FEATURES OF DETERMINATION OF PROGNOSTIC AND DIAGNICALLY SIGNIFICANT MARKERS IN THE BLOOD OF PATIENTS WITH PULMONARY TUBERCULOSIS

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Abstract. *Throughout the world, the epidemiological situation with regard to tuberculosis remains difficult. Purpose of the study: to study the immunobiological properties of the organism in newly diagnosed patients with pulmonary tuberculosis. We conducted a study on the presence of prognostic and diagnostically significant markers in the blood serum of 30 patients with a verified diagnosis. A comparative group contains 15 practically healthy persons. All patients underwent general clinical, bacterioscopic examination, chest X-ray. It is determined the concentration of cytokines - TNF- α and CRP, VGF.*

Conclusions: A moderate increase in the level of CRP allows early detection of the course of the pathological process and as certain the formation of a systemic inflammatory response to activate phagocytosis. Correlation of a decrease in the concentration of TNF- α in the blood serum in patients with recurrent disease secondary to an increase in the level of VEGF in the same patients.

Keywords: *blood markers, pulmonary tuberculosis, immunity, cytocynes.*

The relevance of research. Throughout the world, the epidemiological situation regarding tuberculosis remains difficult. There is no country where tuberculosis has ever been eliminated. Tuberculosis is common in every part of the world.

In the Republic of Uzbekistan, the incidence of tuberculosis in recent years remains at a high level. In 2021, the incidence rate was 35.2 per 100,000 populations. The effectiveness of treatment of all newly diagnosed tuberculosis patients in the Republic of Uzbekistan in 2021 was 87.6%.

In these conditions, the problem of increasing the effectiveness of treatment of patients with pulmonary tuberculosis, taking into account the peculiarities of immunobiological properties in various variants of the course of tuberculosis in newly diagnosed patients, becomes highly relevant. To date, they remain little studied, and it is of interest to compare the features of the clinical course of the process and the effectiveness of treatment of pulmonary tuberculosis in the presence of MBT resistance to ALD, taking into account the immunobiological properties and reactivity of the human body. The body's protective response to infection, including MBT, manifests itself in the form of systemic inflammatory response syndrome, which is currently under the close attention of many researchers around the world. The balance of the complex of its constituent reactions or, on the contrary, the violation of their physiological control determines the development and nature of the course of tuberculous inflammation (the predominance of exudative or productive tissue reactions).

Aim. To study the immunobiological properties of the body in newly diagnosed patients with pulmonary tuberculosis.

Materials and methods. In order to study the immunobiological features and clinical course of pulmonary tuberculosis in newly diagnosed patients, we conducted a study for the presence of prognostic and diagnostically significant markers in the blood serum of 30 patients with a verified diagnosis of pulmonary tuberculosis. Comparative group is 15 practically healthy people.

The general clinical examination included a general blood and urine test, a biochemical blood test, and general diagnostic minimums. Upon admission to the clinic, a bacterioscopic examination, sputum culture, and X-ray examinations were performed.

The concentrations of cytokines TNF-α and CRB, VGF in blood serum were determined . Along with the standard examination, all study participants were additionally assessed for the "basal" level of TNF-α and CRP, VGF in the blood serum by enzyme-linked immunosorbent assay.

Results. Of the 30 patients studied: 7 (23.3±7.7%) were women, 23 (76.7±7.7%) were men; the average age for women is 40.5±3.18 years, for men - 47.1±2.38 years. Focal pulmonary tuberculosis was verified in 7 (23.3±7.7%) patients, infiltrative tuberculosis - in 14 (46.7±8.9%), fibrous-cavernous tuberculosis - in 6 (20.0±7.7 %), cavernous tuberculosis - in 2 (6.7±2.2%); and 1 (3.3±1.2%) had tuberculous bronchoadenitis. The patients were divided into 2 groups: group 1 - newly diagnosed patients (13 people - 43.3±9.0%); group 2 - those who returned again with a relapse of the disease (17 people - 56.7±9.0%); Group 3 – 15 practically healthy people.

