Виявлення специфічних антитіл з використанням злитого протеїну MPT83-MPT63 при деструктивному туберкульозі легень

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Вступ. Розроблення ефективних тест-систем для діагностики туберкульозу і дослідження факторів, що впливають на їх чутливість і специфічність, є актуальним завданням з огляду на нестабільну епідеміологічну ситуацію з туберкульозу в Україні та світі.

Метою роботи було визначити клінічні та лабораторні особливості перебігу туберкульозу легень, що можуть впливати на чутливість виявлення протитуберкульозних антитіл класу G у сироватці крові людини за допомогою імуноензимної тест-системи «IB-Chem Anti-Mycobacterium tuberculosis», розробленої в Інституті біохімії ім. О.В Палладіна НАН України, в якій використовується рекомбінантний злитий протеїн MPT83-MPT63 як антиген.

Методи дослідження. Сироватки крові 62 хворих на хіміорезистентний туберкульоз легень перевірено на наявність протитуберкульозних антитіл за допомогою методу непрямого твердофазного імуноензимного аналізу та проаналізовано залежність одержаних результатів від клінічних симптомів та ознак туберкульозу легень, частоти виявлення різних варіантів антибіотикорезистентності, клітинного складу та біохімічних показників крові.

Результати. Встановлено, що чутливість імуноензимної тест-системи «IB-Chem Anti-Mycobacterium tuberculosis» для визначення в крові людини протитуберкульозних антитіл класу G з використанням рекомбінантного злитого протеїну MPT83-MPT63 становила 71.0 %, а у випадку наявності деструктивної форми туберкульозу легень її чутливість досягла 81.3 %.

Висновки. Отже, озроблена тест-система може бути рекомендована для використання передусім при діагностиці туберкульозу у людей з деструктивними змінами у легенях, при яких діагностична ефективність цієї тест-системи є максимальною.

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Detection of specific antibodies using MPT83-MPT63 fusion protein in patients with destructive pulmonary tuberculosis

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Introduction. The development of effective test systems for tuberculosis (TB) diagnosis and the study of factors influencing their sensitivity and specificity constitute urgent tasks given the unstable epidemiological situation with TB in Ukraine and the world.

The study aimed to determine the clinical and laboratory features of pulmonary TB that may affect the sensitivity of IgG TB antibody detection in human serum by the immuno-enzyme test system "IB-Chem Anti-Mycobacterium tuberculosis", which was developed at the Palladin Institute of Biochemistry of the NAS of Ukraine and uses recombinant fusion protein MPT83-MPT63 as an antigen.

Methods. Sera of 62 patients with chemoresistant pulmonary TB were tested for TB antibodies using an indirect enzyme-linked immunosorbent assay. Dependence of the obtained results on clinical symptoms and signs of pulmonary TB, the frequency of different types of antibiotic resistance, cellular and biochemical parameters of blood were analysed.

Results. The sensitivity of the immuno-enzyme test system "IB-Chem Anti-Mycobacterium tuberculosis" for detecting human IgG TB antibodies using recombinant fusion protein MPT83-MPT63 was found to constitute 71.0%; it reached 81.3% in patients with destructive pulmonary TB.

Conclusion. The developed test system should primarily be used for the TB diagnosis in patients with destructive changes in the lungs where the diagnostic efficiency of this test system reaches high indicators.

***Keywords**:* Diagnosis of tuberculosis, immuno-enzyme test system, tuberculosis antibodies, recombinant fusion protein, destructive forms of pulmonary tuberculosis.

The frequency of false-positive or false-negative diagnoses of tuberculosis (TB) at the stage of primary diagnostics has always prevailed in medical practice [1]. In particular, in 2013, the rate of diagnostic errors reached 99.5% at medical and obstetric points in Ukraine; up to 90% in rural outpatient clinics and district hospitals; up to 70% in central district hospitals; up to 50% in the offices of city and regional general practitioners; up to 3-5% in the offices of phthisiologists [2, 3]. A set of clinical, X-ray, microbiological and immunological methods, including skin and *in vitro* tests were used for TB diagnosis. *In vitro* tests have significant value in the diagnosis of patients with atypical clinical signs of the disease and negative results of the pathogen isolation, which often complicates TB diagnosis [4].

