The restorative effect of immunoglobulin G on indicators of cellular immunity in patients with co-infection drug-resistant tuberculosis/HIV with a CD4 lymphocyte count from 200 to 50 cells/μL

Objective — to study the restorative effect of immunoglobulin G (IgG) in patients on indicators of cellular immunity in patients with drug-resistant tuberculosis (DR-TB)/HIV co-infection at the level of CD4 + lymphocytes from 200 to 50 cells/μL.

Materials and methods. The study involved 52 patients aged 20 to 55 years, the average age was (37.2 ± 7.8) years. All patients were HIV-positive with laboratory-confirmed DR-TB with mycobacterium resistance to first- and second-line drugs. Patients on DR-TB/HIV were divided as follows: Group 1 (control) — 26 patients with DR-TB/HIV with the CD4 lymphocyte count from 200 to 50 cells/μL, who received standard treatment with second-line antimycobacterial therapy (AMBT) and antiretroviral therapy (ARVT); Group 2 (basic) — 26 patients with DR-TB/HIV with a CD4 lymphocyte count from 200 to 50 cells/μL, who also received standard treatment of second-line AMBD and ARVT, with the addition of complex therapy with intravenous IgG.

Results and discussion. In patients of Group 2, at the end of the 20th month of treatment, the normalization of T-helpers level was noted in 69.2 % of people, which reached the average values of (830.0 ± 15.2) cells/μL, with an increase to a subnormal level (408.0 ± 1.6) cells/μL in 30.8 % of patients (p < 0.05). Normalization of the T-helpers indicator in Group 1 was noted on the 20th month only in 42.3 % of people, and it amounted to (670.0 ± 14.5) cells/μL (p < 0.05). The relative (percentage) level of the T-helpers indicator in the 20th month reached normal values in 76.9 % of patients and 46.2 % of patients in Groups 2 and 1, respectively (p < 0.05). The absolute number of T-suppressors at the end of the 20th month decreased to the norm in 76.9 % of patients and 46.2 % of patients in Groups 2 and 1, respectively (p < 0.05). In patients of Group 2, on the 20th month of treatment, the physiological level of the T-suppressors indicator (19—35) % was reached in 65.4 % of cases (p < 0.05); in Group 1, the normal level was registered in 38.5 % of patients (p < 0.05). The CD4+/CD8+ Ratio was below the norm in 100 % of patients of Group 1 during 14 months inclusive, and only on the 20th month it reached the norm in 23.1 % of patients (p < 0.05). Group 2 patients had normalization in 57.7 % of cases at the 20th month (p < 0.05).

Conclusions. The additional use of intravenous IgG in the complex treatment of patients with DR-TB/HIV contributed to a faster recovery of the cellular link of immunity, which makes it possible to prescribe antiretroviral therapy to patients with DR-TB/HIV at an earlier time, prevent the development of systemic inflammation and improve the prognosis for survival.

Keywords
Tuberculosis, HIV, drug-resistant tuberculosis.
Drug-resistant tuberculosis (DR-TB) remains a serious challenge for the health care system in Ukraine and around the world [1, 4, 6]. The World Health Organization (WHO) ranks Ukraine among the thirty countries in the world with the highest burden of tuberculosis (TB) [2, 14], and among the twenty countries with the prevalence of multidrug-resistant tuberculosis (MDR-TB) and tuberculosis with extensive drug resistance (XDR-TB), which account for 86% of all cases of DR-TB in the world [3, 5, 7, 14].

According to WHO global estimates, in 2020 the global rate of successful treatment of patients with MDR-TB was 57%, and 39% for XDR-TB [1, 14]. The effectiveness indicator of MDR-TB treatment in Ukraine in 2020 was 51%, and XDR-TB — 34.4% [9, 10, 14].

A still unresolved issue in TB control in Ukraine is the constant increase in morbidity and mortality from DR-TB co-infection with the human immunodeficiency virus (HIV) [11]. The complexity of the epidemiological situation with DR-TB/HIV co-infection is multifactorial in nature, on the one hand, it is due to the peculiarities of the clinical course of DR-TB in patients with HIV with pronounced immunosuppression, which often becomes aggressive, progresses rapidly and is accompanied by a generalized tuberculosis infection, which leads to a fatal outcome. The severity of immunosuppression is also a favorable basis for the development of the immune system recovery syndrome (IRS), which jeopardizes the antimycobacterial therapy (AMBT) scheme, leads to the late appointment of antiretroviral therapy (ARVT), and, as a result, the failure treatment of DR-TB/HIV [12, 13].

