# A PROGNOSTIC MODEL FOR THE PREDICTION OF GENERALIZED CHRONIC PERIODONTITIS IN PATIENTS WITH METABOLIC SYNDROME

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The pathogenesis of periodontitis involves a complex inflammatory cascade initiated by biofilm bacteria. The susceptibility to or the risk of developing the disease is determined by the body's response to the invasion, specifically, by the strength of the inflammatory response and the differential activation of immune pathways. In this paper, we propose a model for predicting the risk of severe chronic generalized periodontitis (GCP) in patients with metabolic syndrome based on the levels of tumor necrosis factor alpha (TNF-α) in the periodontal pocket exudate. The analysis of oral cavity cytokine profiles conducted in 537 patients with GCP and comorbid metabolic syndrome showed that increased TNF-α correlated with the severity of GCP: higher levels of TNF-α were observed in patients whose condition was more severe. The prognostic model built in Statistica. 10 allowed us to use TNF-α as a prognostic criterium for GCP severity. We determined the cut-off point above which a high risk of severe GCP can be concluded with 91.2% sensitivity and 70.8% specificity. The spreadsheet in Microsoft Excel 2010 automatically computed the risk of severe GCP from a patient's TNF-α concentrations in the PP, which makes the model convenient for routine clinical use in dentistry.

**Keywords:** periodontitis, metabolic syndrome, cytokines, tumor necrosis factor, TNF, prognostic model

**Author contribution:** Petrukhina NB and Shikh EV conceived the study, analyzed and interpreted the obtained data; Zorina OA analyzed and interpreted the obtained data; Kartysheva EV collected the samples, analyzed the literature and interpreted the obtained data; Kudryavtsev AV prepared the manuscript draft and analyze the literature.

**Compliance with ethical standards:** the study was approved by the Ethics Committee of I.M.Sechenov First Moscow State Medical University (Protocol No. 10–15 dated November 18, 2015). All patients gave informed consent to participate.

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# ПРОГНОСТИЧЕСКАЯ МОДЕЛЬ ДЛЯ ОЦЕНКИ ХРОНИЧЕСКОГО ГЕНЕРАЛИЗОВАННОГО ПАРОДОНТИТА У ПАЦИЕНТОВ С МЕТАБОЛИЧЕСКИМ СИНДРОМОМ

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Патогенез пародонтита включает сложный иммунный воспалительный каскад, который инициируется бактериями биопленки, а восприимчивость или вероятность развития заболевания определяется реакцией организма человека, в частности, величиной воспалительного ответа и дифференциальной активацией иммунных путей. Цель исследования — разработать прогностическую модель для оценки риска развития тяжелой степени хронического генерализованного пародонтита в зависимости от содержания фактора некроза опухоли-α (ФНО-α) в экссудате пародонтального кармана (ПК) пациента. При клинико-инструментальном обследовании 537 пациентов с хроническим генерализованным пародонтитом и метаболическим синдромом установлено, что уровень повышения ФНО-α в содержимом ПК коррелировал со степенью тяжести хронического генерализованного пародонтита (ХГП): более высокие значения цитокина соответствовали более тяжелой степени. Разработанная в программе Statistica.10 прогностическая модель дала возможность использовать уровень ФНО-α в содержимом ПК пациента в качестве прогностического критерия течения ХГП. Определено критическое значение, при превышении которого с диагностической чувствительностью 91,2% и специфичностью 70,8% можно заключить о высоком риске развития тяжелой степени ХГП. Созданное окно в программе Microsoft Excel 2010 позволяет автоматически рассчитывать риск развития тяжелой степени ХГП в зависимости от индивидуального значения концентрации ФНО-α в содержимом ПК пациента, что делает данную модель удобной для применения врачами-стоматологами.

**Ключевые слова:** пародонтит, метаболический синдром, цитокины, фактор некроза опухоли, ФНО, прогностическая модель

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Tumor necrosis factor alpha (TNF-α) is one of the early proinflammatory cytokines that plays a key role in periodontal tissue destruction [1]. Clinical studies have demonstrated that elevated TNF-α is a risk factor for the progression of periodontal diseases. TNF-α mediates periodontal tissue destruction via at least two different pathways. Firstly, it stimulates production of osteoclasts that cause alveolar bone resorption [2, 3]. Secondly, TNF-α promotes the body's early immune response to periodontal pathogens and regulates synthesis of matrix metalloproteinases (MMP) capable of damaging connective tissue. Besides, it is reported that TNF-α levels are increased systemically in patients with obesity or metabolic syndrome [4]. Adipose tissue cells secrete TNF-α; therefore, accumulation of excess fat leads to chronic systemic inflammation [5, 6]. It has been shown that TNF-α levels also correlate with insulin resistance [7]. TNF-α is a paracrine mediator: its local activity is aimed at reducing sensitivity of adipocytes to insulin [8]. There is a reciprocal connection between periodontal disease and