All patients studied underwent quantitative determination of prognostic and diagnostically significant markers - C-reactive protein (CRP), vascular endothelial growth factor VEGF and alpha-TNF (tumor necrosis factor).

Quantitative determination of C-reactive protein (CRP) attracts close attention in various types of pathology. This is due to the fact that stimulation of CRP synthesis is one of the earliest reactions in the formation of a systemic inflammatory response, is induced by proinflammatory cytokines and creates conditions for the activation of phagocytosis of various pathogens.

The question of the normal level of CRP in the blood is fundamentally important. For a long time it was believed that in healthy people the concentration of CRP does not exceed 10 mg/l. However, in recent years, most authors indicate that in practically healthy people the level of CRP in the blood serum fluctuates between 0-3 m g/l, in 90% of healthy donors the level of CRP does not exceed 3 mg/l, and in 99% it is less than 10 mg/l.

An increase in the level of CRP in 80.7% of patients with pulmonary tuberculosis allows us to consider this indicator as one of the most informative in assessing the activity of the process in this pathology. CRP values in the range of 4-5 mg/l are early indicators of process activity, often preceding clinical manifestations of the disease. The range of increased levels of CRP in tuberculosis patients ranges from 4-5 to 200 mg/l and clearly correlates with such parameters of the severity of the process as the severity of intoxication, the presence and massiveness of bacterial excretion, the prevalence of the process, the presence or absence of decay. A moderate increase in CRP levels of no more than 30-40 mg/l before the start of chemotherapy makes a beneficial effect of treatment more likely. With effective treatment, after 3 months of intensive chemotherapy, the level of CRP decreases significantly and approaches the upper limit of subclinical. interval, indicating that the inflammatory potential remains at a much lower (compared to the initial) level. In case of ineffective treatment, CRP values do not change significantly over the course of 3 months of intensive chemotherapy.