On the other hand, the development of DR-TB in HIV-infected patients deepens the damage of the immune system, contributing to the progression of opportunistic infections, such as cryptococcal meningitis, herpetic infections, toxoplasmosis, cytomegalovirus infection, pneumocystis pneumonia, which can lead to fatal consequences, even in remote periods treatment [10, 13].

In this regard, the search for various methods of pathogenetic influence, capable of restoring indicators of the immune status of patients with DR-TB/HIV, continues. One of these methods is the use of intravenous immunoglobulin G (IgG), the active component of which are antibodies with specific activity against various infectious agents (viruses, bacteria, Mycobacterium tuberculosis (MTB)) [9—13]. The study of the immunological aspects of the use of intravenous IgG as an option to restore the immune system in patients with DR-TB/HIV is relevant as a way to increase the effectiveness of treatment and reduce mortality among patients with DR-TB/HIV.

Objective — to study the restorative effect of IgG in patients on indicators of cellular immunity in patients with DR-TB/HIV co-infection at the level of CD4+ lymphocytes from 200 to 50 cells/μL.

Materials and methods

The study involved 52 patients aged 20 to 55 years, the average age was (37.2 ± 7.8) years. All patients were HIV-positive with laboratory-confirmed DR-TB with mycobacterium resistance to first- and second-line drugs. Patients on DR-TB/HIV were homogeneous in terms of previous treatment history, adherence level, and resistance profile. Depending on the treatment scheme, they were distributed as follows:

• Group 1 (control) — 26 patients with DR-TB/HIV who received standard treatment with second-line antimycobacterial drugs (AMBD) with the appointment of ARVT in the course of treatment;

• Group 2 (basic) — 26 patients with DR-TB/HIV who additionally received intravenous IgG in a complex of standard AMBT and ARVT.

Intravenous immunoglobulin IgG (5% solution for intravenous drip administration of 50 ml) is an immunologically active protein fraction (the ratio of subclasses of immunoglobulin G in the preparation: IgG1: 43—75 %, IgG2: 16—48 %, IgG3: 1.7—7.5 %, IgG4: 0.8—11.7 %), the maximum content of immunoglobulin G in the preparation is 25 μg/ml. The drug does not contain preservatives and antibiotics, it does not contain antibodies to HIV-1, HIV-2, hepatitis C virus, surface antigen of hepatitis B virus. The active component of the drug is antibodies that have specific activity against various pathogens — viruses and bacteria, including hepatitis A and B, herpes, chicken pox, influenza, measles, mumps, polio, rubella, whooping cough, staphylococcus, Escherichia coli, pneumococci, Mycobacterium tuberculosis. Immunoglobulin G is indicated for replacement immunotherapy in the treatment of primary and secondary immunodeficiency states and related diseases, as well as for the treatment and prevention of diseases caused by bacterial and viral infections [11].

Intravenous IgG was prescribed according to the following scheme: before the start of AMBT at the rate of 0.4 g/kg, intravenous drip in the afternoon; on the second day, second-line AMBD was added, according to sensitivity; and after 2 weeks, ARVT was added. Subsequent injections of IgG were performed every 4 weeks for 3 months, then on the 5th and 8th months of the intensive phase, and on the 14th and 20th months of the continuation phase of DR-TB/HIV treatment.
Design of the study: simple, open, randomized. Inclusion criteria:
- the patient’s consent to participate in the study;
- newly diagnosed DR-TB against the background of HIV infection;
- age of patients from 20 to 55 years;
- patients with DR-TB/HIV who have not previously taken second-line AMBD and ARVT.
Exclusion criteria:
- refusal of the patient;
- repeated cases of DR-TB against the background of HIV infection;
- patients who were previously treated with second-line drugs;
- patients who interrupted treatment and were transferred to palliative treatment in the previous case;
- patients who were in the terminal phase of DR-TB and HIV infection;
- presence of acute kidney or liver failure;
- mental disorders.