metabolic syndrome. The severity of systemic inflammation in patients with metabolic syndrome can affect local inflammation in the periodontium, while the products of periodontal inflammation can stimulate secretion of systemic cytokines. In this study we propose a prognostic model for predicting the risk of severe generalized chronic periodontitis based on TNF-α concentrations in the exudate from a periodontal pocket (PP).

## METHODS

We examined 537 patients (243 females and 294 males; 45.25% vs. 54.75%, respectively) aged 35 to 65 years with clinically diagnosed generalized chronic periodontitis (GCP) and metabolic syndrome. The patients were distributed into 3 age groups: group 1 included patients aged 35–44 years, with the mean age of 41.7 ± 2.1; group 2 consisted of patients aged 45–54 years, with the mean age of 52.2 ± 1.2; group 3 comprised individuals aged 55–65 years, with the mean age of 63.4 ± 1.1. Our study included patients of both sexes aged 35 to 65 years, with clinically diagnosed GCP, comorbid metabolic syndrome and a body mass index ≥ 25 kg/m$^2$, who gave written informed consent to participate. The following exclusion criteria were applied: age under 35 years; hematologic disorders; diseases of the central nervous system, both congenital and acquired; malignancies (cancers, sarcomas); decompensated chronic conditions (myocardial infarction, systemic thromboembolism); pregnancy.

Exudate samples were collected onto filter paper strips introduced into the PP for 30 s. Then, the strips were transferred into Eppendorf tubes containing 1 ml of sterile normal saline and left there for 40 min. After that, the strips were taken out with tweezers, and the content of the Eppendorf tubes was analyzed. TNF-α levels were measured using ELISA kits by BIOSOURCE (Europe S. A.; Belgium); spectrophotometry was done by a microplate reader at 450 nm wavelength. Cytokine concentrations were determined from a standard curve and expressed as pg/ml.

The next step was to create a prognostic model for predicting the risk of developing severe GCP based on TNF-α concentrations. A primary data matrix was generated in Statistica.10 (StatSoft; USA). Model coefficients were calculated in the output spreadsheet and included into the mathematical expression. Then, a ROC curve was constructed and a cut-off point was determined. The cut-off point allows using the model for practical tasks: new data can be assigned to one of the 2 classes depending on their position relative to the cut-off point. Besides, we applied the ROC-curve analysis to assess the diagnostic efficacy of our model by calculating the AUC value (Area Under Curve) using a trapezoidal rule.

## RESULTS

Based on the obtained TNF-α concentrations (Table 1), a prognostic model was built for predicting the risk of developing severe GCP.

The mathematical expression below can be used to calculate the risk of severe periodontal tissue destruction based on the TNF-α concentrations in the PP. The measured TNF-α concentrations should be plugged into the following formula:

$$W = -3.2 + 1.2 \cdot \log_{10}(Y),$$

where $W$ is a risk of developing severe GCP calculated from the cytokine profile of the oral cavity and $Y$ is a TNF-α concentration in the PP expressed as pg/ml.

**Table 1**. Levels of the key proinflammatory cytokine TNF-α in the PP exudate in patients with GCP and comorbid metabolic syndrome

| Parameter | 35–44 years | | 45–54 years | | 55–65 years | |
|---|---|---|---|---|---|---|
| Sex | M | F | M | F | M | F |
| | Mild GCP | | | | | |
| TNF-α (pg/ml) | 576.80 ± 19.49 | 584.96 ± 21.54 | 611.78 ± 21.67 | 634.57 ± 23.5 | 645.67 ± 23.7 | 678.45 ± 24.9 |
| | Moderate GCP | | | | | |
| TNF-α (pg/ml) | 845.44 ± 32.76 | 876.5 ± 33.7 | 848.34 ± 24.5 | 998.56 ± 21.5* | 945.81 ± 32.33 | 1045.33 ± 31.56 |
| | Severe GCP | | | | | |
| TNF-α (pg/ml) | 878.93 ± 32.11 | 911.23 ± 31.67 | 905.78 ± 35.6 | 1145.87 ± 35.11* | 1234.56 ± 33.17 | 1341.54 ± 33.98 |