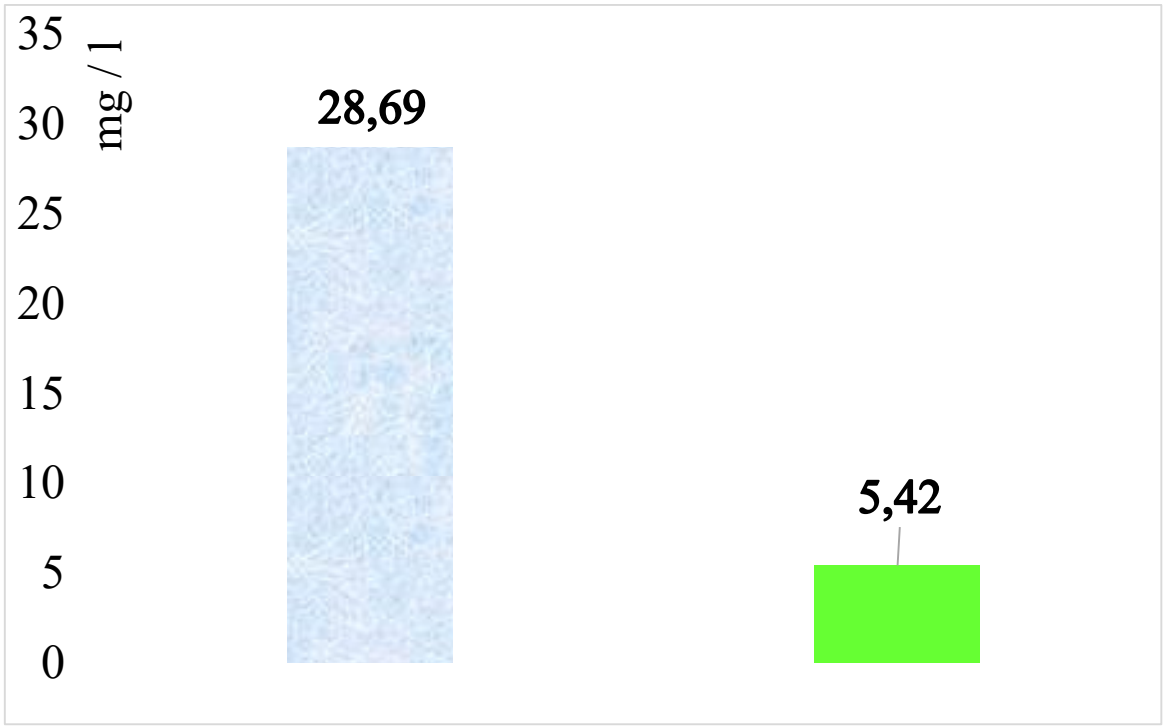

Figure 1. Results of determination of CRP in newly diagnosed (group I) and patients with relapse (group II) pulmonary tuberculosis.

In figure 1 shows the results of determining CRP in newly diagnosed (group 1) and patients with relapse (group 2) pulmonary tuberculosis. Compared to healthy people (CRP concentration 3-5 mg/l), newly diagnosed patients had a significant increase in the marker content in the blood serum (28.69±0.74 mg/l, $p < 0.05$). In patients with recurrent disease, the level of CRP was statistically significantly lower than in newly diagnosed patients with pulmonary tuberculosis (5.42±2.73 mg/l, $p < 0.05$), and corresponded to the background values of healthy people. A moderate increase in the level of CRP within the range of no more than 30-40 mg/l allows us to judge the early detection of the pathological process and the formation of a systemic inflammatory response to activate the phagocytosis of mycobacteria. A subsequent decrease in the concentration of CRP in the blood serum in patients with relapse indicates the absence of an acute immune response of the body to the infection.

The well-studied stimulators of angiogenesis include vascular endothelial growth factor (VEGF), a multifunctional protein that plays an important protective role in the body, namely ensuring impaired blood supply to tissues in the event of any damage. In addition, VEGF has an immunoregulatory effect and is involved in the regulation of nerve growth.

Normally, VEGF is contained in tissues in small quantities, but the expression of its gene is significantly activated during hypoxia through the induction of the transcription factor HIF -1 (hypoxia-inducible factor). Many tissue cells synthesize VEGF, including hepatocytes, fibroblasts, epithelial cells, mast cells, and endothelial cells themselves. In addition to the cellular one, there is also an extracellular emergency pool of angiogenic molecules fixed on the matrix. Being in complex with proteins of the extracellular matrix, VEGF is accessible to the action of proteolytic enzymes, which, when activated, are able to convert it into a free form. Tissue VEGF serves to develop collaterals and eliminate ischemia in organs.

Another source of the factor in the body is the peripheral blood, which contains a strategic reserve of VEGF , necessary for immediate implementation in case of damage. Like many other growth factors, VEGF is synthesized by megakaryocytes and is contained in platelet a-granules, from where they are released upon activation by thrombin. Platelet-derived VEGF, produced during blood clotting, accounts for a significant portion of its serum levels.

