MEDICAL UNIVERSITY – PLEVEN

FACULTY OF PUBLIC HEALTH

DEPARTMENT "INFECTIOUS DISEASES, EPIDEMIOLOGY, PARASITOLOGY AND TROPICAL MEDICINE"

DR. IVAYLO NIKOLAEV PAKOV

CHRONIC INFLAMMATION AND IMMUNE RECOVERY IN CONTROLLED HIV INFECTION

A U T O R E F E R A T
OF DISSERTATION

for awarding an educational and scientific degree

„DOCTOR"

Scientific specialty: Infectious diseases

Scientific supervisor: Prof. Dr. Galya Gancheva, MD, PhD

Pleven, 2024

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CONTENTS

ABBREVIATIONS

3-gl –triglycerides
DIC – disseminated intravasal coagulopathy
cells/μL – cells in microliter
NRCL – National Reference Confirmatory Laboratory
NCIPD – National Center for Infectious and Parasitic Diseases
CBC – Complete blood count
WHO – World Health Organization
AIDS – Acquired Immune Deficiency Syndrome
ALE – Average life expectancy
ABC – abacavir
ASAT – aspartataminotransferase
ALAT – alaninaminotransferase
ART – Antiretroviral therapy
BIC – bictegravir
BMI – body mass index
BUN – blood urea nirogen
c/mL – copies RNA/mL
$CD4^+$ – T-helper lymphocytes
$CD8^+$ – T-suppressor lymphocytes
Chol – total cholesterol
CI – Confidence interval
CMV – Cytomegalovirus
Creat – creatinine
CRP – C- reactive protein
DTG – dolutegravir
EACS – European AIDS Clinical Society
Er – erythrocytes
FTC – emtricitabine
Gluc – glucose
HAV – Hepatitis A virus
Hg – hemoglobin
HBV – Hepatitis B virus
HCV – Hepatitis C virus
HIV – Human immunodeficiency virus
hsCRP – high-sensitivity C-reactive protein
HSV – Herpes simplex virus
IL-6 – interleukin 6
LP – late presenting patient
Ly – lymphocytes
max – maximal value
min – minimal value
MSM – men who have sex with men
N – norm
n – number of patients
NA – not available
NRTI – nucleos(t)ide reverse transcriptase inhibitor
OR – odds ratio
PI – protease inhibitor
PI/b – protease inhibitor pharmacologically boosted
PLWH – people living with HIV
PLT – platelets
PrEP – pre-exposure prophylaxis

sd – standart deviation
TAF – tenofovir alafenamide
UNAIDS – Joint United Nations Programme on HIV/AIDS
VL – viral load (HIV-RNA)
3TC – lamivudine

INTRODUCTION

The publication of the first identical cases of pneumocystis pneumonia and Kaposi's sarcoma during the summer of 1981 among men of active age marked the beginning of the 43-year history of the HIV/AIDS pandemic as perhaps the greatest challenge to modern science, public health policy and economy. The intimate mechanisms of transmission, the long latent period to clinical manifestation, simultaneously combining elements of acute viral and chronic non-infectious disease, mediate the global spread of HIV, with over 85 million infected to date and over 40 million lives lost to the virus and related complications.

The combined strategy including an early start of increasingly effective antiretroviral therapy (ART) in HIV-infected people and the introduction of pre-exposure prophylaxis (PrEP) methods among risk groups simultaneously modulates the pandemic potential of the disease and dramatically reduces the new cases of HIV, as well as its associated complications and premature mortality. Compared to 2010 data, the overall incidence of HIV infection has decreased by as much as 38%, and AIDS-related mortality by 43%. This, respectively, leads to a convincing increase in the average life expectancy of PLWH (people living with HIV), approaching the age of the HIV-uninfected population, which outlines the current clinical profile of HIV infection as a controlled chronic disease in the process of general aging. This expands the key global strategy of UNAIDS ("95-95-95"), aiming for 95% of the infected globally to know their HIV status, 95% of them to be on ART and 95% of them to achieve optimal viral suppression and adds a fourth parameter “95” accounting for the quality of life of PLWH, continuously exploring relevant and long-term clinical-laboratory biomarkers of healthy aging.

The results of all long-term clinical studies show that even optimally treated and virologically controlled patients have an increased tendency to develop more chronic, non-infectious and non-HIV/AIDS-related conditions which require a greater number of other non-HIV drugs, compared to the general uninfected with HIV population of the same age. Chronic organ damage is mostly associated with a generalized state of persistent chronic inflammation and abnormal immune activation that cannot be fully controlled by the administration of current ART alone, which can not reach and clear latent viral reservoirs. The concept of "chronic inflammation in the aging process and at the same time an accelerated aging model in the process of chronic inflammation" (in English for short: "inflammaging") appears, leading to progressive age-associated organ dysfunction.

Locally, the combination of the specific geographical location and the key political transition at the end of the 1980s, followed by periodic social and economic crises, places Bulgaria at the crossroads of two HIV/AIDS epidemics – the one in Western and Central Europe with viral transmission mainly through MSM ("men who have sex with men") contacts, and the other in Eastern Europe and Central Asia as endemic for the infection, mainly with blood route of transmission through the injection use of drugs. The intensified migrant crisis, provoked by the military actions on the territory of Ukraine and partly in the Middle East, drastically changes the European and national statistics. The frequency of newly registered HIV cases per 100,000 population in the European union countries in 2022 is 5.1, whereas in 2021 is 3.9. Data for Bulgaria show a frequency of 4.8/100,000 for 2022 and 3.4/100,000 for 2021, which places our country among those with a relatively low prevalence of the infection. For the period 1986-2023, a total of 4,292 persons with HIV infection were confirmed and registered in Bulgaria, with over 2,300 of them being frequently monitored and receiving modern ART at the specialized treatment clinics in the country. The fate of the rest remains unknown. The tends in our country follows the ones in the other economically developed countries with confirmed infected men to be much more than confirmed infected women (approximately a ratio of 4:1) and over 90% with sexual way of transmission.

The local unsolved challenges about the systemic control of HIV largely overlap with the global ones and generally include the persisting high level of stigma and low common health culture among at-risk populations. During the last years, an unpleasant trend of gradually increasing period of HIV status registration and initiation of ART in PLWH is observed (also known as “linkage to care” period), probably due to the lack of sufficiently good prevention programs after the complete withdrawal of the Global Funding for HIV, Malaria and Tuberculosis in 2017. The increasing numbers of late-presenting and elderly patients with more non-HIV-related chronic conditions, require a complex multidisciplinary approach and a more complete clinical-immunological evaluation. Unfortunately, the lack of sufficiently

engaged and well-trained medical staff in the HIV area continues to be a serious barrier of reaching a comprehensive medical care of PLWH. Late diagnosis and presentation for treatment initiation, as well as poor adherence to ART, hide the risk of emerging resistant mutations of the virus, worsening chronical inflammation and immune dysfunction, which further complicate the build of an appropriate diagnostic and therapeutic algorithm.

Achieving optimal immune recovery and balancing chronic inflammation, as well as measuring them with appropriate clinical-laboratory biomarkers, appears to be a key point in aging well with controlled HIV infection. The desire to be in parallel with the above-mentioned challenges and related tasks motivates us to contribute to the present dissertation work by reviewing the features of the HIV patient profile and elucidating the role of age and immune status in initiation of antiretroviral therapy. The comparison of baseline clinical and laboratory parameters between PLWH and uninfected individuals, supplemented by additional, but not widely used in everyday practice biomarkers, considered in the context of all other indicators, would provide a more detailed interpretation of the patient's overall condition and assessment of the chronic condition as the cause of certain clinical events, complications and possibly an accelerated aging pattern.

AIM:

To investigate the nature of chronic inflammation and the extent of immunologic recovery in controlled HIV infection.

TASKS:

1. Study of **demographic, epidemiological and clinical characteristics** of HIV-positive persons undergoing ART.

2. Study on the dynamics of basic **laboratory parameters** in HIV-positive persons undergoing ART (including complete blood count – CBC, metabolic, liver and kidney parameters).

3. Study on the dynamics of the values of **T-helper lymphocytes** ($CD4^+$), **T-cytotoxic lymphocytes** ($CD8^+$), **$CD4^+$: $CD8^+$ ratio** and their role in evaluating immune recovery.

4. To investigate **correlations of biomarkers of inflammation (IL-6, hsCRP and D-dimer)** with HIV-status, age, initial immune status, specific risk factors and clinical-laboratory indicators during ongoing ART.

5. To develop a general **multifactorial model** taking into account essential HIV-related and non-HIV-related parameters, ongoing ART characterisics and the possibility of immune dysfunction, chronic inflammation and risk of complications in the aging process with controlled HIV infection.

DESIGN:

1. Prospective cohort study of demographic, anthropometric, epidemiological indicators, risk behavioral characteristics and clinical symptoms; laboratory, immunological parameters and biomarkers of chronic inflammation of HIV-positive patients (target group), divided and analyzed by subgroups according to **two main criteria – present age and initial immune status:**

1. By age – PLWH under 40 and over 40 years of age

2. By initial immune status – PLWH with initial $CD4^+$ counts <200 cells/μL, $CD4^+$ <350 cells/μL and $CD4^+$ >350 cells/μL.

2. Comparative study of key demographic, anthropometric, epidemiological indicators, risk behavioral characteristics and clinical sympoms; laboratory parameters and biomarkers of chronic inflammation (IL-6, hsCRP, D-dimer) in HIV-positive patients (target group) with HIV-negative individuals (control group).

3. Study of correlations between biomarkers of chronic inflammation (IL-6, hsCRP, D-dimer) and key clinical-laboratory parameters (BMI, multimorbidity, dyslipidemia, $CD8^+$ values and $CD4^+$: $CD8^+$ index), duration of ART and in HIV-positive patients from the target group.

MATERIALS:

Study object: 60 HIV-positive persons (target group) from the Center for Monitoring and Treatment of HIV-positive patients at the Clinic for Infectious Diseases of University Multiprofile Hospital for Active Treatment (UMHAT) "Dr. G. Stranski", Pleven and **30 HIV-negative persons (control group)** from "Ambulatory group practice for primary outpatient medical care - Dr. Elina Stefanova and Dr. Lyudmila Pakova" - Ltd.

1. Inclusion criteria:

1.1. Study (target) group – HIV-positive: 60 persons

- age over 18 years;
- absence of an established current pregnancy among the studied female subjects;
- antiretroviral therapy (ART) intake duration for at least 12 months;
- with achieved optimal viral suppression (known as undetectable viral load VL <40 c/mL), documented in at least 2 invesigations with an interval between them of at least 3 months;
- no evidence of virological and/or immunological failure (lack of effect from the ongoing ART, expressed in failure to achieve or failure to maintain optimal viral suppression and lack of immune recovery – number of T-helper $CD4^+$ cells in lower values than the initial ones);
- no documented presence of acute infectious diseases or other acute conditions (e.g. myocardial infarction, other cardiovascular and cerebrovascular accidents, operative interventions, etc.) or hospitalizations during the last 3 months;

During the study period, three patients from the target HIV group dropped out due to violation of the inclusion criteria - one patient interrupted therapy for more than 6 months, two patients were diagnosed with accompanying acute conditions requiring hospitalization in other clinical units (CVD; orthopedic intervention). In the study remained 57 HIV-positive patients who had been on therapy for an average of 5.24 years (from 1.2 to 13 years).

1.2. Control group – HIV (-) negative: 30 persons

- age over 18 years;
- absence of an established current pregnancy among the female subjects;
- no documented presence of acute infectious diseases or other acute conditions (e.g. myocardial infarction, other cardiovascular and cerebrovascular accidents, operative interventions, etc.) or hospitalizations during the last 3 months;

Two persons dropped out of the control group due to diagnosed acute conditions that necessitated hospitalization. Twenty eight HIV-negative individuals remain in the study.

2. Studied demographic, anthropometric (height, weight, Body Mass Index – BMI), epidemiological indicators, behavioral risk characteristics and clinical indicators; laboratory, immunological and virological indicators; biomarkers of chronic inflammation

2.1. Characteristics and indicators studied in both groups:

2.1.1. Gender

2.1.2. Age

2.1.3. Residence, educational level and professional commitment

2.1.4. Height, weight, body mass index (BMI)

2.1.5. Behavioral risk factors – smoking, chronic alcoholism or other abuses and addictions

2.1.6. Co-infection with other infectious diseases with chronic tendency

2.1.7. Documented accompanying chronic diseases and conditions that need periodic monitoring and therapy by a specialist – incl. cardiovascular diseases, cerebrovascular diseases, chronic lung diseases, metabolic and endocrine disorders such as dyslipidemia, diabetes mellitus or impaired thyroid function, as well as previous oncological diseases

2.1.8. Systemic everyday therapy intake during the last 2 years for the diagnosed chronic diseases

2.1.9. Hospitalizations – number and reasons

2.1.10. Preventive examinations and immunizations

2.1.11. Registration of subjective complaints

2.1.12. Values of CBC, blood glucose, cholesterol, triglycerides, AST, ALT, creatinine, urea

2.1.13. Monitored IL-6 levels; hsCRP; D-dimer (at baseline and 6 months after study entry). A spring-summer and an autumn-winter season were selected consecutively, with strict monitoring for the occurrence of confounding factors that did not meet the inclusion criteria.

