In Russ.) DOI: 10.21518/2079-701X-2018-6-78-82.

10. Stagniyeva I.V., Simbirtsev A.S. Role of cytokine profile in manifestation of pain in rhinosinusitis. *Tsitokiny i vospaleniye*. 2015; 14 (4): 29–34. (In Russ.)

11. Arefieva N.A., Aznabaeva L.F., Sharipova E.R. The role of interleukin 1-beta in pathogenesis and treatment of recurrent suppurative rhinosinusitis. *Vestnik otorinolaringologii*. 2012; (6): 51–52. (In Russ.)

12. Shkurova N.A., Baranova N.I. Immunological aspects of the pathogenesis of rhinosinusitis and medium otitis with diabetes mellitus type 2. *Rossiyskiy immunologicheskiy zhurnal*. 2014; 8 (3): 641–644. (In Russ.)

13. Simbirtsev A.S. *Tsitokiny v patogeneze i lechenii zabolevaniy cheloveka.* (Cytokines in the pathogenesis and treatment of human diseases.) SPb.: Foliant. 2018; 512 p. (In Russ.)

14. Nacharov P.V., Klyachko L.L., Yanov Yu.K. Cytokines in the pathogenesis of otitis media. *Tsitokiny i vospaleniye*. 2016; 15 (1): 5–11. (In Russ.)

15. Bezrukova E.V., Simbirtsev A.S., Kondratieva E.V., Kalashnikova O.V. The study of the levels of cytokines in nasal secretions of patients with various forms of rhinosinusitis. *Tsitokiny i vospaleniye*. 2012; 11 (2): 63–67. (In Russ.)

16. Klimov A.V., Klimov V.V., Shustova V.A., Tazin I.D. Soderzhaniye IL-8, IL-17, IL-22 i IFN-γ v nazal′nom sekrete detey s khronicheskim adenoiditom i retsidiviruyushchim kariyesom. *Meditsinskaya immunologiya.* 2015; 17 (S): 315. (In Russ.)

17. Khaitov R.M. *Immunologiya: struktura i funktsii immunnoy sistemy.* Uchebnoe posobie. (Immunology: structure and function of the immune system. Tutorial.) M.: GEOTAR-Media. 2013; 280 p. (In Russ.)

18. Fedin A.V., Baranova N.I. Indicators of key cytokines in patients with acute bacterial rhinosinusitis. *Mezhdunarodnyy nauchno-issledovatel'skiy zhurnal*. 2013; (7-5): 78–79. (In Russ.)