The most effective *in vitro* tests, which are widely used, are the following [5, 6]:

- ***Microbiological*** confirmation of TB is challenging despite recent advances (it is used in the period from 2 weeks to 2 months and is only conducted if the patient secretes mycobacteria (MBT) with sputum, which is observed in approximately 60% of patients);
- ***Bacterioscopic*** (these express tests are cheap, however, they are time-consuming and their results are based on subjective estimations; improved analogues are based on the use of fluorescent dyes and appropriate microscopes providing automated data analysis);
- ***Molecular techniques***, in particular, polymerase chain reaction (PCR), are less sensitive than microbiological, but more effective than bacterioscopic; they allow obtaining results within a day, but have a high cost and must be performed by qualified specialists).

An immunological diagnostic test is directly related to the patient's immune response. Therefore, the advantage of an immunological test lies in its capacity to demonstrate whether the patient has been previously sensitized to the MBT and had a confirmed infection, without the need to detect the bacillus in the sputum or any other biological sample of the patient. Immunological tests are based on the concept that T cells of individuals previously sensitized by antigens of *M. tuberculosis* (memory T cells) release IFN-gamma when restimulated with *M. tuberculosis*-specific antigens. A new approach follows the availability of two biological tests for the diagnosis of latent TB infection or the progression to active TB – QuantiFERON-TB and T-SPOT-TB, that measure the *in vitro* production of interferon-gamma (IFN-gamma) by blood mononuclear cells in response to *M. tuberculosis* antigens ESAT-6 and CFP10, and reach up to 90% specificity and 99% sensitivity [7, 8]. High cost and inaccessibility for countries with poor economies (and often with high TB widespread rates) are disadvantages of these tests.

There are some researchers whose goal is to develop new rapid TB diagnostics methods in the world. For example, a search for new serum markers of this infection, based on determining antibodies to any MBT antigens. In particular, it is promising to determine circulating microRNAs (miRNAs) as a potential biomarker to detect tuberculosis [9], immunoassays for measuring the reduced CD27 expression in T-cells in blood [10], and determining complex metabolites in the blood using nuclear-magnetic resonance method [11]. Brazilian scientists used new MBT peptide mimotopes for serodiagnosis of TB (IgG antibodies) and achieved 100% sensitivity and specificity when tested on a 100-sera panel [12]. This is a practical and cost-effective approach suitable for large-scale TB screening.

Research shows that anti-TB antibody levels in the blood of patients could be used as quick markers of the TB process. Thus, Italian scientists received encouraging results when detecting anti-TB antibodies using highly purified recombinant protein antigens PstS1, PstS3 and highly purified lipoglycan of the MTB cell wall in the LIODetect®TBST Tuberculosis Rapid Test [13]. The overall sensitivity and specificity of this test were estimated as 73.3% and 97.4% respectively, which is considered a satisfactory result with a good perspective for future use.

South African researchers proposed to use titres of Ig A antibodies against six MTB antigens (Apa, NarL, Rv3019c, PstS1, MTP64, Tpx) and titres of IgM antibodies against LAM in combination with the definition of 5 inflammation markers (neural cell adhesion molecule (NCAM), vitronectin, complement factor H, ferritin and α-2 macroglobulin) to increase

the sensitivity of diagnostic TB serologic IgA test [14]. This has improved the diagnosis of the disease resulting in a level of sensitivity of 95.2% and specificity of 89.3%. However, it is likely that such a test will not be cheap.

Serological tests for detecting anti-TB antibodies of different classes (IgA, IgM, IgG) remain relevant in developing countries during the diagnostic studies, despite their relatively low sensitivity. Recombinant analogues of *M.tuberculosis* antigens are especially promising, as they allow standardizing ELISA tests. [15]. However, those serological tests have several limitations: lack of correlation between the level of specific anti-TB antibodies in the blood and the severity of the disease, uninformative results in the case of immune suppression of any origin, including AIDS, lack of ability to differentiate latent and active forms of infection, and ELISA kit's relatively low sensitivity (60-70%). At the same time, anti-TB antibody test kits are low-cost, easy and fast to use (within a few hours), and therefore widely used in countries with widespread pulmonary tuberculosis, so research to develop and improve these tests is still relevant.

In particular, serological tests for determining antibodies to MBT antigens MPT63, MPT64 [16], and MPT83 [17, 18] have a good prospect for widespread use.

A new ELISA diagnostic "IB-Chem Anti-*Mycobacterium tuberculosis*" for detecting IgG anti-TB antibodies in the serum of patients with tuberculosis was developed at the Palladin Institute of Biochemistry of the NAS of Ukraine, which uses recombinant fusion protein MPT83-MPT63 containing high immunogenic antigens of M. tuberculosis and M. Bovis that are absent in most other (non-pathogenic) mycobacteria, [19, 20].