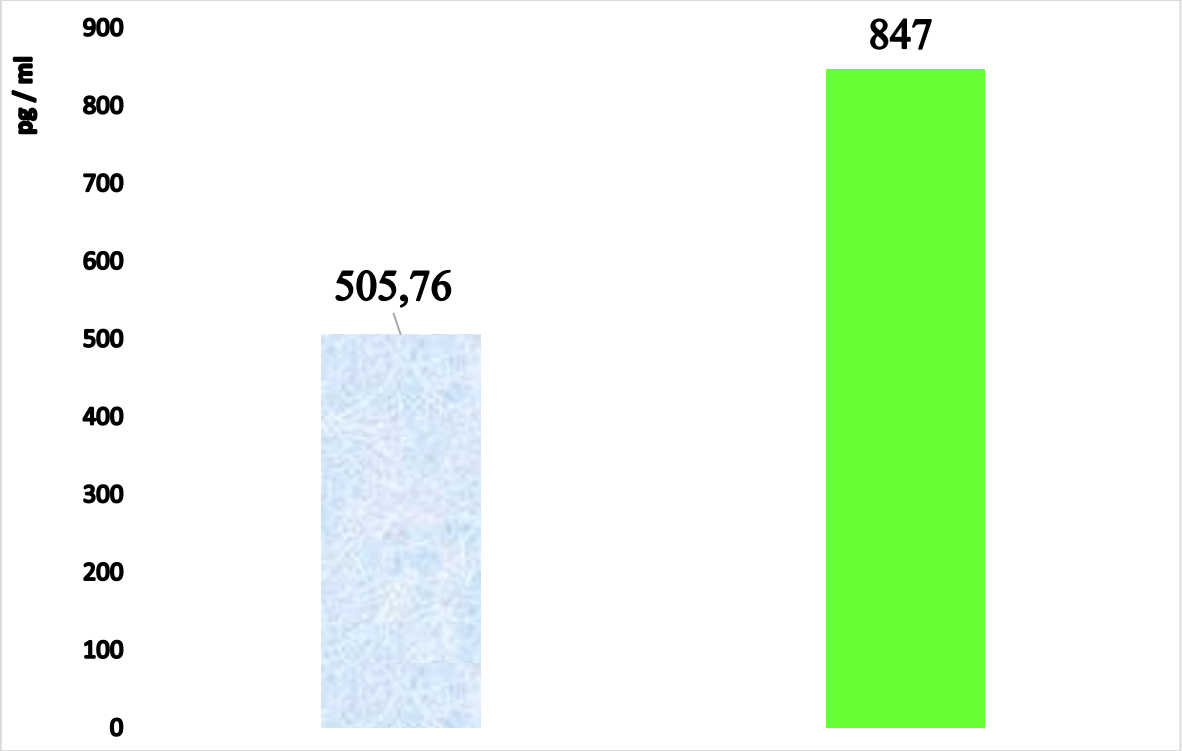

Figure 2. Results of VEGF determination in newly diagnosed (group I) and patients with relapse (group 2) pulmonary tuberculosis.

In Fig. 2. The results of determining VEGF in newly diagnosed and patients with recurrent pulmonary tuberculosis are presented. Compared to healthy people (VEGF concentration 100-200 pg/ml), all studied patients had a significant increase in the level of marker in the blood serum. At the same time, in patients with recurrent disease, the level of VEGF was statistically significantly higher than in newly diagnosed patients with pulmonary tuberculosis (847.0±182.3 pg/ml and 505.76±103.06 pg/ml, respectively, p <0. 05). A significant increase in the content of VEGF in the blood serum indicates the activation of reparative processes in lung tissue in the disease under study. At the same time, relapses of pulmonary tuberculosis induce an even greater increase in the production of VEGF factor, which can serve as a diagnostic and prognostic sign for the early detection of the pathological process.

Tumor necrosis factor-alpha (TNF-α) is a key mediator and cytokine of the immune response. The main producers of TNF-α are activated macrophages. Activation of macrophages by lymphocytes may help limit infection. However, constant stimulation due to the persistence of the pathogen serves as a chronic antigenic stimulus and can lead to tissue damage as a result of the release of a number of products by macrophages: monokines (TNF-α, IL-1β, IL-6, IL-18 and others), reactive oxygen metabolites and hydrolase enzymes. The bactericidal mechanism of activated macrophages is based on the formation of highly active metabolites of nitric oxide (NO), toxic to bacteria. Dysregulation of cytokines and their receptors can cause anemia, fever, tissue damage, hypercalcemia and leukocytosis in patients with tuberculosis and other chronic

inflammatory diseases. IL-1 and TNF-α released from activated macrophages are strong pyrogens and induce an acute phase inflammatory response.