2.2. Characteristics and indicators studied only in the HIV (+) group:

2.2.1. Specific epidemiological features mediating HIV transmission.

2.2.2. Presence of opportunistic and/or other HIV-defining conditions

2.2.3. Baseline viral load (VL), T-helper lymphocytes ($CD4^+$), T-cytotoxic lymphocytes ($CD8^+$), $CD4^+$: $CD8^+$ ratio

2.2.4. Differentiation of PLWH with immunological features of late-presentdtion ($CD4^+$ <350 cells/μL), and/or with advanced immunodeficiency ($CD4^+$ <200 cells/μL)

2.2.5. Periodically tracked dynamics in VL values; $CD4^+$; $CD8^+$; $CD4^+$: $CD8^+$

Since, after extensive literature review, no single consensus was established in the guidelines regarding the definition of precise limits measuring a restored and/or optimal ratio ($CD4^+$:$CD8^+$) in immunocompromised individuals, we adopted an index >0.8 as optimal regarding the accompanying condition, and <0.4 for extremely unsatisfactory. An index >1.0 is considered normal regarding the reference values, it is not appropriate to be considered equally for the HIV-infected and non-HIV-infected population.

METHODS:

1. Sociological method of gathering information.

1.1. Interview – an additional developed questionnaire is attached to collect data on demographic and social characteristics of the persons studied. Questionnaire cards and an informed consent form tailored to patients from the target and control groups were created.

2. Documentary method (medical documents) for collecting data on the health status of the persons – filling in demographic, biometric, laboratory indicators, comorbidity and risk factors in the questionnaires by the persons in the two studied groups.

3. Epidemiological method – epidemiological analysis.

4. Clinical method – the subjects/patients are clinically examined (taking a detailed history and physical examination).

5. Laboratory methods – examination of venous blood for non-specific hematological and biochemical indicators (complete blood count, total cholesterol, triglycerides and blood glucose) according to conventional laboratory methods – for the target and control groups.

5.1. Complete blood count (CBC) – through hematology analyzer MEDONIC M32, Boule. **Reference values: hemoglobin (Hg) – males135-180 g/L; females 120-160 g/L] erythrocytes (Er) – m. 4.4-5.9 x 1012/L; g. 3.7-5.3 x 1012/L] leukocytes (WBC) – 3.5-10.5 x 109/L; platelets (PLT) – 130-360 x 109/L.**

5.2. Biochemical parameters: biochemical analyzer Cobas 6000, Roche. **Blood glucose (Glucose)** – hexokinase method. **Reference values: 4.1-6.1 mmol/L. Total cholesterol (Chol)** – by enzymatic colorimetric method CHOD-PAP. **Reference values: <5.2 mmol/L. Triglycerides (3-gl)** – by enzymatic colorimetric method GPO-PAP. **Reference values: <2.3 mmol/L. ASAT** – by IFCC method, 370C. **Reference values: 0-40 U/L. ALAT** – by IFCC method, 370C. **Reference values: 0-40 U/L. Serum creatinine (Creat)** – by Jaffe's method – kinetic. **Reference values: m. 80-115 μmol/L; f. 53-97 μmol/L. Blood urea (BUN)** – by urease/ GLDH test. **Reference values: 2.8-8.1 mmol/L.**

6. Serological tests – carried out in the Microbiological Laboratory of University Hospital "Dr. G. Stranski" - Ltd, Pleven:

6.1. HBs Ag – by Vidas HBs Ag Ultra (HBs) qualitative enzyme-linked fluorescence assay (ELFA) performed with an automated mini Vidas system of Bio Merioux (France). The result is automatically analyzed and expressed with an index calculated using a standard. **Reference values: < 0.13.**

6.2. anti HCV – by Vidas anti-HCV Ultra (HCV) qualitative enzyme-linked fluorescence test (ELFA) performed with an automated mini Vidas system of Bio Merioux (France). The result is automatically analyzed and expressed with an index calculated using a standard. **Reference values: < 1.00.**

7. Examination of specific immunological indicators ($CD4^+$, $CD8^+$, $CD4^+$: $CD8^+$) and viral load (VL) – only for the target group of HIV-positive persons.

7.1. Viral load (VL) - the test is performed at the NRCL of HIV/AIDS at NCIPD – Sofia, Bulgaria. Each blood sample is accompanied by a coupon for laboratory testing according to Annex No. 4 of Ordinance No. 47 of December 11, 2009 on the conditions and procedures for testing, reporting and reporting infection with the acquired immune deficiency syndrome virus, issued by the Ministry of Health, Pron. SA. no. 103 of December 29, 2009, and Publ. SA. no. 5 of January 14, 2011. Blood samples are transported within 24 hours. The investigation is being performed out by Real-time PCR and is used as monitoring of the success of antiretroviral therapy and presence of resistance to antiretroviral drugs, by sequencing and genotyping of the viral gene.

7.2. Immunophenotyping of lymphocyte subpopulations (incl. $CD4^+$, $CD8^+$) - the study is performed at the Laboratory of Immunology at UMHAT "Dr. G. Stranski" - Pleven. To study lymphocyte subpopulations, flow cytometry of peripheral blood lymphocytes was performed. All samples are processed within 2-24 hours of collection. Leukocytes were analyzed by dual-laser flow cytometer FACS Calibur cytometer (Becton Dickinson, Heidelberg, Germany) and Cell Pro Software (becton Dickinson). ***Reference values***: **T - helper lymphocytes ($CD4^+$): 700 – 1100 cells /µL; T – cytotoxic / suppressor lymphocytes ($CD8^+$): 500 – 900 cells /µL; $CD4^+$: $CD8^+$ ratio: 1.0 – 1.5.**

8. Examination of pro-inflammatory biomarkers (IL-6, D-dimer, hsCRP) using established laboratory methods – for the target and control groups was carried out in the Clinical Laboratory of University Multiprofile Hospital for Active Treatment (UMHAT) "Dr. G. Stranski", Pleven with Roche Diagnostics tests and analyzer Cobas E 411.

8.1. Roche test for determination of D-dimer: Immunoturbidimetric test. The reagent in the test contains latex particles coated with antibodies against DD. The Antigen-Antibody complex produced by the addition of DD samples to the reagent causes the reaction mixture to become cloudy. The change in absorbance is proportional to the concentration of DD. **Reference values: < 5.00 µg/mL.**

8.2. Roche test for determination of hsCRP: Immunoturbidimetric test. Human CRP agglutinates with latex particles coated with anti-CRP antibodies. The precipitate was determined turbidimetrically and the absorbance was proportional to the CRP concentration. **Reference values: 0.00-5.00 µg/mL.**

8.3. Roche test for determination of IL-6: The test uses specific antibodies against IL-6, one of which is labeled with a ruthenium complex and coated in streptavidin microparticles. In the presence of antigen in the sample, a sandwich complex is formed. On the surface of an electrode to which a voltage is applied, microparticles are magnetically captured, and unbound substances are removed. The resulting chemiluminescent emission is measured by a photomultiplier. **Reference values: 0.00-7.00 pg/mL.**

9. Statistical methods

The data were entered and processed with the statistical packages IBM SPSS Statistics 19.0 and MS Excel v. 2010. The following methods of medical statistics were used:

9.1. Determination of prevalences of quality characteristics;

9.2. Determination of central tendency indicators for quantitative variables – calculation of average arithmetic values (average); calculation of mode (mode) and median (median) for uneven variation series.

9.3. Determination of dispersion indicators – standard deviations (sd) and confidence intervals (CI);

9.4. Comparison of mean values and qualitative features (t-test). Statistical significance – at $p < 0.05$.

9.5. One-way analysis of variance with unequal complex (ANOVA). Statistical significance was accepted at $p < 0.05$.

9.6. Correlation analysis of qualitative alternative signs (φ-coefficient – by modified Pearson correlation coefficient formula). When using the φ-coefficient for correlation assessment, a 5-level scale was used: weak correlation dependence at $\varphi<0.3$; moderate – at $0.31<\varphi<0.5$; significant at $0.51<\varphi<0.7$; large - at $0.71<\varphi<0.9$; extremely large correlation dependence at $\varphi>0.9$.

9.7. Criterion for factor impact (OR) – risk of impact of factor studies is accepted at OR >1.0, with the degree of risk increasing as the OR increases.

9.8. Tables and figures are used to visualize the statistical results.

RESULTS

Chapter I. Results of studies of demographic, anthropometric, epidemiological and clinical indicators

1. Studies of demographic, anthropometric, epidemiological, behavioral and clinical indicators of HIV patients (N_1 =57)

Demographic and anthropometric characteristics – mainly age 30-39 years (***Fig. 1***); predominantly male (p<0.0005); urban residence, secondary education; a diverse professional spectrum; excess BMI (47%). All studied patients were Bulgarian citizens, with 55/57 (96.49%) from Northern Bulgaria (***Fig. 2***).

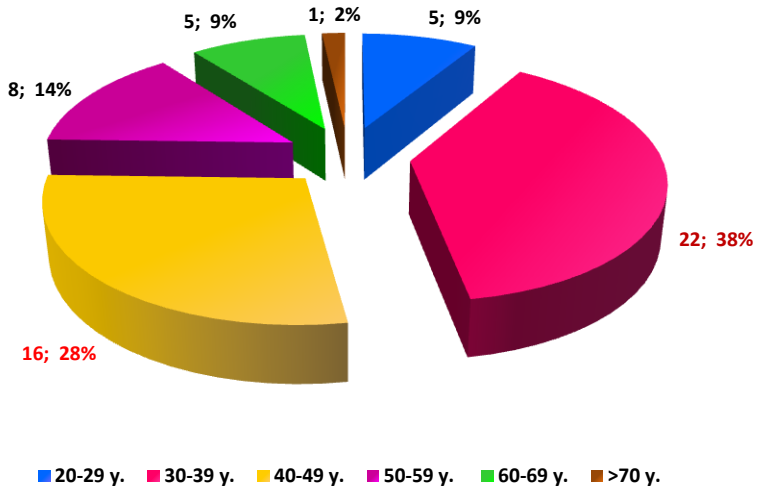


Fig. 1. Age structure of the target HIV group (N_1=57)

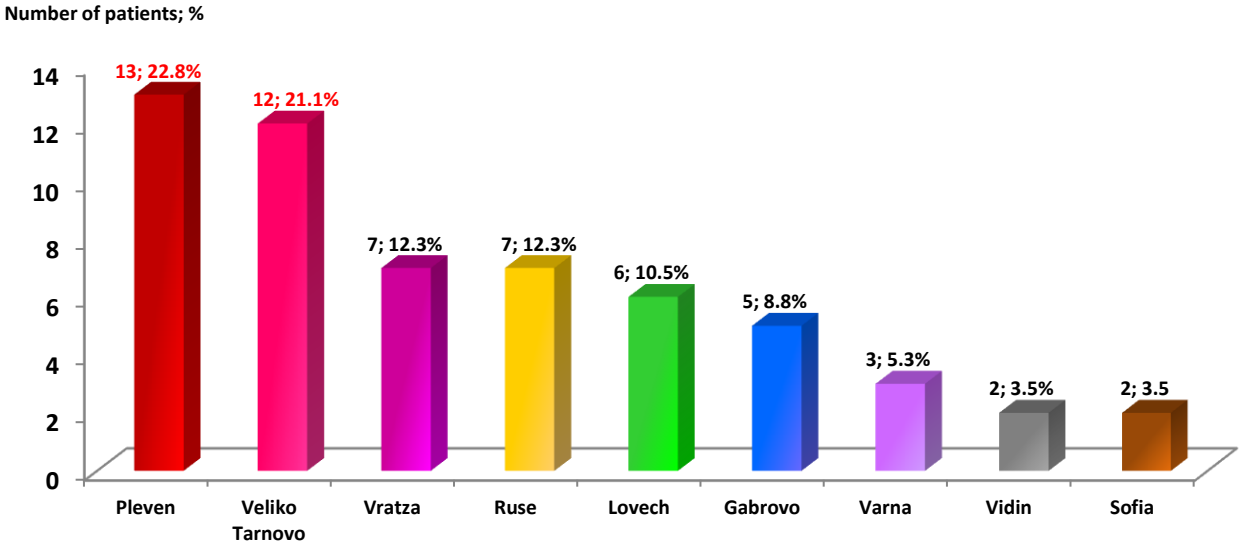


Fig. 2. Distribution by place of residence in the target HIV group (N1=57) – number and prevalence

Epidemiological characteristics – MSM (56.14%); passively detected (68.42%) (p<0.0005); time of infection before detection – average 3 years.

Behavioral characteristics – regular smokers (70.18%); weekly alcohol consumption (38.60%); regular psychoactive substances (10.53%).

Clinical characteristics – co-infections (35.09%) (***Fig. 3***); comorbidities (73.68%) (***Fig. 4***); multimorbidity (26.32%); polypharmacy (12.28%); 19.30% – without OPL; hospitalized in the last 2 years – 56.14%; preventive examinations – 42.11%; mandatory vaccines – 100%; recommended vaccines – 43.86%; most common complaints – increased stress (59.65%), weight gain (45.61%), general weakness and fatigue with usual efforts (36.84% each) (***Fig. 5***).