The experimental test system "IB-Chem Anti-*Mycobacterium tuberculosis"* is not inferior to the existing diagnostic serological tests in terms of reliability and informativeness; it meets the basic requirements for the use of ELISA test kits in practice. Previous studies have shown that the sensitivity of this test system was 70% and specificity was 95.5% [21].

A search for factors, that may affect the sensitivity of anti-TB antibodies detection, is an important and relevant task, which can increase the effectiveness of pulmonary TB diagnostics.

The main hypothesis of the research was an assumption that peculiarities of the patient's organism and clinical features of the tuberculosis process may affect the sensitivity of the test for detecting anti-TB antibodies in the blood.

This study aimed to determine clinical and laboratory features of pulmonary TB progression in patients, which may affect the results of anti-TB antibody detection using the new ELISA kit "IB-Chem Anti-*Mycobacterium tuberculosis"* based on recombinant fusion protein MPT83-MPT63.

Materials and methods

New ELISA MPT83-MPT63 test-system "IB-Chem Anti-Mycobacterium tuberculosis" developed at Palladin Institute of Biochemistry of the NAS of Ukraine was used. An indirect solid-phase enzyme-linked immunosorbent assay was performed on a Biotek multi-well spectrophotometer (USA). A negative test result indicated either the absence of anti-TB antibodies in the sample or that the concentration of these antibodies was below the sensitivity of the assay.

Venous blood sera samples (n=62) of patients infected with *M.tuberculosis*, who were treated in the TB department of the State Organization "National Institute of Phthisiology and Pulmonology named after F.G. Yanovsky National Academy of Medical Sciences of Ukraine" and had a diagnosis of TB confirmed by microbiological methods and/or PCR were used. The patients were informed and accepted a written agreement allowing the use of their biological material for research purposes. Blood sera samples have been used as primary antibodies for the ELISA test. The average age of patients was (35.2 ± 1.6); patients included were 33 men (53.2%) and 29 women (46.8%). HIV-positive patients were not included in the study. Concomitant chronic diseases were observed in 13 patients (21.0%), including 5 patients with diabetes mellitus (8.1%), 6 patients with chronic hepatitis (9.7%), 2 patients with chronic gastritis (3, 2%), 2 patients with chronic pancreatitis (3.2%) and 1 patient with gout (1.6%). Signs of intoxication were found in 36 patients (58.1%), and

bronchopulmonary dysplasia – in 41 patients (66.2%). Limited infiltrative-focal changes in the lungs were X-ray confirmed in 16 patients (25.8%), common changes – in 19 (30.7%), bilateral process – in 27 (43.5%), dissemination in the lungs – in 19 (30.7%), structural pulmonary changes – in 48 patients (77.4%).

Resistance of mycobacteria to anti-TB drugs was noted in all patients: in 21 patients (33.9%), TB resistance only to rifampicin (RifTB) was observed; in 29 patients (46.8%) – multidrug-resistant tuberculosis (MDR-TB), where the pathogen was resistant to at least two of the most effective drugs – isoniazid and rifampicin; in 12 patients (19.4%) – extensively drug-resistant TB (XDR-TB) where the pathogen was resistant to at least isoniazid and rifampicin, as well as any fluoroquinolone and at least one of the three drugs – amikacin, kanamycin or capreomycin.

A positive result of the "IB-Chem Anti-Mycobacterium tuberculosis" test system was obtained for 44 people (71.0%), who formed group I, and negative for 18 patients (29.0%) who formed group II. Groups did not differ significantly in the age and sex composition of patients.

Test results in both groups were analysed depending on the listed clinical characteristics of pulmonary TB and data from laboratory general hospital studies (blood cell count, erythrocyte sedimentation rate (ESR), biochemical parameters – blood aspirate aminotransferase (AST), alanine aminotransferases (ALT), creatinine, total protein and sugar), which were performed on VitaLab Selectra E (Netherlands) and Mingray 3000 (China) with Elitech Diagnostic reagents (France)). The control group consisted of 30 conditionally healthy blood donors.