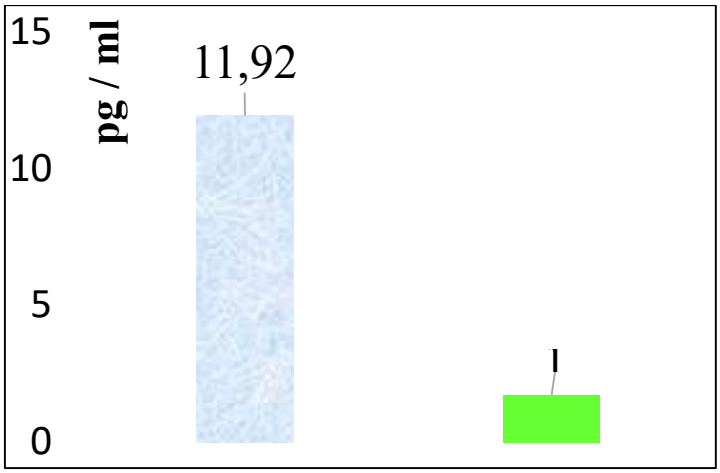

Figure 3. Results of determination of TNF-α in newly diagnosed (group I) and patients with relapse (group 2) pulmonary tuberculosis.

In Fig. 3. The results of determining TNF-α in newly diagnosed and patients with recurrent pulmonary tuberculosis are presented. Compared to healthy people (TNF-α concentration 0.5-1 pg/ml), all studied patients had a significant increase in the marker content in the blood serum. At the same time, in patients with relapse of the disease, the level of TNF-α was statistically significantly lower than in newly diagnosed patients with pulmonary tuberculosis (1.72±0.24 pg/ml and 11.92±2.49 pg/ml, respectively, $p < 0.05$). It should be noted that there is a direct relationship between the levels of VEGF and TNF-α. VEGF expression is induced by proangiogenic factors, in particular cytokines. Thus, a decrease in the concentration of TNF-α in the blood serum in patients with recurrent disease (group 2) directly correlates with an increase in the level of VEGF in these same patients. Determination of the TNF-α marker in the blood serum of patients with pulmonary tuberculosis must be carried out in parallel with the detection of VEGF expression, which can serve as a diagnostic and prognostic sign for the early detection of the pathological process.

All patients underwent a single determination of adenosine deaminase activity in blood serum and pleural fluid (in patients with exudative pleurisy).

Of the 67 patients with suspected pulmonary tuberculosis, 19 (28.3%) were diagnosed with community-acquired pneumonia (CAP), 48 patients with TB, of which 17 (25.4%) had infiltrative tuberculosis, 12 had disseminated pulmonary tuberculosis, 11 (16.4%) had tuberculomas, 8 (1 1.9%) had exudative pleurisy. The presence of destructive changes in the lung tissue was determined in 17 (25.4%) patients. It was found that in patients with CAP, the activity of ADA in the blood serum significantly exceeded the control by 2.1 times ($P<0.001$). In patients with TBL, ADA activity was 3.6 times higher than in the control group ($P<0.001$), and compared to the VP group – 1.7 times higher ($P<0.001$). In the VP and TBL groups there were significant fluctuations in ADA activity from the mean value. The average value in the group of patients with CAP was 46.6±2.9 IU/l (Fig. 4).