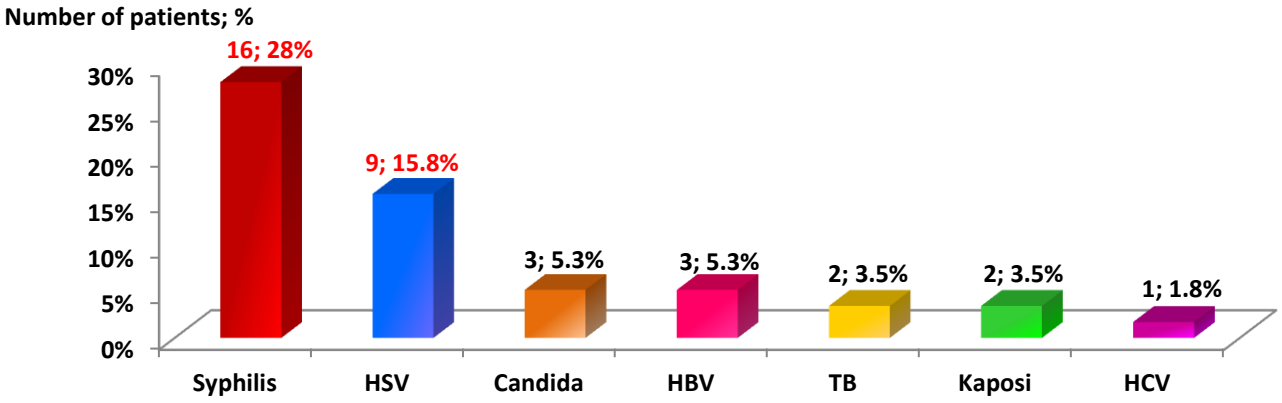


Fig. 3. Opportunistic and co-infections in the target HIV group (N_1=57) – number and prevalence

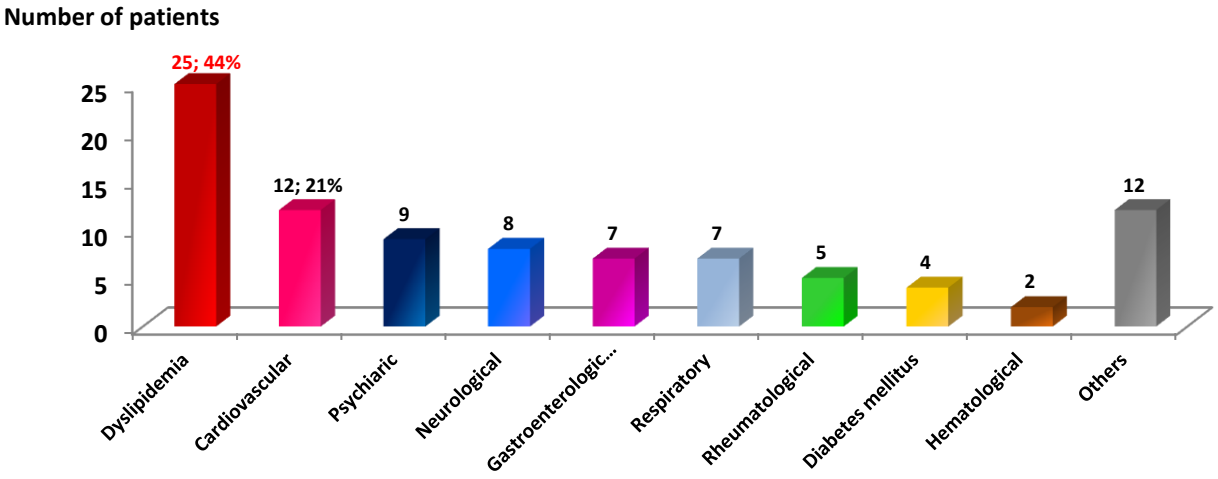


Fig. 4. Number of PLWH with comorbidities in the target HIV group (N_1=57)

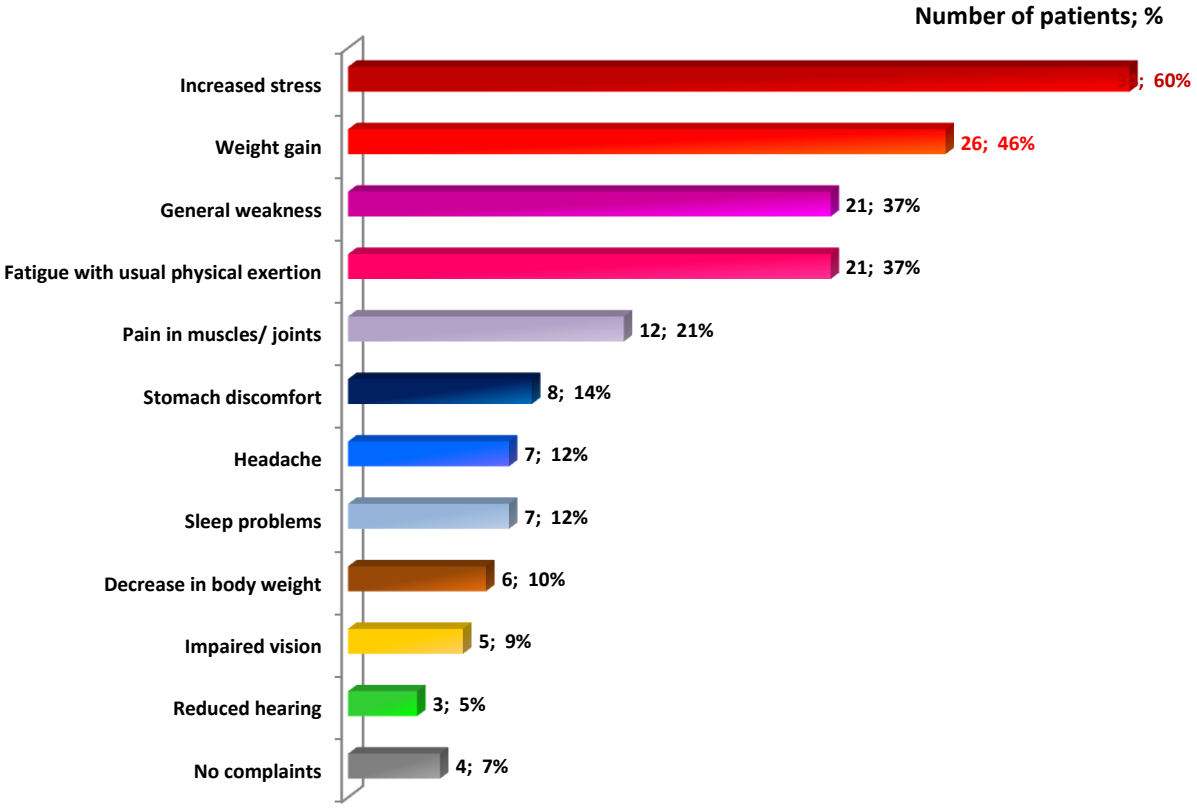


Fig. 5. Number and prevalence of patients in the target HIV group (N_1=57) reporting subjective complaints

2. Comparative study of demographic, anthropometric, epidemiological, behavioral and clinical characteristics of patients with HIV infection (N1 = 57) with individuals from the control group (N2 = 28)

Demographic, anthropometric characteristics – significantly older in the control group; higher educational qualifications of the control subjects ($p<0.025$), without significant anthropometric differences (***Fig. 6***).

Behavioral characteristics – more PLWH smokers ($p<0.0005$) (***Fig. 7***).

Clinical characteristics – no significant differences regarding comorbidities, but psychiatric, neurological, gastroenterological and respiratory diseases only in the target group (***Fig. 8***); multimorbidity more in PLWH ($p<0.025$); more frequent hospitalizations in the target group; preventive examinations – persons from the control group are more motivated and with more recommended vaccines (***Fig. 9***); significantly more frequent increased stress, weight gain, general weakness and fatigue with usual physical exertion in PLWH ($p<0.0005$) (***Fig. 10***).

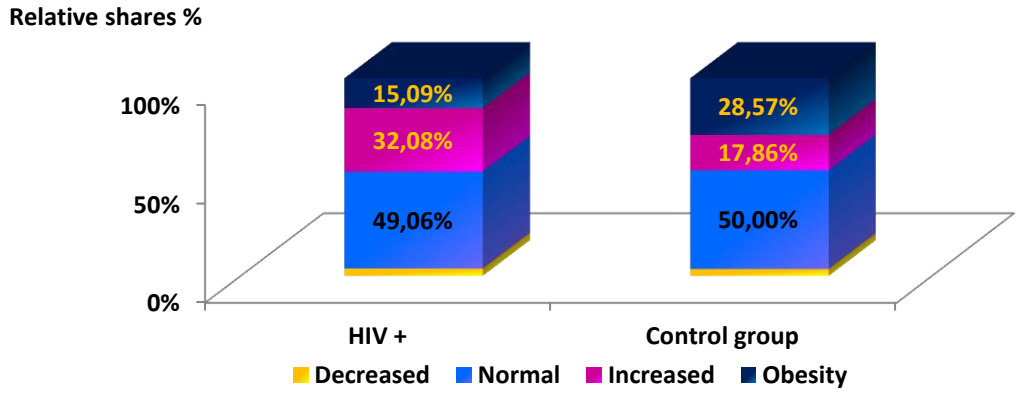


Fig. 6. Prevalence of persons with different body weights in the target group ($N_1 = 57$) and the control ($N_2 = 28$) groups

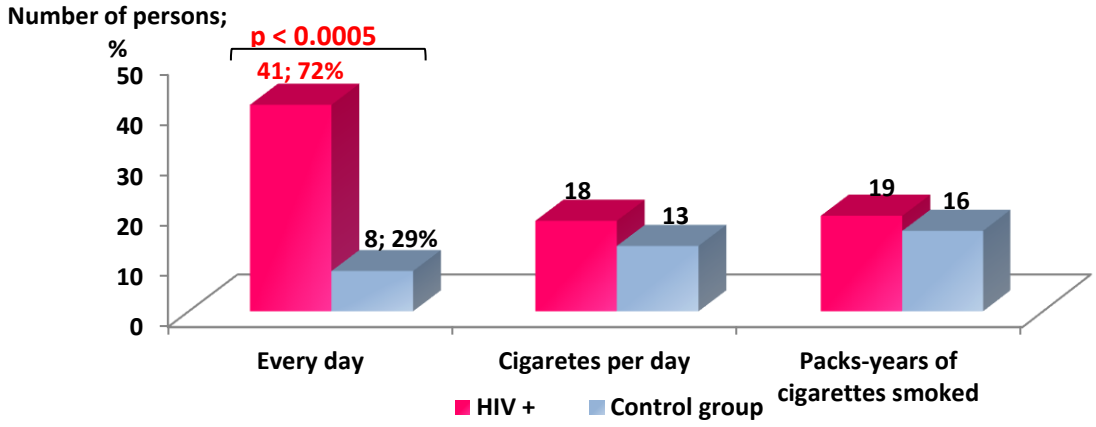


Fig. 7. Characteristics of smoking in the target ($N_1 = 57$) and control (N2 = 28) groups

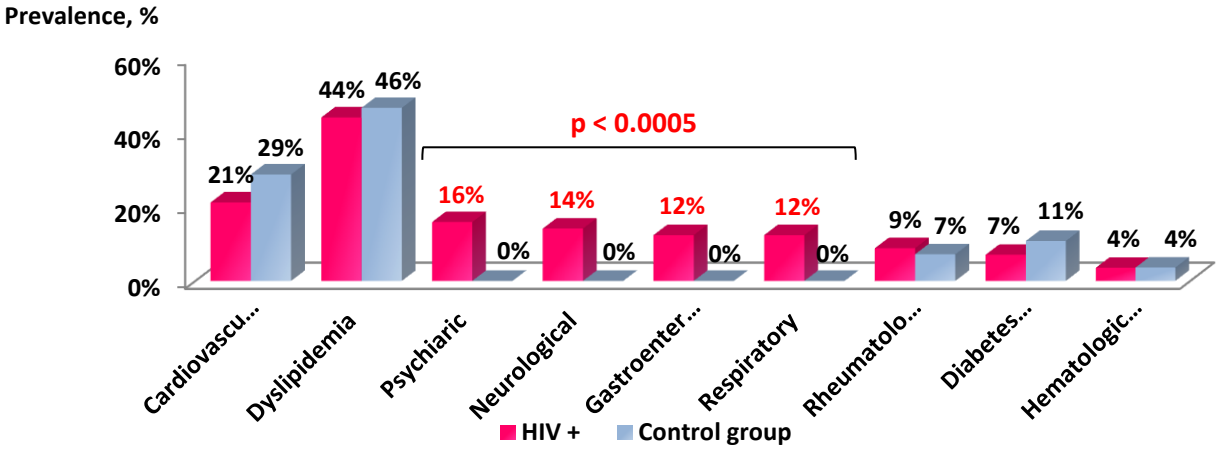


Fig. 8. Prevalence of persons with comorbidities in the target ($N_1 = 57$) and control ($N_2 = 28$) groups

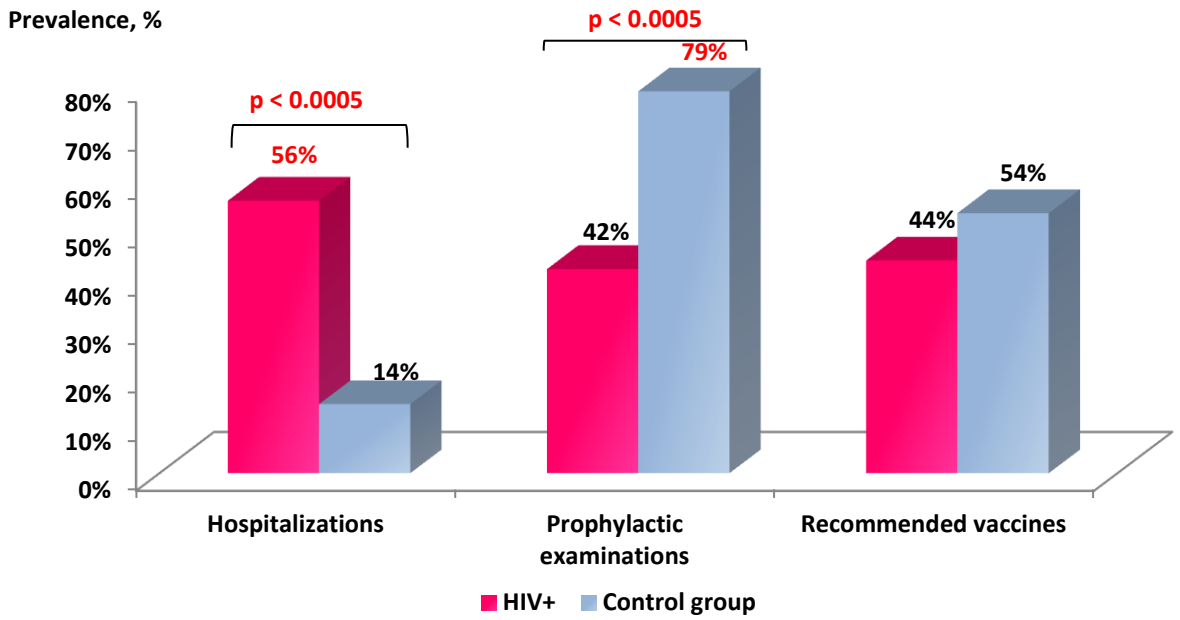


Fig. 9. Prevalence of persons with hospitalizations, preventive examinations and vaccinations in the target (N_1 = 57) and control (N_2 = 28) groups