Statistical data processing was performed using standard software MO Excel and Origin 8.0. The obtained results of the study were checked for the normality of value distribution and further analysed using parametric (Student's t-test) or non-parametric (the Wilcoxon signed-rank test) statistic methods. All results were presented as $M \pm m$, where M is the arithmetic mean and m is the arithmetic mean error, indicating that n is the number of examined patients in the group, as well as in the form of proportions and percentages indicating the confidence interval (CI) using a test of one binomial proportion. When comparing two proportions, a test of two binomial proportions was used. Differences were considered statistically significant for $p < 0.05$.

Results

Specific antibodies against recombinant fusion protein MPT83-MPT63 were detected using the test system "IB-Chem Anti-*Mycobacterium tuberculosis*" in the sera of 44 of 62 patients, thus, the sensitivity of the experimental test system reached 71.0%. Patients' age, gender, and presence of concomitant diseases have not affected the study.

The percentage of patients with intoxication or bronchopulmonary syndrome did not differ in both groups (Table 1). In addition, there was no probable difference between groups I and II in the following parameters: the number of patients with a limited/widespread, one-sided/bilateral process in the lungs, with/without TB dissemination in the lungs. This indicated that the presence of intoxication and bronchopulmonary syndrome, as well as the degree of TB lung damage and the presence of TB dissemination, did not have any significant connection with a positive/negative result of the diagnostic test. However, a significant number of group I patients – 39 out of 44 (88.6%) – had destructive changes in the lungs, while only 9 out of 18 (50.0%) of group II patients, $p = 0.002$, had the destructive process (Table 1). Thus, there were more patients with the destruction of pulmonary tissue among group I patients (with a positive test) than among group II patients (with a negative test).

Regardless of the results of the test for detecting anti-TB antibodies, in patients of both groups, leucocytosis and monocytosis were detected, which is characteristic of the typical TB process (Table 2).

Biochemical blood parameters, which characterize hepatocyte functions (ALT, AST), kidneys (creatinine), as well as blood glucose and protein levels, did not differ significantly in groups, so, did not affect the results of the test for TB antibodies in patients.

To determine the special clinical characteristics of patients with destructive lung chang-

Table 1

Distribution of clinical symptoms and signs of pulmonary TB in patients depending on the results of the test system "IB-Chem Anti-*Mycobacterium tuberculosis*"

Indicators	I group (n = 44)			II group (n = 18)			p
	abs.	%	CI (%)	abs.	%	CI (%)	
Intoxication +	27	61.4	45.5-75.6	9	50.0	26.0-74.0	> 0.05
Bronchopulmonary syndrome +	31	70.5	54.8-83.2	10	55.6	30.8-78.5	> 0.05
Infiltrative-focal pulmonary changes: - limited,	9	20.5	9.8-35.3	7	38.9	17.3-64.2	> 0.05
- widespread	15	34.1	20.5-49.9	4	22.2	6.4-47.6	> 0.05
Bilateral process in the lungs	20	45.5	30.4-61.2	7	38.9	17.3-64.2	> 0.05
Dissemination +	15	34.1	20.5-49.9	4	22.2	6.4-47.6	> 0.05
Destruction +	39	88.6	75.4-96.2	9	50.0	26.0-74.0	= 0.002

es, which can also affect the sensitivity of the diagnostic test. In the further research, all examined patients with tuberculosis (62 patients) were divided into two groups: group "D+" included 48 patients (77.4%) with destructive changes in the lungs, and group "D-" consisted of 14 patients (22.6%)) without acute pulmonary structural changes. The groups did not differ in patients' age and gender distribution.

Anti-TB antibodies were detected using the test system "IB-Chem Anti-*Mycobacterium tuberculosis"* in a significantly higher number of patients of group "D+" (39 of 48 patients – 81.3%, CI = 67.4-91.1) than in group "D-" (5 of 14 patients – 35.7%, CI = 12.8-64.9), p = 0.001. Thus, the sensitivity of the test in group "D+" was probably higher (81.3%) than in group "D-" (35.7%). Patients of group "D+" characterized polyresistant infection strains (MRTB, XDR-TB), while in patients of group "D" monoresistant mycobacteria strains were found, more often rifampin resistance only (Table 3).

Bronchopulmonary syndrome and widespread infiltrative-focal changes in the lungs, were probably more often observed in group "D+" (Table 4). Accordingly, limited infiltrative-focal changes were more often observed in patients of group "D-". Intoxication syndrome and lung dissemination occurred in both groups with equal frequency.

