UDC 616.24-002.5-053.2-085.37.036-07
DOI: 10.14739/2310-1210.2022.2.249347

The effect of immunomodulator azoximer bromide on the cytokine profile in a complex therapy for children with newly diagnosed tuberculosis

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

Aim. To analyze the effect of immunomodulator azoximer bromide on the serum cytokine profile (IL-2, IL-6, IL-4, IL-10) in the complex therapy for children with newly diagnosed tuberculosis (TB).

Materials and methods. The study included 51 newly diagnosed TB children, who were divided into 2 groups: 26 children received immunomodulator azoximer bromide (main group) in combined therapy with concurrent antimycobacterial therapy (AMBT) and 25 children were assigned to only AMBT (control group). The main group children received azoximer bromide (immunomodulator) adjunctive therapy for immunological changes correction concurrently with the standard AMBT: children under the age of 10 years – orally 6 mg twice a day, children aged over 10 years – 12 mg twice a day; the treatment course – 14 days. The serum cytokine profile (IL-2, IL-6, IL-4, IL-10) was examined on the basis of the Immunological Department in the Training Medical and Laboratory Center of ZSMU by solid-phase enzyme-linked immunosorbent assay (ELISA) using a device ELISA – reader Sirio S with respective kits (Bender MedSystems GmbH, Austria, pcg/ml) before AMBT initiation and at the end of AMBT maintenance phase (MF). The study results were processed on a personal computer using the statistical package of the licensed program Statistica, version 13 (Copyright 1984–2018 TIBCO Software Inc. All rights reserved, License No. JPZ8041382130ARCN10-J).

Results. At the AMBT initiation, newly diagnosed TB children of both groups showed a clear imbalance between pro- and anti-inflammatory cytokines towards pro-inflammatory ones with a predominance and high activity of Th1 type cellular immune response. The ratios of IL-2/IL-10 and IL-6/IL-10 pointed to the normalized balance in the regulatory system of pro- and anti-inflammatory cytokines in the main group children upon the MF of AMBT completion. The ratio of IL-2/IL-10 dropped 7.3-fold at the treatment completion relative to that at the initiation of treatment, however, compared to the control group at the end of treatment, it was 7.2 times less. The controls did not show significant on-treatment dynamics of the IL-2/IL-10 and IL-6/IL-10 ratios, the IL-2/IL-10 ratio was 12.8 times higher than in the healthy children group at the treatment completion.

Conclusions. The use of immunomodulator azoximer bromide in combination therapy for newly diagnosed TB children helps to normalize all cytokine profiles and the balance in the regulatory system of pro- and anti-inflammatory cytokines after the AMBT completion. Therefore, the effect of immunomodulator azoximer bromide on the cytokine profile in the treatment for newly diagnosed TB children is substantial, which would increase the AMBT effectiveness among this patient group as a whole.

Key words: tuberculosis, children, immunomodulator, cytokines.

Zaporozhye medical journal 2022; 24 (2), 187-190

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Вплив імуномодулятора азоксимеру броміду в комплексній терапії дітей із новими випадками захворювання на туберкульоз на показники цитокінового профілю

Ю. В. Мирончук, О. М. Разнатовська

Мета роботи – проаналізувати вплив імуномодулятора азоксимеру броміду в комплексному лікуванні дітей із новими випадками захворювання на туберкульоз (ТБ) на показники цитокінового профілю (IL-2, IL-6, IL-4, IL-10) у сироватці крові.

Матеріали та методи. У дослідження залучили 51 дитину з новими випадками захворювання на ТБ, їх поділили на 2 групи: 26 хворих, які в комплексному лікуванні на тлі антимікобактеріальної терапії (АМБТ) отримували імуномодулятор азоксимеру бромід (основна група); 25 пацієнтів, які отримувати тільки АМБТ (контрольна група). У дітей основної групи на тлі стандартної АМБТ для корекції імунологічних змін додатково застосовували азоксимеру бромід (імуномодулятор): у дітей віком до 10 років – внутрішньо по 6 мг двічі на добу, в дітей віком понад 10 років – по 12 мг двічі на добу; курс лікування – 14 діб. Дослідження показників цитокінового профілю (IL-2, IL-6, IL-4, IL-10) у сироватці крові здійснили на базі імунологічного відділу навчального медико-лабораторного центру ЗДМУ методом твердофазного імуноферментного аналізу на обладнанні імуноферментний рідер Sirio S із застосуванням набору Bender MedSystems GmbH (Austria, пкг/мл) на початку АМБТ і по завершенню підтримувальної фази (ПФ) АМБТ. Результати дослідження опрацювали на персональному комп'ютері, використавши програму Statistica, версія 13 (Copyright 1984–2018 TIBCO Software Inc. All rights reserved. Ліцензія № JPZ8041382130ARCN10-J).