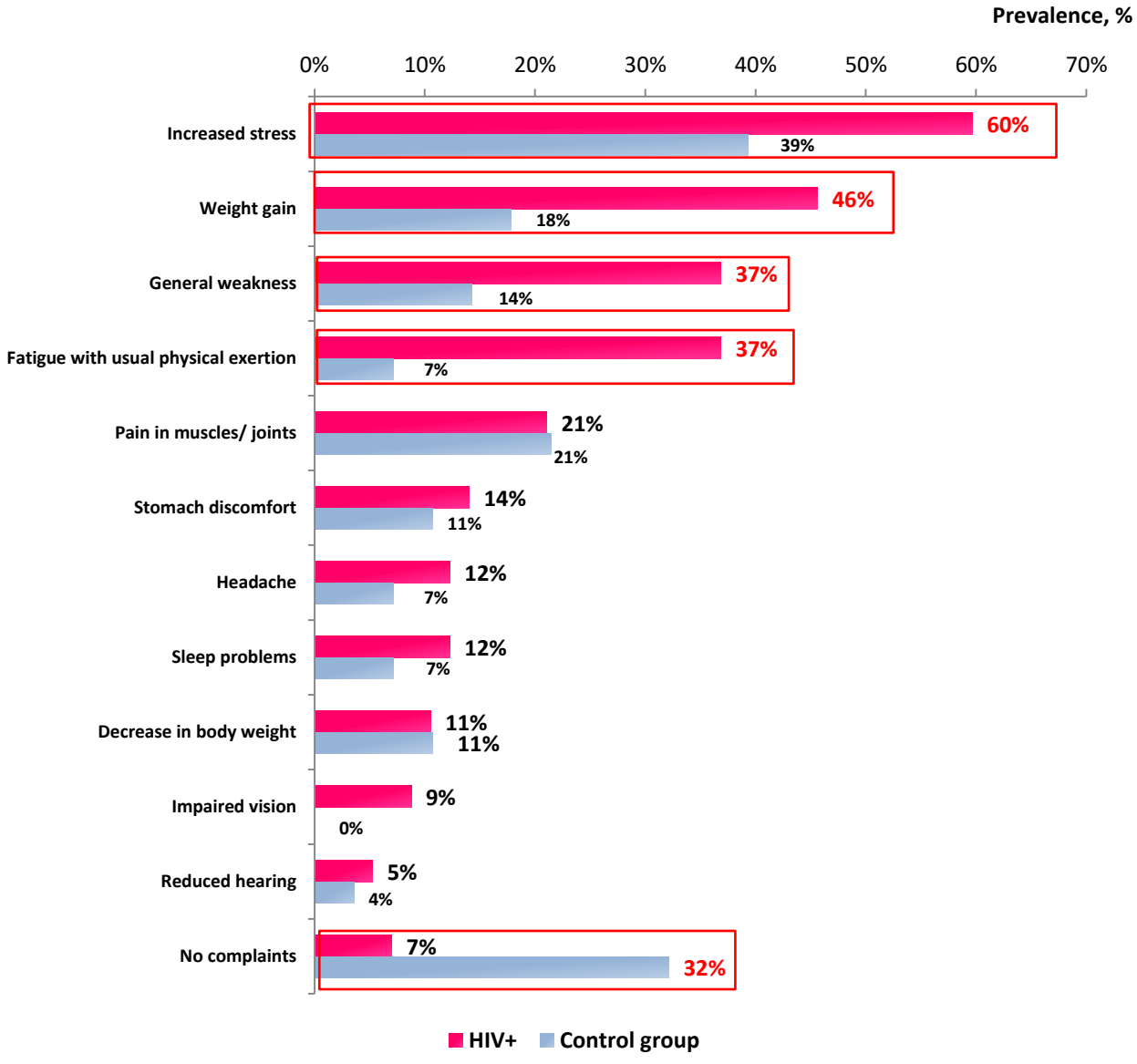


Fig. 10. Prevalence of persons with different subjective complaints in the target group (N_1 = 57) and the control (N_2 = 28) groups

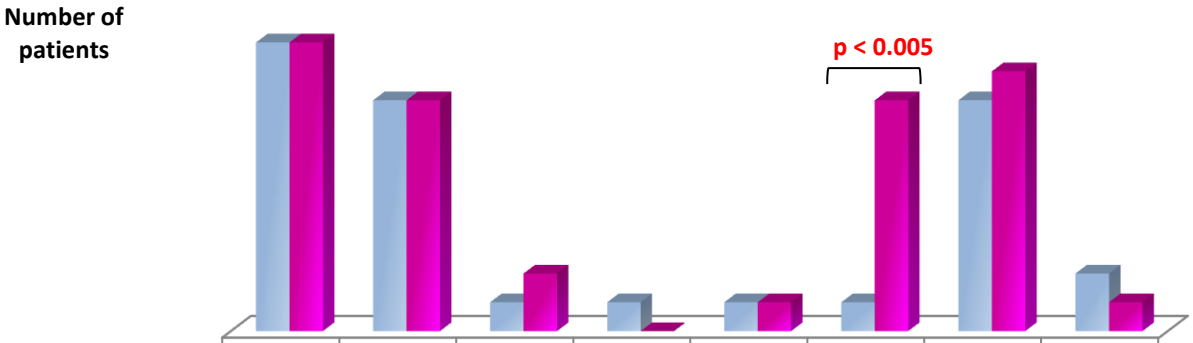
3. Comparative study of demographic, anthropometric, epidemiological, behavioral and clinical indicators in patients with HIV infection aged 20 to 40 years (n_1 = 27) and over 40 years (n_2 = 30)

Demographic and anthropometric characteristics – predominant male gender and urban residence in both age groups; higher educational qualification in the younger group; without significant differences in anthropometric characteristics.

Epidemiological characteristics – significantly higher relative proportion in younger MSM in younger ($p<0.05$), heterosexual men – in older ($p<0.025$); in both groups the passively detected ones predominate.

Behavioral characteristics – regular smokers predominate in both groups, but without a significant difference; use of psychoactive substances – only among younger people.

Clinical characteristics – no significant differences regarding the frequency of co-infections in the two groups, but new herpes infections after more than two years of ART – only in the younger ones ($p<0.005$) (***Fig. 11***); significantly more often comorbidities and multimorbidity over 40 years of age; dyslipidemia after 2 years ART – also in the group over 40 years ($p<0.0005$) (***Fig. 12***); more hospitalizations – in the same group. No significant differences regarding preventive examinations and immunizations (***Fig. 13***). Regarding complaints – significantly more often increased stress, general weakness and frequent stomach discomfort, and joint and muscle pain, fatigue during usual efforts - in the elderly ($p<0.05$) (***Fig. 14***).



	All coinfections	Syphilis	HBV	HCV	Tuberculosis	HSV2, VZV	With 1 coinfection	With 2 coinfections
To 40 y.	10	8	1	1	1	1	8	2
Over 40 y.	10	8	2	0	1	8	9	1

Fig. 11. Number of HIV patients aged up to (n_1 = 27) and over 40 years (n_2 = 30) with various co-infections

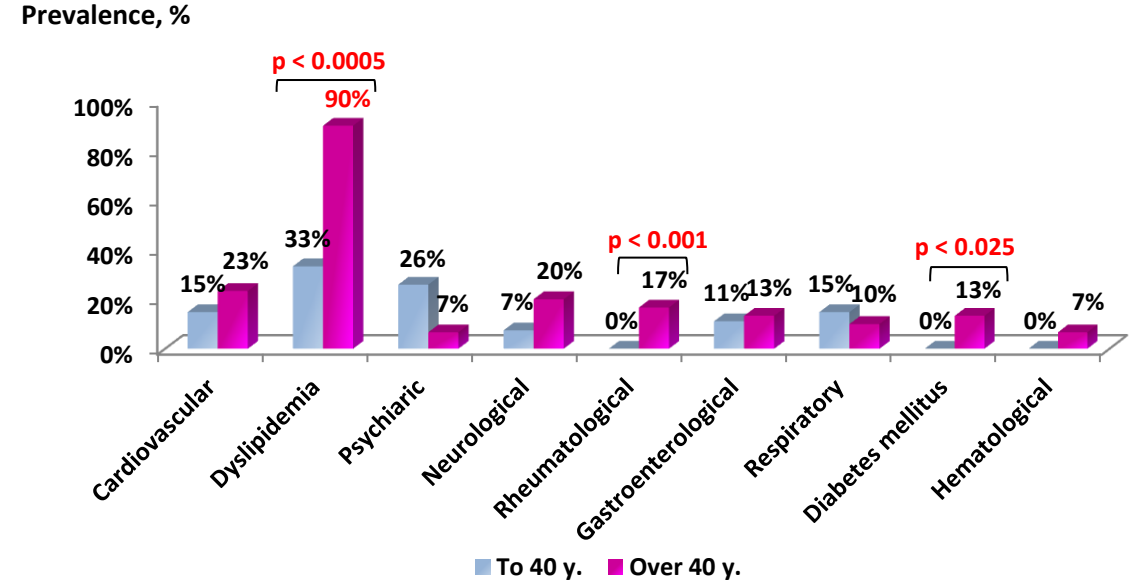


Fig. 12. Prevalence of HIV patients aged up to (n_1 = 27) and over 40 years (n_2 = 30) with various comorbidities

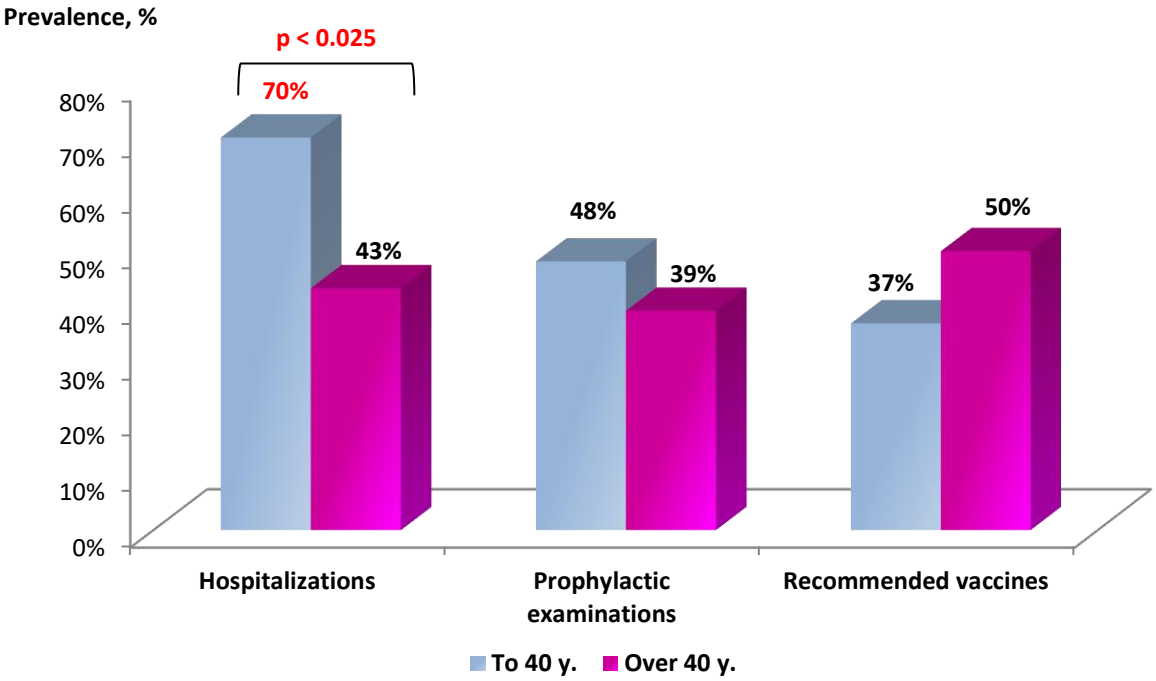


Fig. 13. Prevalence of HIV patients aged up to n_1 = 27) and over 40 years (n_2 = 30) with hospitalizations, preventive examinations and vaccinations