Lymphocytosis, which was observed in patients of group "D-" (Table 5) and was associated with increased activity and lymphocyte count, was also a more favourable sign in people with TB than low lymphocyte count [23, 24].

The groups of "D+" and "D-" patients with or without lung destruction respectively, have shown a difference in the quantitative composition of blood cells (Table 5). Thus, leucocytosis in the blood was determined mainly in patients with group "D+". While in patients of the group "D-" with no pathological structural changes in the lungs, a higher number of lymphocytes was observed in comparison with group "D+", which is a sign of an active

Table 2

Indicators of laboratory blood tests in patients with pulmonary TB depending on the "IB-Chem Anti-*Mycobacterium tuberculosis"* test result, (M ± m)

Blood test parameters	Healthy donors group (n=30)	Pulmonary TB groups	
		I (n=44)	II (n=18)
WBC (10^9/L)	6.4 ± 0.3	8.0 ± 0.4*	7.3 ± 0.7*
Lymphocytes (%)	32.5 ± 1.3	30.6 ±2.0	33.9 ± 2.8
Granulocytes (%)	61.4 ± 1.3	59.6 ± 2.3	56.3 ± 3.6
Monocytes (%)	6.2 ± 0.4	8.4 ± 0.6*	9.3 ± 0.9*
ESR (mm/h)	7.2 ± 1.8	7.8 ± 1.0	8.8 ± 1.7

Note. * - statistically significant difference between the indicator of the group of patients in comparison with the indicator of the group of healthy donor people ($p < 0,05$); ESR – erythrocytes sedimentation rate.

Table 3

Antibiotic resistance in patients with pulmonary TB, depending on the presence of structural pulmonary changes

Antibiotic resistance types	Group "D+" (n = 48)			Group "D-" (n = 14)			p
	abs.	%	CI (%)	abs.	%	CI (%)	
Monoresistant infection strains (RifTB)	13	27.1	15.3-41.8	8	57.1	28.9-82.3	= 0.041
Polyresistant infection strains (MDR-TB, XDR TB)	35	72.9	58.2-84.7	6	42.9	17.7-71.1	= 0.041

Note: RifTB – rifampicin-resistant tuberculosis; MDR-TB – multidrug-resistant tuberculosis (resistant at least to rifampicin and isoniazid); XDR-TB – Extensively drug-resistant TB.

Table 4

Clinical symptoms and signs of pulmonary TB in patients, depending on the presence of structural pulmonary changes

Indicators	Group "D+" (n = 48)			Group "D-" (n = 14)			p
	abs.	%	CI (%)	abs.	%	CI (%)	
Intoxication +	30	62.5	47.4-76.1	6	42.9	17.7-71.1	> 0.05
Bronchopulmonary syndrome +	35	72.9	58.2-84.7	6	42.9	17.7-71.1	= 0.041
Infiltrative-focal pulmonary changes: - limited,	8	16.7	7.5-30.2	8	57.1	28.9-82.3	= 0.005
– common	18	37.5	24.0-52.6	1	7.1	0.2-33.9	= 0.002
Bilateral process in the lungs	22	45.8	31.4-60.8	5	35.7	12.8-64.9	> 0.05
Dissemination +	15	31.3	18.7-46.3	4	28.6	8.4-58.1	> 0.05

Table 5

Indicators of laboratory blood tests in patients with pulmonary TB, depending on the presence of structural pulmonary changes (M ± m)

Blood test parameters	Healthy donors group (n=30)	Pulmonary TB groups		P (differences between groups of TB patients)
		"D+" (n=48)	"D-" (n=14)	
WBC (10^9/L)	6.4 ± 0.3	8.2 ± 0.3*	6.4 ± 0.8	= 0.054
Lymphocytes (%)	32.5 ± 1.3	29.0 ±1.5	40.3 ± 4.4	= 0.027
Granulocytes (%)	61.4 ± 1.3	60.7 ± 2.0	51.7 ± 4.4*	= 0.078
Monocytes (%)	6.2 ± 0.4	8.3 ± 0.5*	9.9 ± 1.1*	> 0.05
ESR (mm/h)	7.2 ± 1.8	9.0 ± 1.6	6.7 ± 0.9	> 0.05

Note. * - statistically significant difference between the indicator of the group of patients in comparison with the indicator of the group of healthy donor people ($p < 0,05$); ESR – erythrocytes sedimentation rate.

immune response to infection, and a reduced number of granulocytes compared to normal. The number of monocytes was increased in both groups, and no differences in ESR were observed.