Результати. На початку АМБТ у дітей із новими випадками захворювання на ТБ обох груп визначали виражений дисбаланс про- та протизапальних цитокінів у бік прозапальних цитокінів із переважанням та високою активністю Th1-типу клітинної відповіді імунітету. Коефіцієнт IL-2/IL-10 після завершення лікування знизився в 7,3 раза щодо показника на початку лікування. Показник основної групи після завершення лікування у 7,2 раза нижчий порівняно контрольною групою. У контрольній групі вірогідну динаміку коефіцієнтів IL-2/IL-10 та IL-6/IL-10 у процесі лікування не спостерігали. Коефіцієнт IL-2/IL-10 після завершення лікування у 12,8 раза вищий, ніж у групі здорових дітей.

Висновки. Застосування імуномодулятора азоксимеру броміду в комплексній терапії дітей із новими випадками захворювання на ТБ після завершення ПФ АМБТ сприяє нормалізації всіх показників цитокінового профілю з нормалізацією стану балансу в регуляторній системі про- та протизапальних цитокінів. Тому вплив імуномодулятора азоксимеру броміду в комплексній терапії дітей із новими випадками захворювання на туберкульоз на показники цитокінового профілю є вагомим, дає змогу підвищити ефективність антимікобактеріальної терапії в цього контингенту хворих загалом.

Ключові слова: туберкульоз, діти, імуномодулятор, цитокіни.

Запорізький медичний журнал. 2022. Т. 24, № 2(131). С. 187-190

According to the Center for Public Health of the Ministry of Health of Ukraine [1], there was a 1.1 % increase in the incidence of tuberculosis (TB) among Ukrainian children aged 0–14 years (from 8.9 to 9.0 per 100 000) and a 5.3 % increase – among adolescents aged 15–17 years (from 19.0 to 20.0 per 100 000) in 2019. About 1 million children are diagnosed with TB worldwide annually, including 200 000 deaths [2]. Dodd P. J. et al. [3] have reported TB as a major cause of mortality among children younger than 5 years of age, especially if left untreated or improperly managed.

Today, pediatric phthisiology faces the most important challenge of increasing therapeutic efficacy. In the age pattern of different population categories, children with notable features of the disease clinical course and certain various alterations in an immunological status are of particular concern.

Cytokines are a group of polypeptide mediators involved in the formation and regulation of protective reactions in the human body [4]. The clinical picture, features of the course and consequences of TB was found to be associated with the severity of imbalance between pro- and anti-inflammatory cytokines [5,6].

There are data on a direct link between the clinical and immunological efficacy of combined treatment for patients with newly diagnosed infiltrative TB. For instance, increased activation of IL-6 gene expression correlates with hindered resorption of infiltrates in lung tissue and prolonged persistence of respiratory symptoms in patients, and IL-2 gene expression is associated with limited lung tissue damage. Based on the data obtained, the authors recommend timely estimating the levels of pro- and anti-inflammatory cytokines in order to administrate an appropriate immunocorrective therapy concurrently with antimycobacterial therapy (AMBT).

The relevance and diagnostic value of determining the levels of cytokines (both pro-inflammatory and anti-inflammatory) in infectious diseases to assess the severity and predict the disease course is evidenced by the scientific work of Yu. G. Prytulina et al. [7]. The authors emphasize the dynamic cytokine profile examination in the treatment process with a view to early correcting the identified changes.

Following a meta-analysis of current data, L. H. Gutiérrez-González et al. [8] concluded that the correct diagnosis of immunological changes, prognosis and the use of potential immunomodulators as adjunct therapy in TB would meet personalized treatment.

The drug azoximer bromide has an immunomodulatory effect and increases the body resistance to various infectious diseases. The mechanism of immunomodulatory action of the drug is based on direct activation of phagocytic cells and natural killers in addition to stimulation of antibody production. The use of this drug activates the monocyte-macrophage system resulting in enhanced migration of neutrophils into an inflammatory site, increases the activity of lysosomal enzymes, improves the ability of phagocytes to capture and kill microbial agents. Azoximer bromide restores immunity in secondary immunodeficiency states, thereby increasing the effectiveness and reducing the length of treatment.