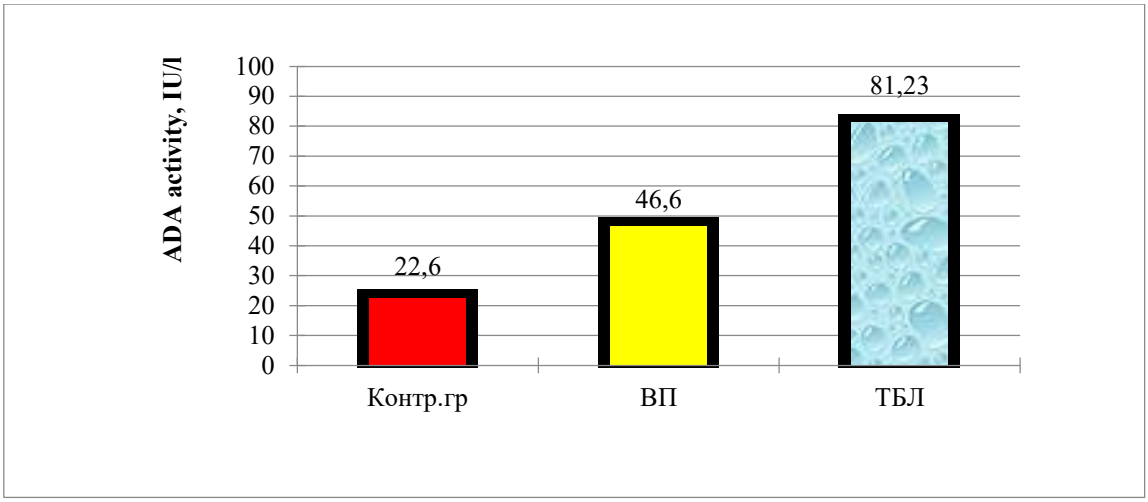

Figure 4. ADA activity in blood serum in patients with CAP and TBL.

The upper limit of the average value of this indicator according to the t-Student table at P <0.05 is 52.8 IU/l, and the lower limit is 40.5 IU/l, while in patients with TBL the average values are 81.2 ± 5, 74 IU/l, upper limit – 92.5 IU/l, lower limit – 69.9 IU/l. The smaller spread of ADA activity in patients with CAP is due to the homogeneity of the infiltrative process in the lungs. In patients with TBL with its various forms - infiltrative, disseminated, tuberculoma, exudative pleurisy - the maximum activity of ADA in the blood serum was recorded in 11 (55%), and the minimum - in 3 (15%).

Of the patients with CAP, the maximum ADA activity was recorded in 11 (57.9%), the minimum – in 3 (15.8%). The point of intersection of the Gaussian curves and the distribution of ADA values in patients with CAP and controls is at the level of 38.3 IU/l. The distribution limit of the ADA activity value between patients with VP and TBL is at the level of 61.8 IU/l. It is important to emphasize that within the group of patients with TBL there was also a difference in the distribution of the intersection of the Gaussian curves of the value of ADA activity in the blood serum. In patients with the infiltrative form of TBL, the distribution limit between EP was 58.4 IU/L, with disseminated TBL – 44.2 IU/L, exudative pleurisy – 77.9 IU/L, tuberculoma – 36.8 IU/L.

The results also showed that ADA activity in the blood serum of patients with CAP differs from controls in greater sensitivity, specificity and diagnostic accuracy, which is also confirmed by the high probability of correct diagnosis or prognosis of the disease. To substantiate the diagnostic significance of the obtained observational results, we analyzed the indicators of sensitivity, specificity, diagnostic accuracy, as well as calculated the posterior probability based on the Bays theory.

Thus, when determining the activity of ADA in the blood serum in 9 patients with CAP (47.6%), true positive results were found, in 5 (26.3%) false negative results were found, the sensitivity was 64.3%. A true negative result was observed in 4 patients (21.1%), a false positive result in 2 (10.5%). That is, the specificity was 80.0%, and the diagnostic accuracy was 68.2%. In the group of TBL patients, 21 (43.8%) had a true positive result, 3 (6.3%) had a false negative result. Sensitivity in this group was 82.9%, and a true negative result was detected in 22 (45.8%) patients. Simple calculations showed that the specificity of the method in patients in this group was 87.3%. A false-positive result among patients with TBL was detected in 2 (4.2%), which made it possible to determine the diagnostic accuracy of high ADA activity in blood serum (up to

77.1%). It should be noted that prognostic and diagnostic indicators in patients with various forms of TBL also differed significantly from those in CAP.