Immunological diagnostics with the determination of the level of lymphocytes subgroups (CD3+, CD4+, CD8+, CD4+/CD8+) was carried out in the clinical laboratory of the Odesa Regional AIDS Center, using an AQUIOSCL flow cytometry device manufactured by Beckman Coulter (certified according to ISO 15189 standard) at the beginning and after 3—20 months of treatment. This is a direct volumetric method for a single platform. The sample was processed using two multitasking probes: One probe pricked the lid and prepared the sample into a 96 pits microplate, while the other aspirate prepared the sample for analysis. While the first sample was incubated, the system continued to prepare additional samples and add them to the queue. Whole blood (140 μL) was added to every pit; then, specific white blood cell staining was performed by incubating whole blood with a monoclonal antibody reagent. Then the red blood cells were removed by their lysis without washing, and the remaining leukocytes were analyzed by flow cytometry. A 488 nm solidstate diode laser was used to measure the light diffraction, fluorescence, and electron volume, which estimated the relative size of cells. It was used a readytouse antibodies mixture (AQUIOS Tetra1 panel, Beckman Coulter): CD45isothiocyanate fluorescein (FITC) (clone B3,821F4A)/CD4phycoerythrin(PE) (clone SFCI12T4D11)/CD8phycoerythrin TexasRedX (ECD) (clone SFCI21Ch3Cyrin3 5 (clone UCHT1) for staining of the cells. The strategy of gating includes side and forward scattering, SS/CD45 strob ing and electric volume (EV) evaluation with 2 parameters EV/SS that promote purification and better restoration of total lymphocytes.

Statistical analysis was performed using the Statistica 10.0 software (Dell Software, Austin, TX, USA). It was determined whether there is a significant difference concerning frequency of studied criteria between the two studied groups. Quantitative indicators in the text and tables are presented in the form M ± m (M — arithmetic average and m — standard deviation); quality indicator a represented in the form Q ± mq (Q is the frequency of occurrence of the trait and mq is the standard deviation). Statistical significance was assumed at the p < 0.05. Kruskal—Wallis, ANOVA, and Chi-square tests were used in this study.

Results and discussion

Before the start of treatment, the level of subpopulations of CD3+ lymphocytes in the blood reached (637.0 ± 31.5) cells/μL in 80.8 % of patients of Group 1 and (629.0 ± 30.7) cells/μL in 84.6 % of Group 2 (p < 0.05), in both cases this indicator was lower than the lower limit of the norm (norm 690–2540 cells/μL), and was (50.2 ± 2.8) % and (50.1 ± 2.6) %, with normal (55—84 %) of patients of Group 1 and Group 2, respectively (Table 1, 2 and Fig. 1).

Later, in (50.0 ± 10.0) % of patients of Group 2 already on the 3rd month of treatment, a normal level of the CD3+ lymphocyte subpopulation in the blood was registered, while in Group 1 only (30.8 ± 9.2) % (p < 0.05); in the 5th month — in (80.8 ± 7.9) % and (46.2 ± 10.0) % of the main and control groups, respectively (p < 0.05); in the 8th month of observation, the restoration of the content of CD3+ cells was determined in 100 % of patients of Group 2, and in (61.5 ± 9.7) % of Group 1 (p < 0.05) (see Table 1, 2 and Fig. 1).

On the 14th and 20th months of complex treatment with the addition of Ig G, no patient of Group 2 had a decrease in the level of CD3+ cells, however, in patients of the control group, the decrease in the content of the indicator was determined in (19.2 ± 7.9) % in the 14th month and in (11.5 ± 6.4) % of people — in the 20th month (p < 0.05) (see Table 1, 2 and Fig. 1).