Reviewing the existing literature, we have not found papers related to immunomodulator azoximer bromide effects on the cytokine profile in TB children, that has determined the choose and relevance of a subject for a study.

Aim

To analyze the effect of immunomodulator azoximer bromide on the serum cytokine profile (IL-2, IL-6, IL-4, IL-10) in the complex therapy for children with newly diagnosed TB.

Materials and methods

The study included 51 newly diagnosed TB children, who were divided into 2 groups: 26 children received immunomodulator azoximer bromide (main group) in combined therapy with concurrent AMBT and 25 children were assigned to only AMBT (control group). The groups were matched by age, sex, AMBT regimen prescribed, and the specific process severity. A comparison group included 30 apparently healthy children.

The main group children received azoximer bromide (immunomodulator) adjunctive therapy for immunological changes correction concurrently with the standard AMBT: children under the age of 10 years – orally 6 mg twice a day, children aged over 10 years – 12 mg twice a day; the treatment course – 14 days.

The serum cytokine profile (IL-2, IL-6, IL-4, IL-10) was examined on the basis of the Immunological Department in the Training Medical and Laboratory Center of ZSMU by solid-phase enzyme-linked immunosorbent assay (ELISA) using a device ELISA –reader Sirio S with respective kits (Bender MedSystems GmbH, Austria, pcg/ml) before AMBT initiation and at the end of AMBT maintenance phase (MF).

The study results were processed on a personal computer using the statistical package of the licensed program Statistica, version 13 (Copyright 1984–2018 TIBCO Software Inc. All rights reserved, License No. JP-Z8041382130ARCN10-J). The normality of continuous data distribution was analyzed using the Shapiro–Wilk test [9]. Normally distributed data were assessed by Student's t-tests for single comparisons. Descriptive statistics of non-normally distributed parameters were presented as medians and interquartile ranges, Me (Q25; Q75) [10] and compared by Mann–Whitney tests [11,12]. A two-tailed P-value of less than 0.05 was considered statistically significant.

Results

Generalized clinical forms of TB (infiltrative and disseminated) prevailed among newly diagnosed TB children of both groups: 17 (65.3 %) persons of the main group and 18 (72.0 %) controls. The dynamic pattern of serum cytokine profiles (*Table 1*) in newly diagnosed TB children did not differ significantly between the group of combination therapy with immunomodulator azoximer bromide and the control one at the AMBT initiation. Nevertheless, a significant difference was found in both groups relative to the comparison group indicators. At the AMBT initiation, newly diagnosed TB children of both groups showed a clear imbalance between pro- and anti-inflammatory cytokines towards pro-inflammatory ones with a predominance and high activity of Th1 type cellular immune response.

Table 1. Dynamics of serum cytokines in newly diagnosed TB children on the immunomodulator azoximer bromide adjunctive therapy, Me (Q25; Q75)

Serum cytokine profile indicators, pcg/ml	Comparison group, n = 30	Control group, n = 25		Main group, n = 26	
		At the AMBT initiation	At the MF of AMBT completion	At the AMBT initiation	At the MF of AMBT completion
IL-6	1.58 (1.45; 1.78)	0.10 (0.05; 0.19)*	0.09 (0.07; 0.13)*	0.12 (0.07; 0.19)*	1.48 (0.79; 1.69)#,**
IL-4	1.74 (1.54; 1.94)	0.67 (0.36; 1.01)*	0.78 (0.45; 1.31)*	0.70 (0.36; 1.12)*	1.72 (1.03; 2.06)#,**
IL-2	0.30 (0.24; 0.35)	1.73 (1.09; 4.12)*	1.22 (0.95; 2.01)*	1.52 (1.05; 2.65)*	0.36 (0.22; 1.29)*,#,**
IL-10	3.47 (2.88; 3.68)	0.94 (0.41; 1.69)*	0.75 (0.41; 1.01)*	0.94 (0.37; 1.78)*	3.56 (2.21; 4.26)*,#,**

*: indicators with significant difference relative to the comparison group (P < 0.05); #: significant difference of the indicator at the MF of AMBT completion compared with that at the AMBT initiation (P < 0.05); **: indicators with significant difference between the main and control groups (P < 0.05).