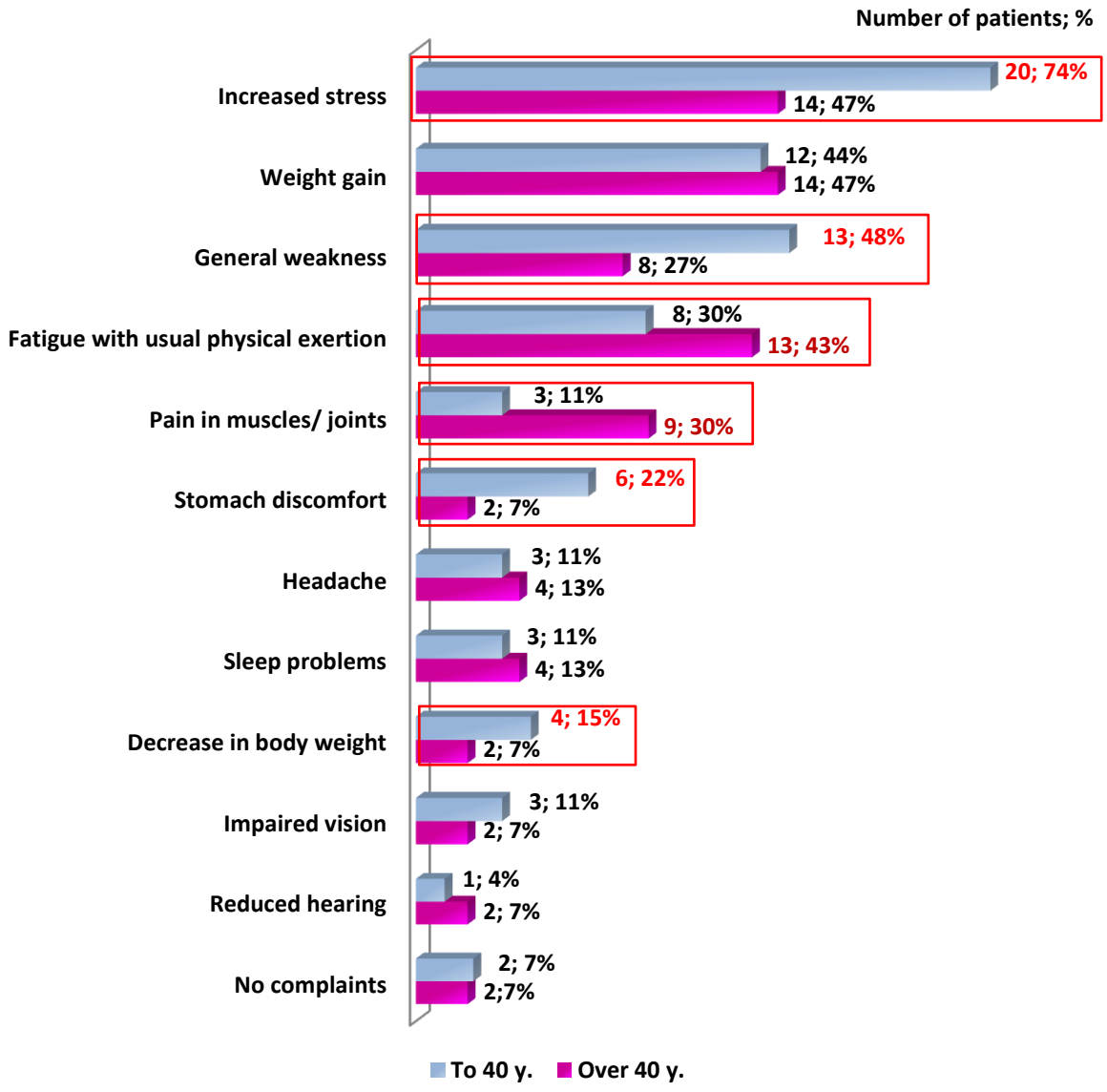


Fig. 14. Prevalence of HIV patients aged up to (n_1 = 27) and over 40 years (n_2 = 30) with various subjective complaints

4. Comparative study of demographic, anthropometric, epidemiological and clinical indicators and behavioral characteristics in patients with HIV infection with different initial immune status ($CD4^+$<200 cells/μL – n_1 = 17, $CD4^+$<350 cells/μL – n_2 = 28 and $CD4^+$>350 cells/μL – n_3 = 29)

Demographic and anthropometric characteristics – predominant male gender and urban residence in all groups; significantly higher BMI in the group with $CD4^+$ >350 cells/μL ($p<0.05$) (***Fig. 15***).

Epidemiological characteristics – in all three groups with different initial immune status, MSM are over 50% ($p>0.05$). The prevalence of passively detected is greater – 92.86% in patients with $CD4^+$<200, 75.86% in those with $CD4^+$<350 and 60.71% in the group with $CD4^+$>350 cells/μL, respectively ($p<0.025$).

Behavioral characteristics – in all three groups regular smokers predominate, regardless of the intensity of smoking.

Clinical characteristics – no significant differences regarding co-infections (***Fig. 16***), comorbidities (***Fig. 17***); hospitalizations, preventive examinations and immunizations (***Fig. 18***). Complaints - increased stress, weight gain, fatigue with usual efforts, but no significant difference between groups; significantly more often sleep problems in patients with CD4+ <350 cells/μL ($p<0.025$) (***Fig. 19***).

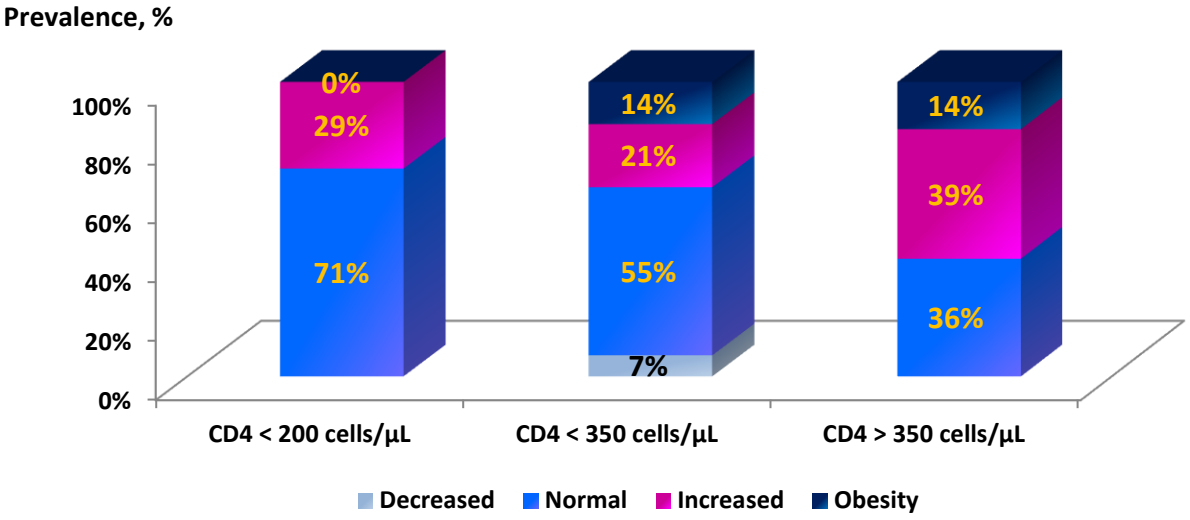
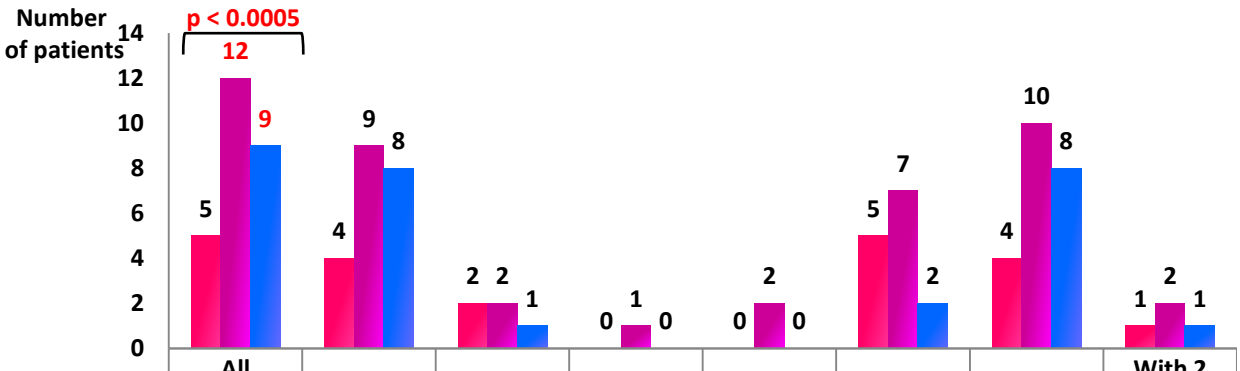


Fig. 15. Body weight in HIV patients with initial no. $CD4^+$ <200 (n_1 =17), <350 (n_2 =28) and >350 cells/μL (n_3 =29)



	All coinfections	Syphilis	HBV	HCV	Tuberculosis	HSV2, VZV	With 1 coinfection	With 2 coinfections
CD4 < 200	5	4	2	0	0	5	4	1
CD4<350	12	9	2	1	2	7	10	2
CD4>350	9	8	1	0	0	2	8	1

Fig. 16. Coinfections in HIV patients with initial $CD4^+$ counts <200 (n_1 = 17), <350 (n_2 = 28) and >350 cells/μL (n_3 = 29) – number of patients

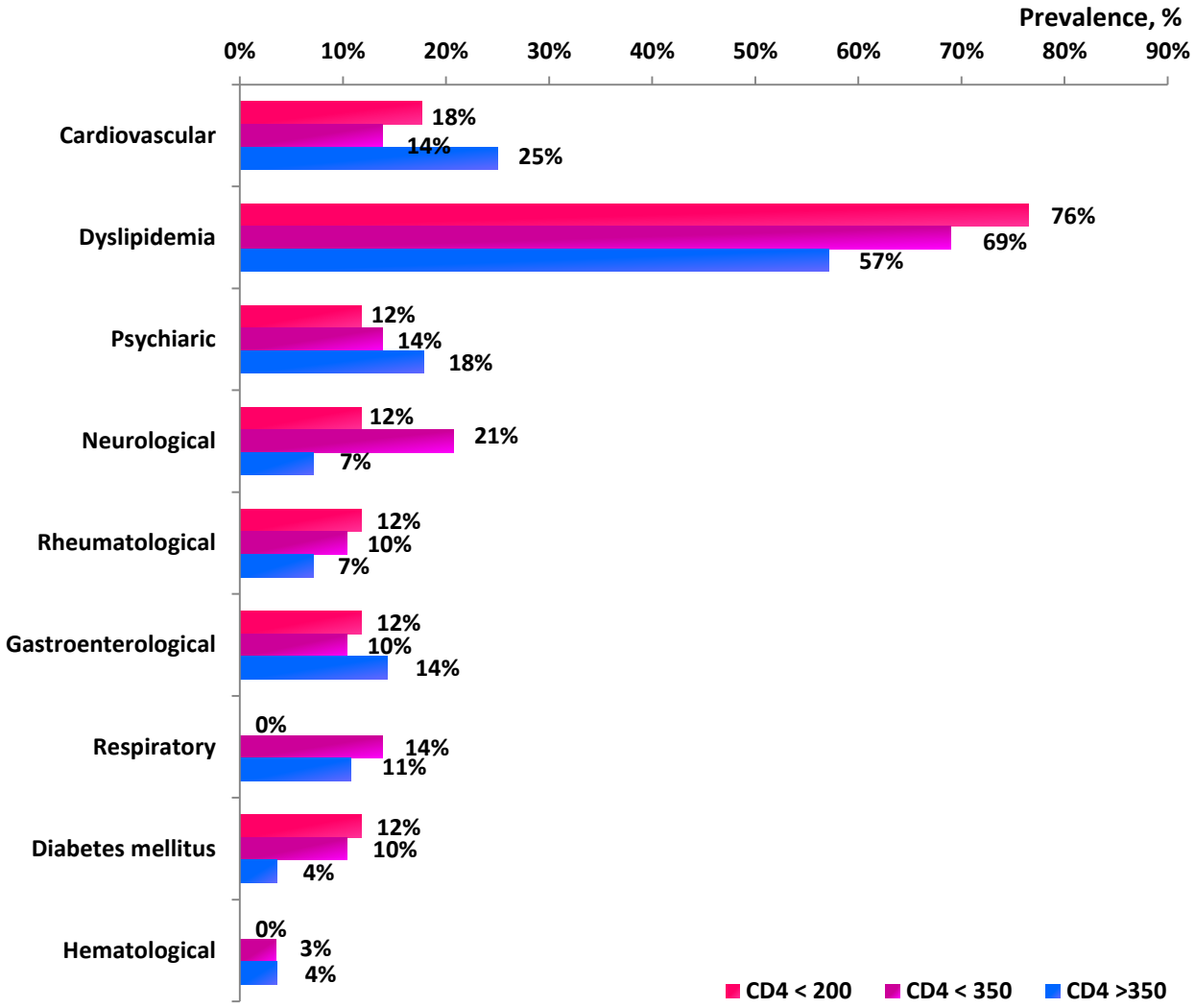


Fig. 17. Comorbidities in HIV patients with an initial CD4$^+$ count <200 (n_1 = 17), <350 (n_2 = 28) and >350 cells/μL (n_3 = 29) – number of patients