**Note:** * — shows that the difference is significant.

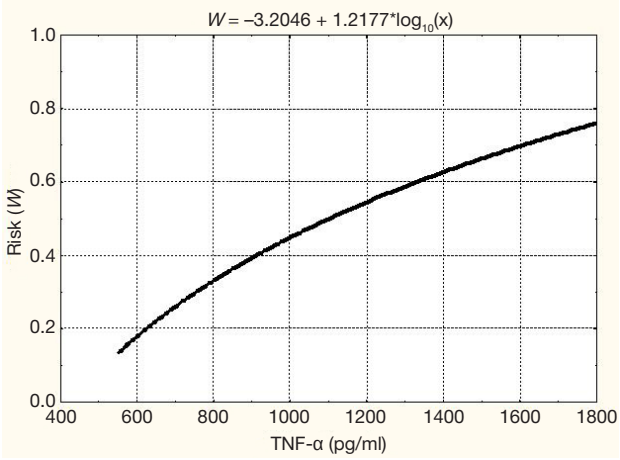


**Fig. 1.** The graph shoes the relationship between the risk of developing severe GCP and the TNF-α concentrations in the PP

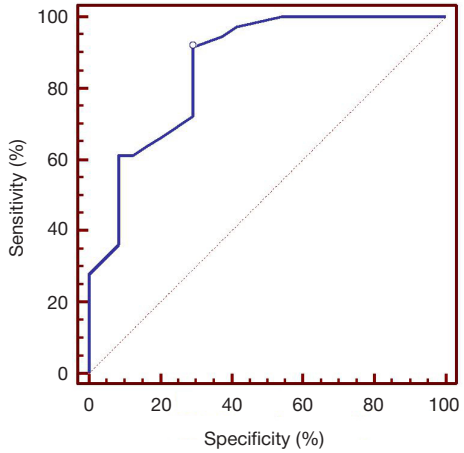


**Fig. 2.** The ROC-curve represents a relationship between the diagnostic sensitivity and specificity in predicting the risk of severe GCP from the cytokine profile of the oral cavity

Fig. 1 is the graphic representation of the relationship between the risk of developing severe GCP and the TNF-α concentrations in the PP. The risk for severe GCP increases as TNF-α levels grow in the PP.

The risk *W* for developing severe GCP was calculated for each study participant from TNF-α concentrations in the PP. Then, the ROC analysis was conducted to determine a critical (cut-off) value for *W* (0.3), above which a high risk for severe GCP could be predicted with maximum specificity and sensitivity.

$W \geq 0.3$ means that the risk for severe GCP is high; $W < 0.3$ means it is low. The diagnostic sensitivity of the method is 91.2%, whereas its specificity is 70.8%.

Fig. 2 features a ROC curve for different values of the prognostic coefficient *W*. Table 2 shows sensitivity and specificity of the method at which $W = 0.3$ had the highest sensitivity and specificity.

The AUC value of $0.862 \pm 0.05$ ($z = 7.3$; $p < 0.001$) and the confidence interval of 0.765–0.959 suggest that *W* has a high diagnostic significance in predicting severe GCP based on the cytokine profile of the oral cavity.

*W* was computed in Microsoft Excel 2010; individual TNF-α concentrations were entered into the highlighted cell (Fig. 3).

## DISCUSSION

TNF-α plays a key role in the pathogenesis of periodontal diseases. When bacterial lipopolysaccharides permeate periodontal tissue, macrophages assisted by CD14-lymphocytes activate a number of innate and adaptive immunity mechanisms through specific receptors. An inadequately strong immune response leads to chronic inflammation and periodontal tissue destruction [9, 10]. Prostaglandin E2, IL1β and TNF-α are key proinflammatory mediators that activate tissue metalloproteinases and thereby stimulate bone resorption by osteoclasts and induce damage to the periodontium [11]. A number of nonimmune periodontal cells, such as epithelial cells and fibroblasts, can recognize and respond to proinflammatory IL1β and TNF-α. Tissue metalloproteinases produced by neutrophils, macrophages, fibroblasts, and osteoclasts promote proteolysis of collagen, gelatin and elastin, destroying the connective tissue components of tooth-supporting structures. Among the members of the TNF superfamily are osteotropic factors, such as the receptor activator of NF-kB ligand (RANKL) and RANK themselves that are synthesized by osteoclasts and promote bone resorption [12]. The binding of the RANK ligand to the RANK receptor is accompanied by a fusion of a few precursor cells into a mature multinucleated osteoclast that immediately starts to destroy bone tissue (Fig. 4).