The level of T-helpers (T-h) (CD3+ CD4+) in all patients of Group 1 and Group 2 in 100 % was significantly lower than the norm. At the end of the 5th month of observation, there was a gradual increase in the indicator to (367.0 ± 18.7) cells/μL in Group 2 and to (217.0 ± 19.4) cells/μL in Group 1 (p < 0.05), on the 14th month of complex treatment, the content of T-h in (30.8 ± 9.2) % of patients of Group 2 normalized, and in (69.2 ± 9.2) % it increased to subnormal values (407.0 ± 2.9) cells/μL, while in none of the patients of the control group did this indicator reach the normal level at the 14th month of treatment (p < 0.01). At the end of the
20th month of observation, there was a normalization of the T-h level in (69.2 ± 9.2)% of Group 2 individuals, which reached average values of (830.0 ± 15.2) cells/μL, while increasing to a subnormal level (408.0 ± 1.6) cells/μL — in (30.8 ± 9.2)% of patients (p < 0.05). However, the normalization of the T-h indicator in the control group was noted within the normal range, which was almost 2 times higher than the similar indicator of the control group (23.1 ± 8.4) % (p < 0.05) (Table 2). In all subsequent months, the tendency towards the normalization of the absolute level of T-s was unchanged — the absolute number of T-s at the end of the 20th month decreased to the norm in 76.9 % of patients and 46.2 % of patients, in Groups 2 and 1, respectively, (p < 0.05) (see Table 2, Fig. 2).

The relative (percentage) level of the T-h indicator reached normal values in (30.8 ± 9.2)% of cases of patients of Group 2 at the 14th month of treatment and in (61.5 ± 9.7)% (p < 0.05) — on the 20th month; in patients of Group 1, the recovery of the indicator was determined only at the end of treatment in (34.6 ± 9.5)% of people at the 20th month (p < 0.05) (see Table 2, Fig. 2).

Before the start of treatment, the absolute number of T-suppressors (T-s) was higher than the norm in 100 % of patients of both groups and was (1059.0 ± 74.2) and (1062.0 ± 72.8) cells/μL (with a norm of 372—974 cells/μL) (Table 1, Fig. 3). Already on the 3rd month, in 12 patients (46.2 ± 13.5)% of Group 2 and 9 patients (38.5 ± 13.5)% of Group 1, the absolute number of T-s was determined only at the end of treatment in (69.2 ± 9.2)% of Group 2 and (65.4 ± 9.7)% of Group 1, the recovery of the indicator was noted within the normal range, which was almost 2 times higher than the similar indicator of the control group (23.1 ± 8.4) % (p < 0.05) (Table 2).
the relative level of T-s was higher than the norm during the first 5 months in 100% of patients of both groups (see Table 2). Further observation showed that the average level of the indicator increased to (71.6 ± 2.5)% (p < 0.05) in Group 1 individuals, while in Group 2, on the contrary, a gradual decrease in the percentage of T-s with from (67.3 ± 3.1)% to (62.4 ± 2.1)% (p < 0.05) (see Table 2, Fig. 3). Starting from the 8th month, patients of Group 2 began to register cases with a normal relative level of T-s in (15.4 ± 7.2)% of people, while there were no such signs in Group 1 (p < 0.001) (see Table 2).

Starting from the 8th month, patients of Group 1 had a tendency to decrease the average percentage level of T-s from (63.8 ± 2.4)% to (56.7 ± 2.1)% at the 20th month (p < 0.05); in persons of Group 2—from (62.1 ± 2.0) to (50.1 ± 1.8)% in the corresponding months (p < 0.05) (see Table 2, Fig. 3).

It should be noted that in patients of Group 2, on the 14th month of treatment, the physiological level of the T-s indicator (19—35%) was reached in (38.5 ± 9.7)% and on the 20th—in (65.4 ± 9.5)% (p < 0.05). In the control group, a normal level was recorded from the beginning of the 14th month of treatment in (23.1 ± 8.4)% of patients, at the 20th month in (38.5 ± 9.7)% (p < 0.05) (see Table 2, Fig. 3).

The immunoregulatory index (CD4+/CD8+) in both groups of patients was lower than the norm before the start of treatment, and was on average (0.08 ± 0.02) cond. un., at the norm (1.2—2.1) cond. un. (Table 1). The reduced level remained in 100% of

<table>
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<td>T-lymphocytes (CD3+), %</td>
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<td>T-suppressors (CD8+), %</td>
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<td>7.7 ± 5.3*</td>
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Notes. * The difference is significant between Group 1 and Group 2 (p < 0.05); ** significant difference between Group 1 and Group 2 (p < 0.01); ^the difference in the dynamics of indicators within the group is significant (p < 0.05); ***the difference in the dynamics of indicators within the group is significant (p < 0.01).
patients of Group 1 for 14 months inclusive, and only on the 20th month it reached the norm in 23.1 % of patients (p < 0.05). Group 2 patients at the 20th month normalization was in 57.7 % of cases (р < 0.05) (see Table 2).