Changes in blood biochemical parameters (ALT, AST, creatinine, glucose and protein levels) between "D+" and "D-" groups were not found.

Discussions

In a previously conducted study, indicators of general specificity and sensitivity of the newly developed test system "IB-Chem Anti-Mycobacterium tuberculosis" and two analogous commercial test systems for detecting IgG antibodies to M.tuberculosis were analysed (test system #1 (Ukraine), test system #2 (Russia)) [21]. The results showed that both commercial diagnostic kits have the same sensitivity rate at 55% (compared to 70% for "IB-Chem

Anti-Mycobacterium tuberculosis"). All 3 diagnostics were characterized by high specificity, which was based on the results of the survey of healthy donors and used the serum of patients with non-tuberculous pulmonary diseases. The specificity of test systems #1 and #2 appeared to be similar and was 92.5% and 90.2% respectively. In the case of "IB-Chem Anti-Mycobacterium tuberculosis") it was higher – 95.5%. The fraction of false-positive results was 4.5%. Thus, we have shown that this test system based on the chimeric protein MPT83-MPT63 is characterized by a reliable value of specificity, and subsequent studies have examined the sensitivity of the ELISA test kit only.

The last study found that the sensitivity of the "IB-Chem Anti-Mycobacterium tuberculosis" test kit for detecting anti-TB IgG antibodies in human blood serum using recombinant fusion protein MPT83-MPT63 reached 71.0%. With a satisfactory specificity of the test, the test sensitivity of about 70% is considered to be not high.

Note that in a similar study by Italian scientists La Manna MP et al. (2021), a similar sensitivity (73.3%) and test specificity (97.4%) are established in determining serum anti-TB antibodies with the use of highly purified recombinant proteins antigens PstS1 and PstS3 antigens and highly purified lipoglycan of MTB cell wall in the LIODetect®TBST Tuberculosis Rapid Test [13]. This indicates certain limitations in the tests of serum anti-TB antibodies definition, which is likely to depend on unclear factors that should be studied further. In this paper, we tried to establish such factors. This will enhance the efficiency of the cheap anti-TB antibody detection tests.

There are other ways to increase the efficiency of serological tests to determine anti-TB antibodies today. For example, increasing the number of antigens used in the test (before Jacobs R, et al. (2022) [14]) in combination with the definition of inflammatory markers, search and the use of several MBT mimotopes to determine the corresponding IgG in the blood of patients [12].

A statistical analysis of factors that could affect this result was conducted. No relation with the following parameters has been found: gender and age of patients, the presence of concomitant diseases, characteristic features of the tuberculosis process, such as intoxication and bronchopulmonary syndrome in patients, the severity of infiltrative-focal changes and the dissemination of the process in the lungs, as well as laboratory cellular indicators and biochemical parameters of the blood.

It was also found that destructive changes in the lungs were most often observed in a group of patients with positive tests. I.e., the sensitivity of the test system was 81.3% in the case of a destructive form of pulmonary tuberculosis.

At the same time, polyresistant infection strains (MRTB, XDR-TB) were more often found in patients with destructive forms of pulmonary tuberculosis. Obviously, the insensitivity of the pathogen to several anti-TB drugs contributed to the destruction of the lungs due to the ineffectiveness of the treatment [3, 22]. Thus, the higher level of anti-TB antibodies in the blood of patients was due to the presence of destruction in the lungs, which is treated long and difficult, and often accompanied by resistance to treatment.

It is logical to assume that the presence of necrotic changes in the lungs, which was accompanied by the destruction of lung tissue and the formation of decay cavities, probably contributed to the direct penetration of mycobacterial antigens into the bloodstream, which in turn contributed to the abundance of anti-TB antibodies.

It was confirmed that the clinical course of the TB was more complicated in the patients with destructive forms of pulmonary TB when the bronchopulmonary syndrome (with a strong cough and sputum secretion) and widespread infiltrative-focal changes in the lungs were observed more often. They are companions of destructive processes in the lungs. Less severe limited infiltrative-focal changes were found more rarely.

Thus, the presence of destruction in the lungs was accompanied by more intense pro-inflammatory blood reactions, which were characterized by leucocytosis with a reduced proportion of lymphocytes (which is a sign of a more severe acute TB inflammation).