Table 2. Dynamics of the cytokine ratios in newly diagnosed TB children on the immunomodulator azoximer bromide adjunctive therapy, Me (Q25; Q75)

Cytokine ratio indicators, r. u.	Comparison group, n = 30	Control group, n = 25		Main group, n = 26	
		At the AMBT initiation	At the MF of AMBT completion	At the AMBT initiation	At the MF of AMBT completion
IL-6/IL-10	0.49 (0.39; 0.54)	0.11 (0.03; 0.22)*	0.13 (0.08; 0.19)*	0.12 (0.06; 0.25)*	0.34 (0.19; 0.44) #,**
IL-2/IL-10	0.13 (0.06; 0.11)	1.56 (0.96; 5.08)*	1.67 (0.98; 2.95)*	1.70 (0.67; 3.67)*	0.23 (0.05; 0.47)*,#,**

*: indicators with significant difference relative to the comparison group (P < 0.05); #: significant difference of the indicator at the MF of AMBT completion compared with that at the AMBT initiation (P < 0.05); **: indicators with significant difference between the main and control groups (P < 0.05).

The serum IL-6 concentration in the main group children at the MF of AMBT completion was 1.48 (0.79; 1.69) pcg/ml, that did not differ significantly from the comparison group (1.58 (1.45; 1.78)) pcg/ml) and it was 12.3 times (P < 0.01) higher than that at the treatment initiation, and 16.4 times higher (P < 0.02) as compared to the control group (0.09 (0.07; 0.13) pcg/ml).

The serum IL-4 level in the main group children significantly increased 2.4-fold (1.72 (1.03; 2.06) pcg/ml) compared to that at the treatment initiation (0.70 (0.36; 1.12) pcg/ml, P < 0.000003), being 2.20-fold higher (P < 0.0003) than in the control group at the MF of AMBT completion (0.78 (0.45; 1.31) pcg/ml). The serum level of IL-4 did not differ significantly between the main and comparison groups.

The serum concentration of IL-10 in the main group children did not differ significantly from that in healthy children at the treatment completion (3.56 (2.21; 4.26) pcg/ml vs. 3.47 (2.88; 3.68) pcg/ml; P > 0.05), but it was 3.7-fold higher (P < 0.000001) than that at the treatment initiation (0.94 (0.37; 1.78) pcg/ml) and 4.7 times (P < 0.000001) as high as that of the control group at the MF of AMBT completion (0.75 (0.41; 1.01) pcg/ml).

Nor were there significant differences in the serum IL-2 concentration between the main group and healthy children at the treatment completion (0.36 (0.22; 1.29) pcg/ml vs. 0.30 (0.24; 0.35) pcg/ml, P < 0.01). Meanwhile, it was 4.2-fold significantly decreased compared to that at the treatment initiation (0.36 (0.22; 1.29 pcg/ml) vs. 1.52 (1.05; 2.65) pcg/ml; P < 0.01), which was 3.4 times (P < 0.02) as low as in the control group at the MF of AMBT completion (1.22 (0.95; 2.01) pcg/ml).

The control group children did not show significant changes in the cytokine profile at the AMBT course completion. Indeed, a downward trend in the serum IL-2 concentration by 1.4 times was revealed at the treatment completion (1.22 (0.95; 2.01) pcg/ml vs. 1.73 (1.09; 4.12) pcg/ml; P > 0.05), which was 4 times (P < 0.0005) the indicator of healthy children (0.30 (0.24; 0.35) pcg/ml). The serum concentrations of IL-6 and IL-4 remained almost at the same level as determined at the treatment initiation, which was 17.5 times (P < 0.000001) and 2.2 times (P < 0.000001) less, respectively, than those in the healthy group children.

A further decrease in the serum concentration of IL-10 was found among the controls at the treatment completion, as it was 4.6 times less than that in the comparison and main groups (0.75 (0.41; 1.01) pcg/ml vs. 3.47 (2.88; 3.68) pcg/ml and 3.56 (2.21; 4.26) pcg/ml, respectively; P < 0.0003).

The ratios of IL-2/IL-10 and IL-6/IL-10 (*Table 2*) pointed to the normalized balance in the regulatory system of pro- and anti-inflammatory cytokines in the main group children upon the MF of AMBT completion. In this vein, the IL-6/IL-10 ratio in the main group did not differ significantly from that in healthy children at the completion of AMBT course, but it was 2.8 times increased compared to that at the treatment initiation (0.34 (0.19; 0.44) r. u. vs. 0.12 (0.06; 0.25) r. u., P < 0.01), being 2.8 times (P < 0.01) as high as in the control group upon the MF of AMBT completion (0.13 (0.08; 0.19) r. u.).