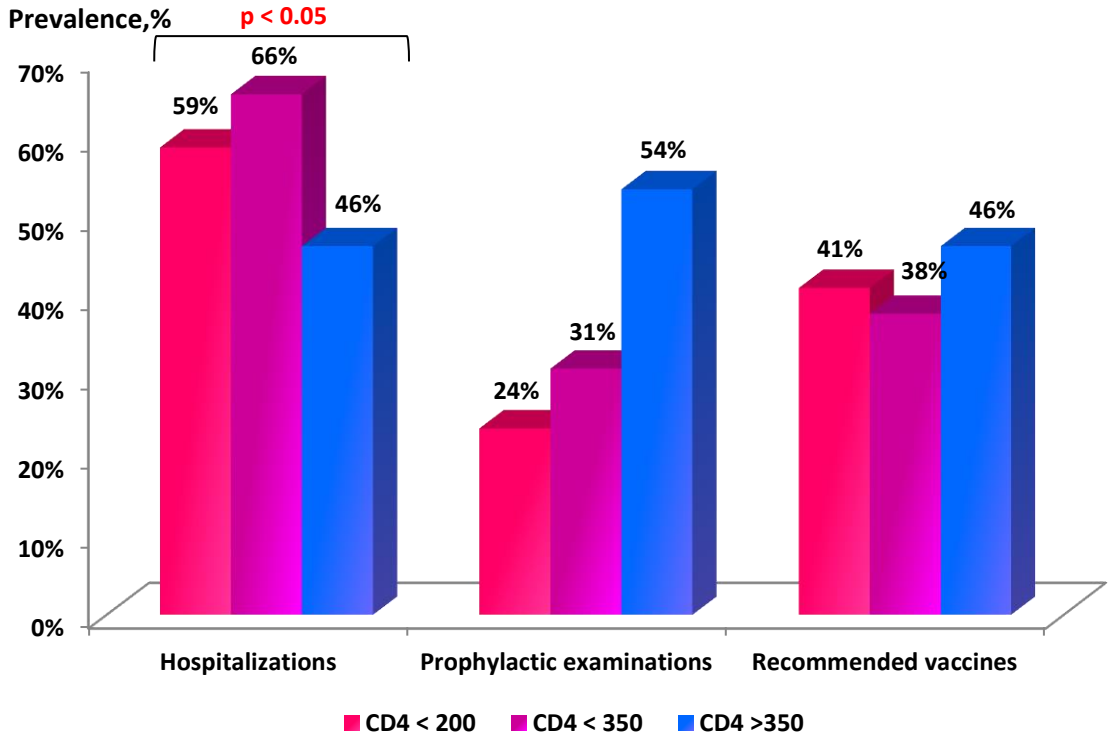


Fig. 18. Hospitalizations, preventive examinations and vaccinations in HIV patients with an initial CD4$^+$ count <200 (n_1 = 17), <350 (n_2 = 28) and >350 cells/μL (n_3 = 29)

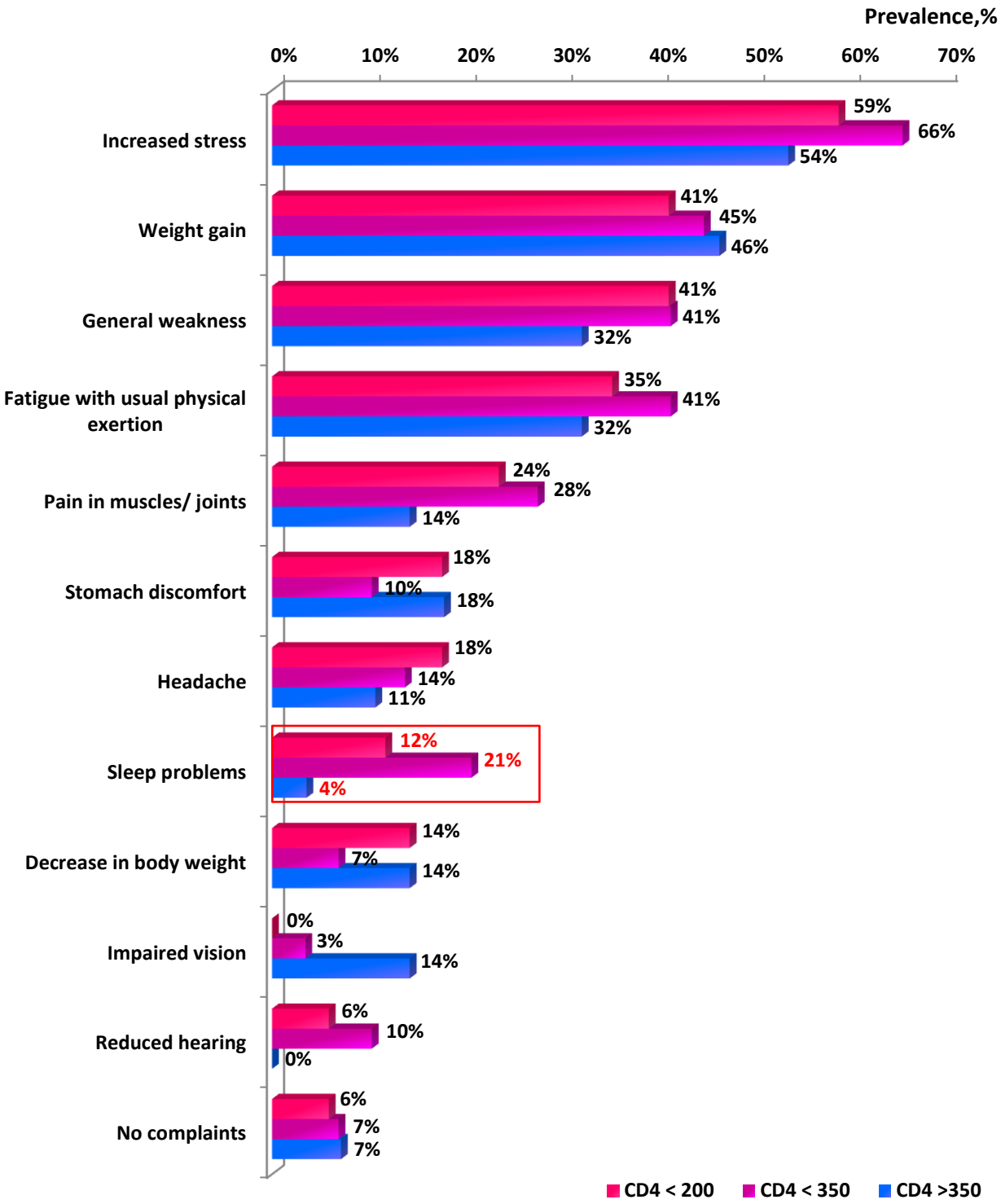


Fig. 19. Complaints in HIV patients with initial $CD4^+$ counts <200 (n_1 = 17), <350 (n_2 = 28) and >350 cells/μL (n_3 = 29)

Chapter II. Studies of laboratory parameters

1. Study of laboratory parameters in patients with HIV (N_1 = 57) (*Table 1*)

1. Complete blood count

1.1. Hemoglobin

At the start of monitoring, hemoglobin ranged from 88 to 165 g/L (mean 137.17 ± 18.90). With low hemoglobin at the start are 22.81%. **The last hemoglobin values ranged from 107 to 166 g/L (mean 144.74 ± 11.88); with low hemoglobin are 14.04%.**

1.2. Erythrocytes

Baseline erythrocyte values ranged from 3.0 to 7.4 x 1012/L (mean 4.78 ± 0.70). With low starting values are 19.23%. **The last values of erythrocytes ranged from 3.6 to 7.1 x 1012/L (mean 4.70 ± 0.57); with a low number of erythrocytes are 22.81%.**

1.3. Mean erythrocyte volume (MCV)

The starting MCV ranged from 63 to 103 (mean 84.94 ± 7.40). With low starting values of MCV are 20.41%, with high – 4.08%. **The last MCV values ranged from 62 to 106 (mean 91.89 ± 7.68); with low MCV are 5.26%, with high – 17.54% of patients.**

1.4. Leukocytes

Baseline leukocyte values ranged from 2.0 to 15.6 x 109/L (mean 6.03 ± 2.68). With low starting values they are 11.32%, with high – 7.55%. **The last values ranged from 3.5 to 12.6 (mean 6.98 ± 1.76); with leukocytosis are 3.51%.**

1.5. Platelets

Starting values ranged from 62 to 526 x 1012/L (mean 227 ± 77). With low starting values are 3.85%, with high – 3.85%. **The last values – from 148 to 388 x 1012/L (average 247±52); no patients with thrombocytopenia, with moderately elevated platelets 5.26%.**

2. Blood glucose

At baseline, blood glucose ranged from 4.2 to 15.6 mmol/L (mean 5.44 ± 1.59). There were 7 patients (13.73%) with elevated blood glucose at the start. **The last blood glucose values ranged from 1.2 to 8.8 mmol/L (mean 5.38 ± 1.14); 1 patient had low blood glucose (1.96%), and 7 had high blood glucose (13.73%).**

3. Total cholesterol

Baseline values ranged from 2.2 to 7.7 mmol/L (mean 4.62 ± 1.21). There were 16 patients (30.77%) with high initial cholesterol. **At the end, total cholesterol varied from 2.4 to 8.8 mmol/L (mean 5.36 ± 1.19) (*Fig. 20*); 33 patients had elevated cholesterol (57.89%) (*Fig. 21*).**

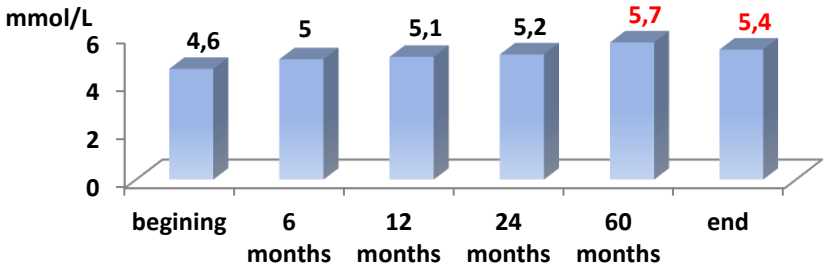


Fig. 20. Mean values (mmol/L) of cholesterol in HIV patients (N_1 = 57, target group)

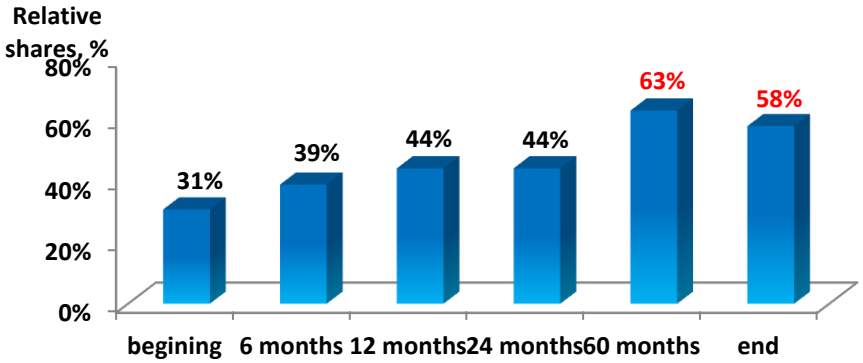


Fig. 21. Relative proportions of HIV patients (N_1 = 57, target group) with elevated cholesterol

4. Triglycerides

Baseline triglyceride values ranged from 0.5 to 4.7 mmol/L (mean 1.54 ± 1.01). With high triglycerides at the beginning are 17.31%. **Last values – from 0.23 to 6.0 mmol/L (average 1.68 ± 1.21); with elevated triglycerides – 15.79%.**

5. ASAT

At baseline, AST ranged from 12 to 199 IU/L (mean 35.8 ± 35.7). With high values at launch are 18.00%. **Last values ranged from 10 to 97 IU/L (mean 24.7 ± 13.6); with elevated ASAT values are 7.02%.**

6. ALAT

Baseline values ranged from 7 to 308 IU/L (mean 43.5 ± 33.4). With high ALAT values at startup are 26.92%. **The last values ranged from 5 to 65 IU/L (mean 23.6 ± 14.5); with elevated ALAT values are 14.04%.**

7. Serum creatinine

At baseline, creatinine ranged from 33 to 105 μmol/L (mean 75.4 ± 14.6). With increased starting creatinine are 5.77%. The last values ranged from 54 to 121 μmol/L (mean 83.7 ± 14.6); increased creatinine in 1.75% of patients.

8. Blood urea nitrogen

Urea at the beginning was from 2.2 to 8.4 mmol/L (mean 4.65 ± 1.34). There were no patients with elevated urea at baseline. **The last values ranged from 2.1 to 8.9 mmol/L (mean 5.02 ± 1.38); with elevated urea are 3.51%.**

Table 1. Laboratory parameters in patients with HIV (N_1 = 57, target group) at baseline and at the end of the survey

	Start mean ± sd min-max	**95% CI**	**End mean ± sd min-max**	**95% CI**	**p**
Hg	137 ± 19 88 - 165	132 - 142	145 ±12 107 - 166	142 - 148	**<0.01**
Er	4.78 ± 0.70 3 - 7.4	4.59 - 4.98	4.70 ± 0.57 3.6 - 7.1	4.55 - 4.85	>0.05
MCV	85 ± 7.4 63 - 103	82.8 - 87.1	92 ± 7.7 62 - 106	89.8 - 93.9	**<0.0005**
WBC	6.0 ± 2.7 2 - 15.6	5.3 - 6.8	7.0 ± 1.8 3.5 - 12.6	6.5 - 7.4	**<0.025**
PLT	227 ± 77 62 - 526	206 - 249	247 ± 52 148 - 388	233 - 261	>0.05
glucose	5.4 ± 1.6 4.2 - 15.6	4.99 - 5.9	5.4 ± 1.1 1.2 - 8.8	5.1 - 5.7	>0.05
Chol	4.6 ± 1.2 2.2 - 7.7	4.3 - 4.96	5.4 ± 1.2 2.4 - 8.8	5.0 - 5.7	**<0.0025**
3-gl	1.5 ± 1.0 0.5 - 4.7	1.3 - 1.8	1.7 ± 1.2 0.2 - 6.0	1.4 - 2.0	>0.05
ASAT	36 ± 35 12 - 199	26 - 46	25 ± 14 10 - 97	21 - 28	**<0.025**
ALAT	44 ± 33 7 - 308	29 - 59	24 ± 15 5 - 65	20 - 28	**<0.01**
Creat	75 ± 15 33 - 105	71 - 80	84 ± 15 54 - 121	80 - 86	**<0.0025**
BUN	4.7 ± 1.3 2.2 - 8.4	4.3 - 5.1	5.0 ± 1.4 2.1 - 8.9	4.5 - 5.4	>0.05

2. Comparative study of laboratory parameters in patients with HIV infection (N_1 = 57) with those of the control group (N_2 = 28)

Complete blood count – no significant difference in the mean values of **hemoglobin** in the two groups and in the relative shares of persons with a low indicator; **erythrocytes** – significantly lower values in the target group ($p<0.01$); **MCV** – significantly higher in the target group ($p<0.005$); **WBC** – without significant differences in the average values and the relative proportions of persons with an abnormal indicator; **PLT** – within reference range and without significant difference in both groups.