This is confirmed by the high probability of correct diagnosis and prognosis (P). Having carried out a comparative study of the estimated indicators of ADA activity in patients with CAP and TBL, we found that in patients with TBL with various forms, the indicators of sensitivity, specificity, diagnostic accuracy, and posterior probability (P) are statistically significantly higher than in patients with CAP.

Consequently, in all forms of TBL, prognostic and diagnostic assessment factors and the marker of lung tissue destruction ADA are higher than in patients with CAP. Significant differences between the intersections of the Gaussian curves of the distribution of indicator values in the blood serum in patients with CAP and TBL make it possible, along with other diagnostic tests, to most likely diagnose and predict not only the development of TBL, but also to determine its nosological forms, which is important for their timely diagnosis and providing adequate therapy.

Assessment of the ADA indicator, taking into account the results of anamnestic, microbiological, radiological, clinical studies and matrices that make up diagnostic sensitivity and accuracy, specificity, posterior probability, allows you to differentiate TBL from CAP with infiltrates in the lungs, if tuberculosis is suspected, as well as predict the severity of the pathological process in the lungs of patients with various forms of TBL.

Conclusion. For early diagnosis of pulmonary tuberculosis, in addition to the general diagnostic minimum (GDM), it is necessary to use additional modern research methods (a method for determining the level of adenosine deaminase activity, C-reactive protein, interleukins, TNF-α and angiogenesis. The results of the study help to specify the tactics for further management of patients.

In a comparative aspect, the activity of adenosine deaminase (ADA) in blood serum was studied, which was 55.3 μg/ml. Indicators of the acute phase of inflammation factors (C-reactive protein, TNF-α and angionases (VEGF) were 4.83 μg/ml, 44.2 pg/ml and 8.47 pg/ml, respectively. To substantiate the diagnostic significance of the obtained results, a analysis of indicators of sensitivity, specificity, diagnostic accuracy, as well as calculation of posterior probability based on the Bays theory. Thus, in 21 cases (43.8%) there was a true positive result, in 2 (4.2%) - a false positive result, in 3 (6.3%) - false negative, and a true negative result was detected in 22 (45.8%) patients. The sensitivity of the method was 82.9%, specificity - 87.3%, diagnostic accuracy - up to 77.1%, which confirms the establishment correct diagnosis. The technique can be used in an outpatient setting, in the presence of a centrifuge at 1500 rpm, a conventional photocolorimeter or spectrophotometer. Reagents, except ADA, are not in short supply. The technique is easily reproducible, analysis results can be obtained within 3 hours.

The most informative method for diagnosing MTB for the first time and early relapse is the determination of adenosine deaminase in combination with C-reactive protein, TNF-α and angionases (VEGF) in the process of relapse of pulmonary tuberculosis in the infiltrative form.
The ADA test showed high diagnostic significance when carrying out diagnostic measures in determining the tuberculosis process during differential diagnosis in pulmonology departments. Thus, there is another chance to reduce diagnostic time and begin adequate treatment as early as possible.

Conclusion. A moderate increase in the level of CRP in initially diagnosed patients with pulmonary tuberculosis was found to be no more than 30-40 mg/l, which allows early detection of the course of the pathological process and the formation of a systemic inflammatory response to activate phagocytosis.

Correlation of a decrease in the concentration of TNF in the blood serum in patients who returned for a second time with a relapse of the disease with an increase in the level of VEGF in the same patients. Determination of the TNFa marker in the blood serum of patients with pulmonary tuberculosis must be carried out in parallel with the detection of VEGF expression, which increases the diagnostic and prognostic value of the studied markers for the early detection of the pathological process.

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