In summary, the increased sensitivity of the test system "IB-Chem Anti-*Mycobacterium tuberculosis"* for detecting anti-TB antibodies in patients' sera with a more severe destructive process in the lungs may be due to the destruction of lung tissue and the formation of decay cavities, which results in mycobacterial antigens leak into the blood with the following formation of specific anti-tuberculosis antibodies by immune cells. At the same time, the identified differences in other studied clinical signs and laboratory parameters, which are associated with the presence of the structural pulmonary changes, are secondary.

In conclusions:

1. The sensitivity of the "IB-Chem Anti-*Mycobacterium tuberculosis"* ELISA test kit for the anti-TB class G immunoglobulins detection in human blood serum using recombinant fusion protein MPT83-MPT63 achieves 71.0%; it is higher in the case of a destructive form of pulmonary TB system and reaches up to 81.3%.
2. The new diagnostic test system "IB-Chem Anti-*Mycobacterium tuberculosis"* can be advised primarily for the serological diagnosis of TB in patients with structural pulmonary changes in the lungs where the diagnostic efficiency of this test system reaches high indicators.

References

1. Melnyk VM, Novozhylova IO, Matusevych VH, Yukhymets VO, Lynnyk MI, Tarasenko OR, Arefieva LV, Prykhodko AM, Bushura IV. The role of primary care facilities in the detection, treatment and prevention of tuberculosis in health care reform and improvement of anti-tuberculosis measures. Kyiv: State Institution "National Institute of Phthisiology and Pulmonology named after F. G. Yanovsky", National Academy of Medical Sciences of Ukraine, 2017. 44 p. (In Ukrainian).
2. Feshchenko YuI. Current trends in the study of tuberculosis. *Ukr Pulmonol Journal*. 2019; 1: 8-24. (In Ukrainian).
3. Feshchenko YuI, Melnyk VM, Opanasenko MS. Ineffective treatment of patients with pulmonary tuberculosis and its prevention. K.: Lira-K, 2019. 246 p. (In Ukrainian).
4. Order of the Ministry of Health of Ukraine dated 25.02.2020 № 530 "On approval of health standards for tuberculosis". https://phc.org.ua/sites/default/files/users/user90/Nakaz_MOZ_vid_25.02.2020_530_Standarty_medopomogy_pry_TB.pdf (In Ukrainian).
5. Feshchenko YuI, Zhurylo OA, Barbova AI. Laboratory diagnostics of tuberculous infection. Kyiv: BCB "Medicine", 2017. 304 p. (In Ukrainian).
6. Tendolkar MS, Tyagi R, Handa A. Review of advances in diagnosis and treatment of pulmonary tuberculosis. *Indian J Tuberc*. 2021; 68(4):510-515. DOI: 10.1016/j.ijtb.2021.07.002.
7. World Health Organization (2018). Latent tuberculosis infection: updated and consolidated guidelines for programmatic management. Available at https://apps.who.int/iris/handle/10665/260233.
8. Rekalova OM, Bilogortseva OI, Koval NG. Immunological methods for tuberculosis diagnosis. *Tuberculosis, lung diseases, HIV infection.* 2017; 1: 75-83. (In Ukrainian).
9. Pattnaik B, Patnaik N, Mittal S, Mohan A, Agrawal A, Guleria R, Madan K. Micro RNAs as potential biomarkers in tuberculosis: A systematic review. *Noncoding RNA Res*. 2022; 7(1):16-26. DOI: 10.1016/j.ncrna.2021.12.005.
10. Müller C, Rumetshofer R, Winkler HM, Bécède M, Kneussl M, Winkler S. Loss of T cells expressing CD27 at the site of active tuberculosis - A prospective diagnostic study. *Tuberculosis (Edinb)*. 2020; 125:102009. DOI: 10.1016/j.tube.2020.102009.
11. Conde R, Laires R, Gonçalves LG, Rizvi A, Barroso C, Villar M, Macedo R, Simões MJ, Gaddam S, Lamosa P, Puchades-Carrasco L, Pineda-Lucena A, Patel AB, Mande SC, Barnejee S, Matzapetakis M, Coelho AV. Discovery of serum biomarkers for diagnosis of tuberculosis by NMR metabolomics including cross-validation with a second cohort. *Biomed J.* 2021; S2319-4170(21)00094-9. DOI: 10.1016/j.bj.2021.07.006. Epub ahead of print.
12. Serena NN, Boschero RA, Hospinal-Santiani M, Pacce VD, Costa JMDV, Magalhães FB, Wiedmar C, Alban SM, Soccol CR, Thomaz-Soccol V. High-performance immune diagnosis of tuberculosis: Use of phage display and synthetic peptide in an optimized experimental design. *J Immunol Methods*. 2022; 503:113242. DOI: 10.1016/j.jim.2022.113242.
13. La Manna MP, Tamburini B, Orlando V, Badami GD, Di Carlo P, Cascio A, Singh M, Dieli F, Caccamo N. LIODetect®TB-ST: Evaluation of novel blood test for a rapid diagnosis of active pulmonary and extrapulmonary tuberculosis in IGRA confirmed patients. *Tuberculosis (Edinb)*. 2021; 130:102119. DOI: 10.1016/j.tube.2021.102119.
14. Jacobs R, Awoniyi DO, Baumann R, Stanley K, McAnda S, Kaempfer S, Malherbe ST, Singh M, Walzl G, Chegou NN; AE-TBC Consortium. Concurrent evaluation of cytokines improves the accuracy of antibodies