Blood glucose – within the reference range and without a significant difference in the relative shares of individuals with abnormal indicators in the groups.

Total cholesterol – average 5.36 ± 1.19 mmol/L in the target group and average 5.34 ± 0.96 mmol/L in the control group, with relative proportions of individuals with elevated cholesterol – 57.89% in the target group and 53.57% in the control group ($p>0.05$) (***Fig . 22***).

Triglycerides – no significant difference between the target group (1.68 ± 1.21 mmol/L) and the control group (1.34 ± 1.04 mmol/L) ($p>0.05$), but the relative proportion of patients with elevated triglycerides was higher in the target group (15.79% vs. 3.57%, respectively) ($p<0.005$) (***Fig. 22***).

ASAT – variation of serum enzyme levels within reference range in both groups, but significantly higher mean values in PLWH; with no significant difference in the relative proportions of individuals with elevated enzyme in the two groups.

ALAT – a characteristic identical to that of ASAT.

Creatinine – significantly higher mean value in the target group ($p<0.025$), but towards the upper limit of the reference range.

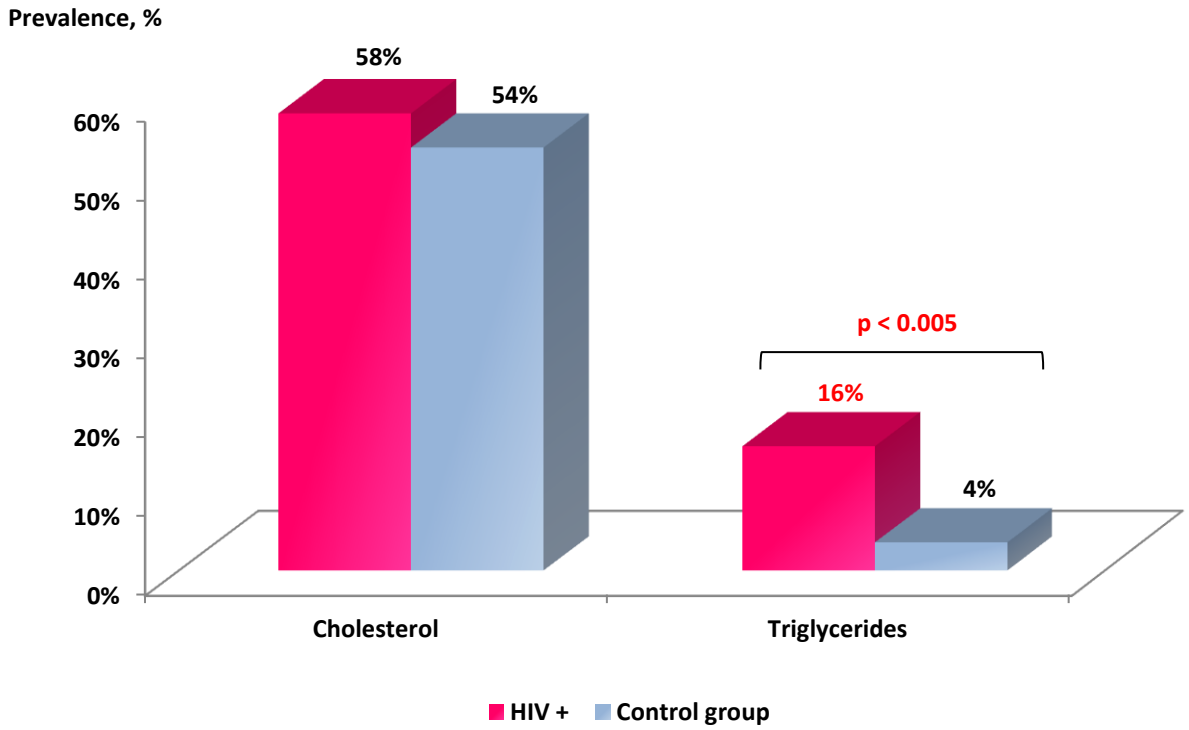


Fig. 22. Relative proportions of PLWH with elevated cholesterol and triglycerides in the target HIV (N_1=57) and control group (N_2=28)

3. Comparative study of laboratory parameters in patients with HIV infection aged 20 to 40 years (n_1 = 27) and over 40 years (n_2 = 30) (*Table 2*)

Complete blood count – no significant difference in the average values of **hemoglobin** in the two groups and in the prevalence of persons with an abnormal parameter; no significant difference in the dynamics of the parameter in the two groups; **erythrocytes, MCV, WBC** and **PLT** – identical ratios between the two groups as for hemoglobin.

Blood glucose – in dynamics without a reliable increase in the average values in both groups, but significantly more clearly in the group over 40 years old (p<0.005).

Total cholesterol – distinct dynamics towards an increase in the average value at the end of the study compared to the beginning (significantly higher in the group over 40) (p<0.001) (***Fig. 23***) and a significantly higher relative proportion with an increased indicator in the same group (p<0.0005) (***Fig. 24***).

Triglycerides – within reference range, with transiently higher mean values in over 40 group, significantly higher proportion of patients with elevated at baseline in PLWH over 40 (***Fig. 25***).

ASAT – variation of serum levels of the enzyme in the reference range in both groups, with no significant differences in the mean values and in the relative proportions of individuals with elevated enzyme between the two groups.

ALAT – significantly transiently higher mean values in the over 40 group (p<0.025), but without significant difference in the relative shares of individuals with elevated enzyme between the two groups.

Creatinine – no significant difference in mean values between the two groups.

Blood urea – transiently higher mean values at 6th and 60th month from baseline in the over 40 group.

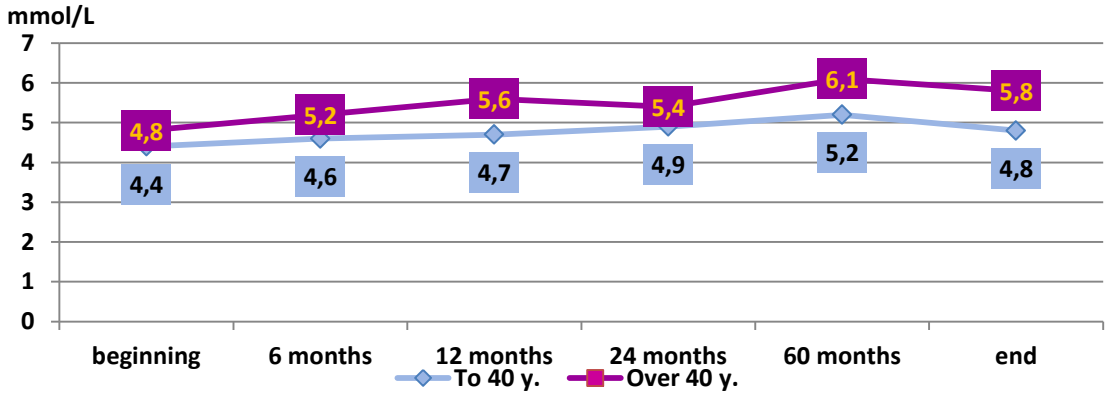


Fig. 23. Dynamics of average values (mmol/L) of cholesterol in PLWH up to (n_1 = 27) and over 40 years (n_2 = 30)

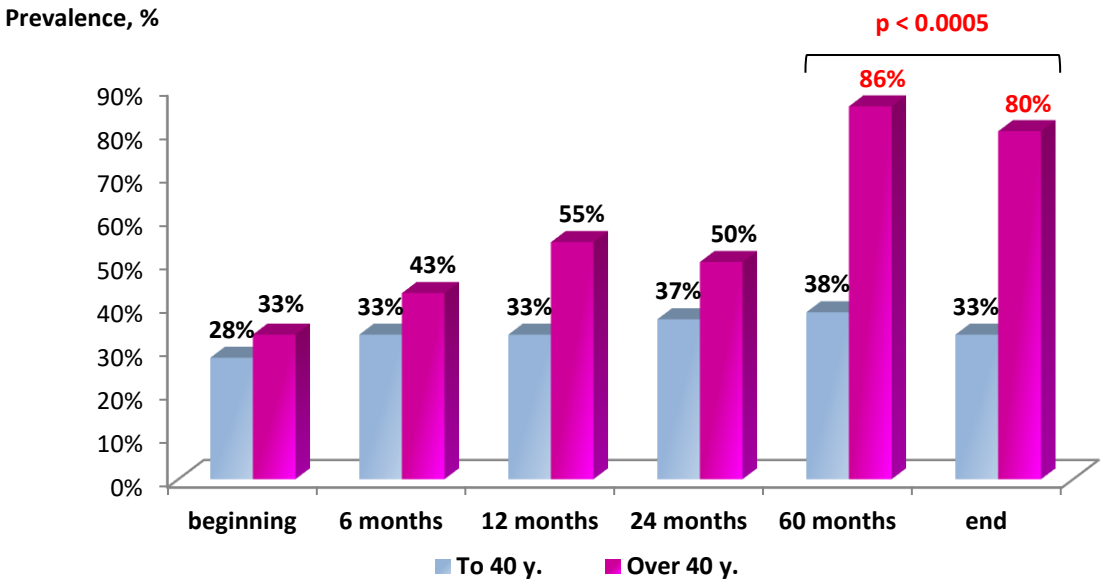


Fig. 24. Dynamics of the prevalence of PLWH aged 20 to 40 years (n_1 = 27) and over 40 years (n_2 = 30) with elevated cholesterol

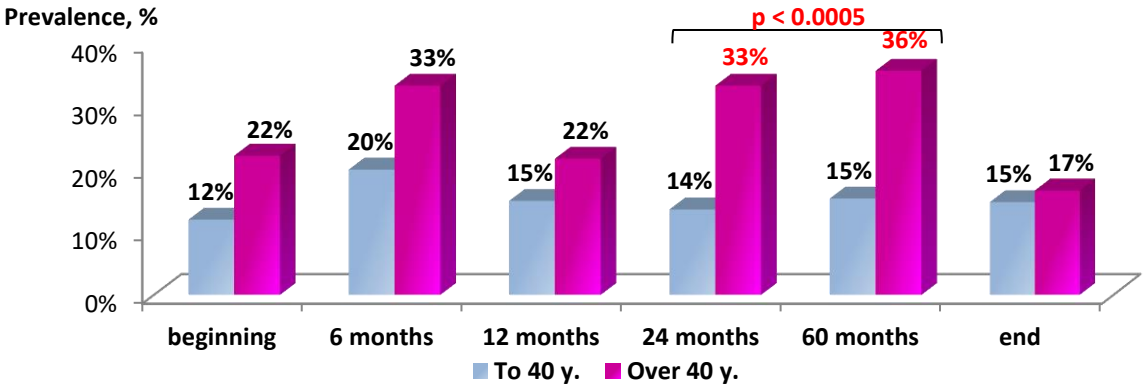


Fig. 25. Dynamics of the prevalence of PLWH aged 20 to 40. (n_1 = 27) and over 40 years (n_2 = 30) with elevated triglycerides

Table 2. Dynamics of laboratory parameters in patients with HIV aged 20-40 years and over 40 years

	Term / Age	Start mean ± sd min-max	95% CI	End mean ± sd min-max	95% CI	p
Hg	**20-40 y.**	135 ± 21 88 - 165	127 - 144	144 ±10.7 114 - 161	140 - 149	**<0.005**
	>40 y.	139 ±16.9 92 - 159	132 - 145	145 ±13 107 - 166	140 - 150	>0.05
Er	**20-40 y.**	4.8 ± 0.9 3 - 7.4	4.5 - 5.2	4.7 ± 0.6 3.7 – 7.1	4.5 - 5.0	>0.05
	>40 y.	4.7 ± 0.4 3.6 - 5.4	4.6 - 4.9	4.7 ± 0.5 3.6 - 5.9	4.5 - 4.8	>0.05
MCV	**20-40 y.**	84 ± 8.8 63 - 103	80 - 88	91 ± 9 62 - 104	88 - 95	**<0.005**
	>40 y.	86 ± 5.9 63 - 94	83 - 88	93 ± 6.2 67 - 106	90 - 95	**<0.0005**
WBC	**20-40 y.**	6.4 ± 2.9 2 - 15.6	5.2 - 7.6	7.0 ± 1.8 3.5 - 12.6	6.3 - 7.8	**<0.025**
	>40 y.	5.7 ± 2.5 2.2 - 12.2	4.7 - 6.7	6.9 ± 1.7 4.5 - 11.1	6.3 - 7.5	**<0.025**
PLT	**20-40 y.**	228 ± 84 62 - 526	194 - 263	238 ± 46 148 - 341	220 - 257	>0.05
	>40 y.	226 ± 70 82 - 401	199 - 254	255 ± 56 166 - 388	206 - 248	>0.05
glucose	**20-40 y.**	5.1 ± 0.5 4.4 - 6.4	4.9 - 5.3	4.9 ± 1.0 1.2 - 6.2	4.5 - 5.3	>0.05
	>40 y.	5.7 ± 2.1 4.2 - 15.6	4.9 - 6.6	5.8 ± 1.1 4.1 - 8.8	5.4 - 6.2	>0.05
Chol	**20-40 y.**	4.4 ± 1.3 2.2 - 7.7	3.9 - 5.0	4.8 ± 1.2 2.4 - 8.8	4.4 - 5.3	>0.05
	>40 y.	4.8 ± 1.1 2.6 - 6.8	4.4 - 5.2	5.8 ± 1.0 3.2 - 7.9	5.5 - 6.2	**<0.0005**
3-gl	**20-40 y.**	1.3 ± 0.9 0.5 - 4.7	0.9 - 1.7	1.5 ± 1.1 0.2 - 5.5	1.0 - 1.5	>0.05
	>40 y.	1.7 ± 1.1 0.7 - 4.3	1.3 - 2.2	1.9 ± 1.3 0.6 - 6.0	1.4 - 2.4	>0.05
ASAT	**20-40 y.**	36 ± 35 13 - 153	21 - 52	24 ± 8.4 13 - 44	21 - 27	>0.05
	>40 y.	35 ± 36.1 12 - 199	21 - 50	25 ± 17.1 10 - 97	19 - 32	>0.05
ALAT	**20-40 y.**	38 ± 39 7 - 176	22 - 55	22 ± 12 5 - 53	17 - 27	**<0.05**
	>40 y.	48 ± 64 10 - 308	23 - 74	25 ± 16 7 - 65	19 - 31	**<0.05**
Creat	**20-40 y.**	74 ± 16 33 - 105	68 - 81	82 ± 14 54 - 113	76 - 87	**<0.05**
	>40 y.	76 ± 13 49 - 102	71 - 82	86 ± 15 58 - 121	80 - 91	**<0.01**
BUN	**20-40 y.**	4.4 ± 1.3 7 - 176	3.8 - 5.0	5.1 ± 1.5 5 - 53	4.5 - 5.7	>0.05
	>40 y.	4.9 ± 1.3 2.3 – 8.4	4.3 - 5.4	5.0 ± 1.2 3.2 - 7.6	4.5 - 5.4	>0.05