In light of this, the study of TNF-α levels in the PP of patients opens new possibilities for predicting the severity of GCP.

## CONCLUSIONS

We have found that elevated TNF-α concentrations in the PP correlate with the severity of GCP in patients with metabolic syndrome: higher TNF-α levels are associated with a more

**Table 2**. Sensitivity and specificity of the method at different values of the prognostic coefficient *W* used to predict the risk of severe GCP from the cytokine profile of the oral cavity

| Parameter *W* | Diagnostic sensitivity | CI for DSn | Diagnostic. specificity | CI for DSp |
|---|---|---|---|---|
| > 0.29 | 94.44 | 81.3 – 99.3 | 62.5 | 40.6 – 81.2 |
| > 0.3* | 91.67 | 77.5 – 98.2 | 70.83 | 48.9 – 87.4 |
| > 0.32 | 86.11 | 70.5 – 95.3 | 70.83 | 48.9 – 87.4 |
| > 0.33 | 83.33 | 67.2 – 93.6 | 70.83 | 48.9 – 87.4 |
| > 0.34 | 80.56 | 64.0 – 91.8 | 70.83 | 48.9 – 87.4 |
| > 0.35 | 72.22 | 54.8 – 85.8 | 70.83 | 48.9 – 87.4 |
| > 0.36 | 69.44 | 51.9 – 83.7 | 75 | 53.3 – 90.2 |
| > 0.38 | 66.67 | 49.0 – 81.4 | 79.17 | 57.8 – 92.9 |
| > 0.4 | 63.89 | 46.2 – 79.2 | 83.33 | 62.6 – 95.3 |
| > 0.41 | 61.11 | 43.5 – 76.9 | 87.5 | 67.6 – 97.3 |
| > 0.45 | 61.11 | 43.5 – 76.9 | 91.67 | 73.0 – 99.0 |
| > 0.46 | 55.56 | 38.1 – 72.1 | 91.67 | 73.0 – 99.0 |
| > 0.5 | 44.44 | 27.9 – 61.9 | 91.67 | 73.0 – 99.0 |
| > 0.51 | 36.11 | 20.8 – 53.8 | 91.67 | 73.0 – 99.0 |
| > 0.52 | 27.78 | 14.2 – 45.2 | 100 | 85.8 – 100.0 |

**Note:** * — marks the cut-off point.


**Fig. 3.** A spreadsheet in Microsoft Excel 2010 used for automatic calculations of the risk for severe GCP based on the oral cavity cytokine profile

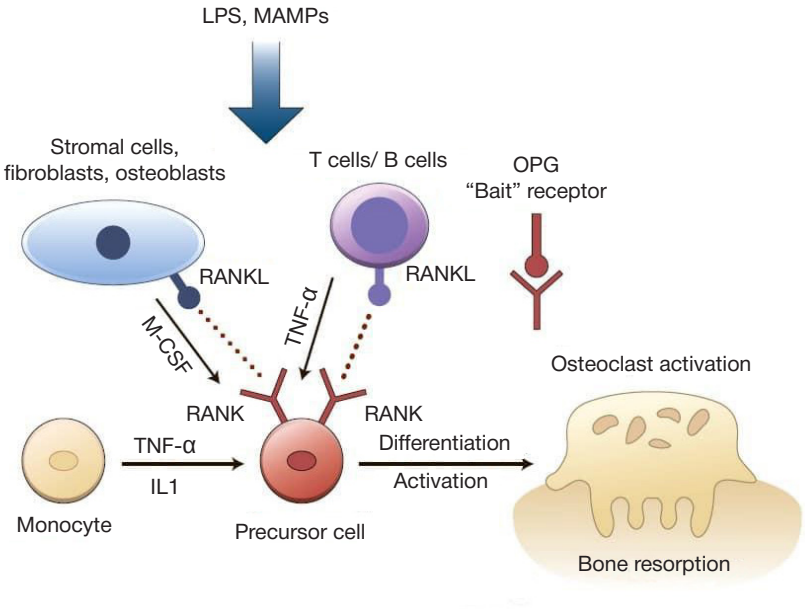


**Fig. 4.** Integration of proinflammatory and osteotropic factors in bone resorption

severe course of the disease. The proposed prognostic model based on the TNF-α concentrations in the PP is a promising and informative noninvasive method that can be used to predict the progression of the disease. The advantages of our model include low costs, availability, fast results, and the simplicity of use, which is crucial for routine dental practice.

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