against Mycobacterium tuberculosis antigens in the diagnosis of active tuberculosis. *Tuberculosis (Edinb)*. 2022; 133:102169. DOI: 10.1016/j.tube.2022.102169.
15. Abebe F, Holm-Hansen C, Wiker HG, Bjune G. Progress in serodiagnosis of *Mycobacterium tuberculosis* infection. *Scand. J. Immunol*. 2007; 66(2-3): 176-191. DOI: 10.1111/j.1365-3083.2007.01978.x
16. Kim JS, Cho E, Mun SJ, Kim S, Kim SY, Kim DG, Son W, Jeon HI, Kim HK, Jeong YJ, Jang S, Kim HS, Yang CS. Multi-Functional MPT Protein as a Therapeutic Agent against Mycobacterium tuberculosis. *Biomedicines.* 2021; 9(5):545. DOI: 10.3390/biomedicines9050545.
17. Ahmad A, Agus R, Massi MN, Natzir R, Madhyastha R, Madhyastha HK, Maruyama M. Cloning and expression of MPT83 gene from Mycobacterium tuberculosis in E. coli BL21 as vaccine candidate of tuberculosis: A preliminary study. *J Genet Eng Biotechnol*. 2018; 16(2):335-340. DOI: 10.1016/j.jgeb.2018.04.001.
18. Ippolito D, Boniotti MB, Fiasconaro M, Fontana S, Boifava M, Pruiti Ciarello F, Amato B, Pacciarini M, Di Marco Lo Presti V. Development and evaluation of a multi-antigen serological assay for the intra-vitam diagnosis of Tuberculosis caused by Mycobacterium Bovis in pigs. *J Immunol Methods.* 2022; 503:113234. DOI: 10.1016/j.jim.2022.113234.
19. Redchuk TA, Korotkevich NV, Kaberniuk AA, Oliinyk OS, Labyntsev AIu, Romaniuk SI, Kolibo DV, Busol VA, Komisarenko SV. Statistical analysis of the distribution of the antibody levels to *Mycobacterium Bovis* antigens for bovine tuberculosis diagnostics. *Cytol Genet*. 2010; 44(5): 280-285.
20. Siromolot AA, Oliinyk OS, Kolibo DV, Gerilovych AP. Improvement and optimization of antigenic composition for serodiagnosis of tuberculosis. *J Veter Med Biotechnol Biosafety*. 2016; 2(4): 11-15.
21. Siromolot AA, Chudina TO, Danilova IS. Rekalova OM, Kolybo DV, Komisarenko SV. Specificity and sensitivity of the new test for the serological evaluation of tuberculosis using MPT83-MPT63 fusion antigen. *Ukr Biochem J*. 2018; 90(6): 41-48.
22. Feshchenko YuI, Todoriko LD, Kuzhko MM, Gumeniuk MI. Pathomorphosis of tuberculosis – the realities of the day and chemoresistance as a sign of its progression. *Ukr Pulmonol Journal*. 2018; 2: 6-10. (In Ukrainian).
23. Cherenko SO. Immunopathogenesis of tuberculosis. *Asthma and allergy*. 2013; 1: 32-37. (In Ukrainian).
24. Fehri SM, Racil H, Habibech S, Bacha S, Cheikhrouhou S, Chaouech N, Chabbou A. Lymphocytopenia in pulmonary tuberculosis: Does it have a clinical value? *European Respiratory Journal*. 2016; 48:PA266; DOI: 10.1183/13993003.congress-2016.PA2667