The ratio of IL-2/IL-10 dropped 7.3-fold at the treatment completion relative to that at the treatment initiation (0.23 (0.05; 0.47) r. u. vs. 1.70 (0.67; 3.67) r. u., P < 0.02) in the main group, but still remained 1.7 times as high (P < 0.01) as in the comparison group (0.13 (0.06; 0.11) r. u.), however, compared to the control group at the end of treatment, it was 7.2 times less (0.23 (0.05; 0.47) r. u. vs. 1.67 (0.98; 2.95) r. u., P < 0.007). The controls did not show significant on-treatment dynamics of the IL-2/IL-10 and IL-6/IL-10 ratios, the IL-2/IL-10 ratio was 12.8 times higher than in the healthy children group (1.67 (0.98; 2.95) r. u. vs. 0.13 (0.06; 0.11) r. u., P < 0.007) at the treatment completion.

Discussion

As is seen from the results, newly diagnosed TB children were characterized by the imbalance in the regulatory system of pro- and anti-inflammatory cytokines with the predominance of Th1 type cellular immune response at the AMBT initiation. Moreover, most children of both TB groups were diagnosed with the generalized clinical forms of the disease (65.3 % and 72.0 %). According to the literature, regardless of the clinical form, generalized destructive TB is accompanied by a clear quantitative predominance of B-lymphocytes with stressed functional activity as well as a predominantly Th2 type cellular response.

Kolosova A. et al. reported the following manifestations of cytokine-mediated suppression of the Th1 immune response in TB patients: hypersecretion of IL-10 (infiltrative form of the specific process) and IL-4 (disseminated form) along with IL-2 deficiency.

Kuzhko M. et al. [13] studying the cytokine profile in newly TB diagnosed patients found an active Th1 immune response (increased serum levels of IL-2 and IFN-γ) with reduced Th2 activity (IL-4 and IL-10) at the treatment initiation. Upon the intensive phase of AMBT completion (2 months after), the opposite changes were revealed: activated Th2 immune response and suppressed Th1 immune response.

We have found the predominance and high activity of Th1 type cellular immune response in newly diagnosed TB children who received only AMBT throughout the course of treatment and upon its completion. This fact has been well reflected in the literature [5].

The researchers demonstrated a tendency to restore balance in the system of pro- and anti-inflammatory cytokines on the AMBT, but none of the cytokine indices returned to normal levels, requiring additional immunocorrective therapy. However, found normal serum levels of pro-inflammatory cytokines (IL-1β, IL-2, TNF-α) in young TB children (under 3 years of age) and older (16–17 years of age) after 3 months of using only AMBT.

The results of our work suggest that the use of immunomodulator azoximer bromide in combination therapy for newly diagnosed TB children helps to normalize all cytokine profiles and the balance in the regulatory system of pro- and anti-inflammatory cytokines after the AMBT completion. To sum up, the findings have found scientific evidence reported in the literature, but there is also a sufficient number of papers which are contrary to the data obtained.

Conclusions

The use of immunomodulator azoximer bromide in combination therapy for newly diagnosed TB children helps to normalize all cytokine profiles and the balance in the regulatory system of pro- and anti-inflammatory cytokines after the AMBT completion. The clear imbalance between pro- and anti-inflammatory cytokines towards pro-inflammatory ones with the predominance and high activity of Th1 type cellular immune response is the characteristic of TB children receiving only AMBT at the treatment course completion. Therefore, the effect of immunomodulator azoximer bromide on the cytokine profile in the treatment for newly diagnosed TB children is substantial, which would increase the AMBT effectiveness among this patient group as a whole.

Prospects for further research. To study the effect of the immunomodulator azoximer bromide on the effectiveness of complex therapy for newly diagnosed TB children.

Conflicts of interest: authors have no conflict of interest to declare.
Конфлікт інтересів: відсутній.

Надійшла до редакції / Received: 27.12.2021
Після доопрацювання / Revised: 11.01.2022
Прийнято до друку / Accepted: 24.01.2022