Conclusions
The use of immunoglobulin G in the complex treatment of patients with DR-TB/HIV (Group 2) contributed to the restoration of cellular immunity due to the increase to a subnormal level of T-h (408,0 ± 1.6) cells/μL — in (30.8 ± 9.2 ) % of patients (p < 0.05) and normalization in (69.2 ± 9.2) % of people with 76.9 % of T-s being reduced to normal.

The additional appointment of intravenous IgG in patients with DR-TB/HIV improves the indicators of the cellular link of immunity in patients with the content of CD4+ lymphocytes in the blood from 200 to 50 cells/μL — in 57.7 % of cases (in controls — 23.1 %).

Intravenous IgG against the background of etiotropic therapy of AMBT and ARVT contributes to the restoration of immunological indicators of cellular immunity due to pathogenetic, immunoreplacement and immunomodulatory effects, which contributes to the improvement of the effectiveness of the treatment of patients on DR-TB/HIV in a state of deep immunosuppression, leads to a decrease in the manifestations of intoxication, enables in more early appointment of ARVT, prevention of interruptions in treatment and complications in the form of development of systemic inflammation and multiple organ failure, improvement of survival prognosis.

Figure 3. Number of patients with normal CD8+ and increased CD8+ (absolute) count according to the treatment strategy

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References
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Відновлювальний вплив імуноглобуліну G на показники клітинного імунітету у хворих на ко-інфекцію лікарсько-стійкий туберкульоз/ВІЛ з рівнем CD4⁻-лімфоцитів від 200 до 50 клітин/мкл

Мета роботи — вивчити відновлювальний вплив імуноглобуліну G (IgG) на показники клітинного імунітету у хворих на ко-інфекцію лікарсько-стійкий туберкульоз (ЛС-ТБ)/ВІЛ зі стійкістю мікобактерій до препаратів першого та другого ряду. Хворих розподілили на дві групи: група 1 (контрольна) — 26 хворих з рівнем CD4⁻-лімфоцитів від 200 до 50 клітин/мкл, які отримували стандартне лікування (АМБП) другого ряду та антиретровірусну терапію (АРВТ), група 2 (основна) — 26 хворих з рівнем CD4⁻-лімфоцитів від 200 до 50 клітин/мкл, які, крім стандартного лікування АМБП другого ряду та АРВТ, отримували терапію внутрішньовенним IgG.

Результати та обговорення. У хворих групи 2 наприкінці 20-го місяця лікування у 69,2 % осіб зафіксовано нормалізацію рівня T-хелперів (у середньому — (830,0 ± 15,2) кл/мкл), у 30,8 % — субнормальний рівень ((408,0 ± 1,6) кл/мкл) (р < 0,05). Нормалізація цього показника у групі 1 (у середньому — (670,0 ± 14,5) кл/мкл) зареєстрована лише у 42,3 % осіб (р < 0,05). Відносний рівень T-хелперів наприкінці 20-го місяця досяг в 100 % хворих групи 2, а у групі 1 — у 35,4 % (р < 0,05). У групі 2 досягнуто фізіологічного рівня цього показника (19—35 %) у 65,4 % випадків (р < 0,05), у групі 1 — у 38,5 % (р < 0,05). Імунорегуляторний індекс (CD4⁺/CD8⁻) у групі 2 був нижчим за норму у 100 % хворих групи 1 протягом 14 міс і лише на 20-й місяць досяг норми у 23,1 % пацієнтів (р < 0,05). У групі 2 на 20-й місяць нормалізація цього показника зафіксована у 57,7 % випадків (р < 0,05).

Висновки. Додаткове застосування внутрішньовенного IgG у комплексному лікуванні хворих на ЛС-ТБ/ВІЛ сприяло швидшему відновленню клітинної ланки імунітету, що дає змогу в ранніші терміни призначати АРВТ хворим на ЛС-ТБ/ВІЛ, запобігти розвитку системного запалення та підвищити прогноз щодо виживання.

Ключові слова: туберкульоз, ВІЛ, лікарсько-стійкий туберкульоз.

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