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References

[1] Public Health Center of the Ministry of Health of Ukraine. (2021). *Statystyka z tuberkulozu [Tuberculosis statistics].* https://www.phc.org.ua/kontrol-zakhvoryuvan/tuberkuloz/statistika-z-tb [in Ukrainian].
[2] Jenkins, H. E., Yuen, C. M., Rodriguez, C. A., Nathavitharana, R. R., McLaughlin, M. M., Donald, P., Marais, B. J., & Becerra, M. C. (2017). Mortality in children diagnosed with tuberculosis: a systematic review and meta-analysis. *The Lancet. Infectious Diseases, 17*(3), 285-295. https://doi.org/10.1016/S1473-3099(16)30474-1
[3] Dodd, P. J., Yuen, C. M., Sismanidis, C., Seddon, J. A., & Jenkins, H. E. (2017). The global burden of tuberculosis mortality in children: a mathematical modelling study. *The Lancet. Global Health, 5*(9), e898-e906. https://doi.org/10.1016/S2214-109X(17)30289-9
[4] Druszczynska, M., Seweryn, M., Wawrocki, S., Kowalewska-Pietrzak, M., Pankowska, A., & Rudnicka, W. (2021). Cytokine Biosignature of Active and Latent Mycobacterium Tuberculosis Infection in Children. *Pathogens, 10*(5), Article 517. https://doi.org/10.3390/pathogens10050517
[5] Piskur, Z. I., Mykolyshyn, L. I., Platonova, I. L., & Lapovets, L. Ye. (2017). Osoblyvosti tsytokinovoho spektra IL-1β, IL-2, IL-10 i TNF-α u ditei, khvorykh na tuberkuloz [Peculiarities of cytokine spectrum IL-1β, IL-2, IL-10 and TNF-α in children with tuberculosis]. *Tuberkuloz, lehenevi khvoroby, VIL-infektsiia*, (1), 42-47. [in Ukrainian].
[6] Hemingway, C., Berk, M., Anderson, S. T., Wright, V. J., Hamilton, S., Eleftherohorinou, H., Kaforou, M., Goldgof, G. M., Hickman, K., Kampmann, B., Schoeman, J., Eley, B., Beatty, D., Pienaar, S., Nicol, M. P., Griffiths, M. J., Waddell, S. J., Newton, S. M., Coin, L. J., Relman, D. A., … Levin, M. (2017). Childhood tuberculosis is associated with decreased abundance of T cell gene transcripts and impaired T cell function. *PLOS ONE, 12*(11), Article e0185973. https://doi.org/10.1371/journal.pone.0185973
[7] Pritulina, Yu. G., Krivoruchko, I. V., Shentsova, V. V., Fil, G. V., Astapchenko, D. S., & Sakharova, L. A. (2014). Prakticheskaya znachimost' analiza tsitokinovogo profilya pri ryade infektsionnykh zabolevanii [The practical significance of cytokine profile analysis of in a number of infectious diseases]. *Aktualna infektolohiia*, (1), 40-44. [in Russian].
[8] Gutiérrez-González, L. H., Juárez, E., Carranza, C., Carreto-Binaghi, L. E., Alejandre, A., Cabello-Gutiérrrez, C., & Gonzalez, Y. (2021). Immunological Aspects of Diagnosis and Management of Childhood Tuberculosis. *Infection and Drug Resistance, 14*, 929-946. https://doi.org/10.2147/IDR.S295798
[9] Le Boedec, K. (2016). Sensitivity and specificity of normality tests and consequences on reference interval accuracy at small sample size: a computer-simulation study. *Veterinary Clinical Pathology, 45*(4), 648-656. https://doi.org/10.1111/vcp.12390
[10] Vetter, T. R. (2017). Descriptive Statistics: Reporting the Answers to the 5 Basic Questions of Who, What, Why, When, Where, and a Sixth, So What? *Anesthesia & Analgesia, 125*(5), 1797-1802. https://doi.org/10.1213/ANE.0000000000002471
[11] Bishara, A. J., & Hittner, J. B. (2012). Testing the significance of a correlation with nonnormal data: Comparison of Pearson, Spearman, transformation, and resampling approaches. *Psychological Methods, 17*(3), 399-417. https://doi.org/10.1037/a0028087
[12] Bakker, M., & Wicherts, J. M. (2014). Outlier removal, sum scores, and the inflation of the type I error rate in independent samples t tests: The power of alternatives and recommendations. *Psychological Methods, 19*(3), 409-427. https://doi.org/10.1037/met0000014
[13] Kuzhko, M. M., Butov, D. O., Stepanenko, H. L., Hrynishyna, O. O., & Maksymenko, O. A. (2012). Vplyv tsytokiniv na moduliatsiiu monoklonalnykh antytil krovi u khvorykh iz vpershe diahnostovanym tuberkulozom lehen [Effect of cytokines on the modulation of the blood monoclonal antibodies in patients with new-onset pulmonary tuberculosis]. *Tuberkuloz, lehenevi khvoroby, VIL-infektsiia,* (3), 29-32. [in Ukrainian].