4. Comparative study of laboratory indicators in patients with HIV infection with different initial immune status ($CD4^+$<200 cells/μL – n_1 = 17, $CD4^+$<350 cells/μL – n_2 = 28 and $CD4^+$>350 cells/μL – n_3 = 29) (*Table 3*)

Complete blood count – significant increase at the end of the mean value of **hemoglobin, MCV, WBC** and **PLT** in PLWH with $CD4^+$ <350 cells/μL ($p<0.05$), of **MCV** – in the groups; a significant increase in the dynamics of the average values in the groups ($p<0.0005$); **erythrocytes** and **PLT** – without significant difference in the mean values between the groups and in dynamics of the parameters.

Blood glucose – no significant difference in the mean values and no significant dynamics of the parameter in the groups.

Total cholesterol – significantly higher mean values in groups with $CD4^+$ <200 and <350 cells/μL; distinct dynamics towards an increase in the average value at the end of the study compared to the beginning (***Fig. 26***) and an increase in the prevalence with an increased parameter in the same groups ($p<0.025$) (***Fig. 27***).

Triglycerides – in the reference range, without significant dynamics in the average values and the relative shares of patients with an increased indicator in the groups.

ASAT – no significant differences in the mean values and in the relative proportions of individuals with an elevated enzyme between the groups; in dynamics, a significant decrease in the mean value in the group with $CD4^+$ <350 cells/μL. ALAT – identical to ASAT relationships.

Creatinine – no significant difference in mean values between groups; in dynamics a reliable increase within the reference range in the group with $CD4^+$ >350 cells/μL.

Blood urea – no significant dynamics in the average values and the relative shares of patients with an increased indicator in the groups.

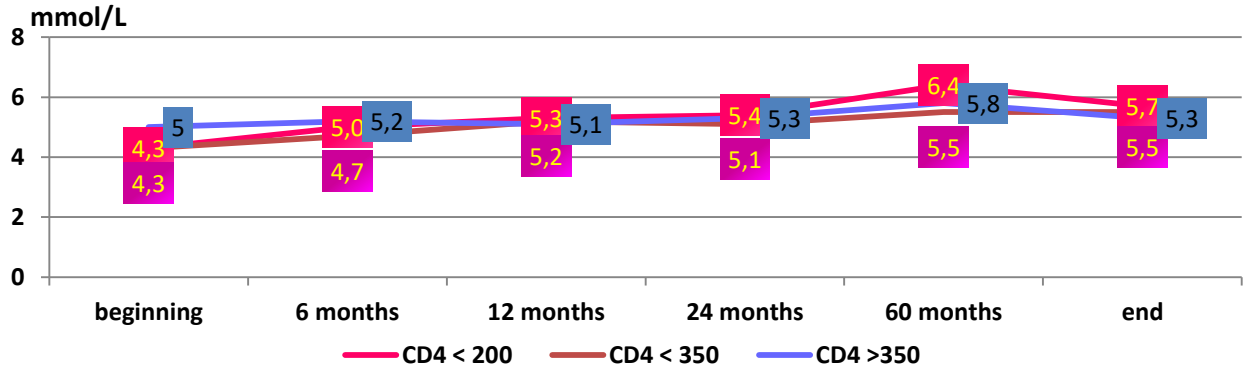


Fig. 26. Dynamics of mean cholesterol values (mmol/L) in PLWH with starting $CD4^+$ <200 (n_1 = 17), <350 (n_2 = 28) and >350 cells/μL (n_3 = 29)

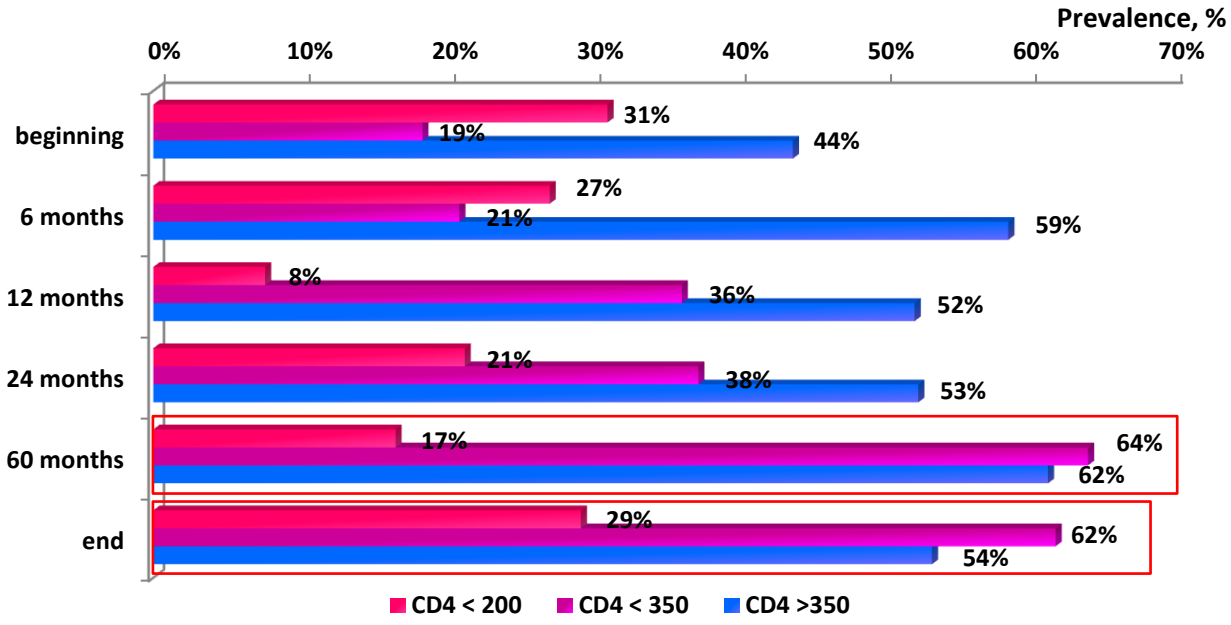


Fig. 27. Dynamics of relative proportions of PLWH with elevated cholesterol with starting $CD4^+$ <200 (n_1 = 17), <350 (n_2 = 28) and >350 cells/μL (n_3 = 29)

Table 3. Dynamics of laboratory indicators (start - end) in PLWH with starting CD4$^+$ <200 (n_1 = 17), <350 (n_2 = 28) and >350 cells/μL (n_3 = 29)

	Term / CD4$^+$	Start mean ± sd min-max	95% CI	End mean ± sd min-max	95% CI	p
Hg	**< 200**	135 ± 21 88 -165	124 - 146	145± 9 125 - 161	140 - 150	**<0.05**
	< 350	134 ± 22 88 - 165	126 - 143	145 ± 10 125 - 166	126 - 139	**<0.025**
	> 350	141 ± 15 102 -162	134 - 147	145± 13 107 - 166	139 - 150	>0.05
Er	**< 200**	4.5 ± 0.7 3.0 - 5.4	4.2 - 4.9	4.5 ± 0.3 3.8 - 5.0	4.4 - 5.0	>0.05
	< 350	4.7 ± 0.6 3.0 - 5.4	4.4 - 4.9	4.6 ± 0.4 3.8 - 5.9	4.4 - 4.8	>0.05
	> 350	4.9 ± 0.8 3.4 - 7.4	4.6 - 5.3	4.8 ± 0.7 3.6 - 7.1	4.5 - 5.1	>0.05
MCV	**< 200**	87 ± 4 82 - 98	84 - 89	94 ± 4 88 - 106	92 - 97	**<0.0005**
	< 350	85 ± 6.3 63 - 98	83 - 88	93 ± 6.6 67 - 106	91 - 96	**<0.0005**
	> 350	85 ± 8.6 63 - 103	81 - 88	90 ± 8.5 62 - 104	87 - 94	**<0.025**
WBC	**< 200**	4.6 ± 2.6 2.0 - 12.2	3.3 - 6.0	6.8 ± 1.5 4.4 - 9.8	6.0 - 7.6	**<0.005**
	< 350	5.3 ± 2.6 2.0 - 12.2	4.4 - 6.3	7.0 ± 1.8 4.4 - 12.6	6.3 - 7.7	**<0.005**
	> 350	6.8 ± 2.6 3.8 - 15.6	5.7 - 7.9	6.9 ± 1.8 3.5 - 11.1	6.3 - 7.6	>0.05
PLT	**< 200**	210 ± 86 62 - 401	164 - 256	243 ± 56 148 - 388	214 - 271	>0.05
	< 350	216 ± 75 62 - 401	187 - 245	245 ± 46 148 - 388	228 - 263	**<0.05**
	> 350	241 ± 79 133 - 526	207 - 274	250 ± 59 166 - 386	227 - 272	>0.05
glucose	**< 200**	5.8 ± 2.8 4.2 - 15.6	4.3 - 7.4	5.7 ± 1.1 4.5 - 8.1	5.1 - 6.4	>0.05
	< 350	5.6 ± 2.1 4.2 - 15.6	4.7 - 6.5	5.6 ± 0.9 4.1 - 8.1	5.2 - 6.0	>0.05
	> 350	5.3 ± 0.7 4.3 - 6.8	5.0 - 5.6	5.2 ± 1.3 1.2 - 8.8	4.6 - 5.7	>0.05
Chol	**< 200**	4.3 ± 1.2 2.2 - 6.5	3.6 - 4.9	5.7 ± 1.3 2.6 - 7.9	5.1 - 6.4	**<0.0025**
	< 350	4.3 ± 1.1 2.2 - 6.5	3.9 - 4.7	5.5 ± 1.2 2.6 - 7.9	5.0 - 5.9	**<0.0005**
	> 350	5.0 ± 1.3 2.9 - 7.7	4.4 - 5.5	5.3 ± 1.2 2.4 - 8.8	4.8 - 5.8	>0.05
3-gl	**< 200**	1.9 ± 1.2 0.7 - 4.7	1.2 - 2.5	1.9 ± 1.4 0.6 - 5.5	1.2 - 2.6	>0.05
	< 350	1.5 ± 1.0 0.5 - 4.7	1.1 - 1.9	1.6 ± 1.1 0.6 - 5.5	1.2 - 2.1	>0.05
	> 350	1.6 ± 1.0 0.6 - 4.2	1.2 - 2.0	1.7 ± 1.3 0.2 - 6.0	1.2 - 2.2	>0.05
ASAT	**< 200**	43 ± 31 16 - 137	26 - 60	29 ± 21 13 - 97	19 - 40	>0.05
	< 350	42 ± 41 15 - 199	26 - 59	27 ± 17 10 - 97	20 - 33	**<0.05**
	> 350	29 ± 28 12 - 153	17 - 41	23 ± 8 13 - 44	20 - 26	>0.05
ALAT	**< 200**	61 ± 53 7 - 308	22 - 99	24 ± 17 5 - 65	16 - 33	**<0.05**
	< 350	54 ± 65 7 - 308	28 - 79	25 ± 17 5 - 65	19 - 32	**<0.025**

	> 350	33 ± 32 7 - 176	18 - 47	22 ± 11 10 - 53	18 - 26	>0.05
Creat	**< 200**	76 ± 15 49 - 105	68 - 84	86 ± 16 58 - 108	78 - 94	**<0.05**
	< 350	76 ± 16 33 - 105	69 - 82	84 ± 16 54 - 121	78 - 90	>0.05
	> 350	75 ± 13 49 - 102	70 - 80	83 ± 13 62 - 113	78 - 88	**<0.025**
BUN	**< 200**	4.4 ± 1.4 2.3 - 7.8	3.6 - 5.2	5.0 ± 1.2 3.2 - 7.6	4..4 - 5.6	>0.05
	< 350	4.7 ± 1.4 2.3 - 8.4	4.1 - 5.3	4.8 ± 1.1 2.1 - 7.6	4.4 - 5.2	>0.05
	> 350	4.6 ± 1.3 2.2 - 8.0	4.1 - 5.2	5.2 ± 1.6 3.3 - 8.9	4.6 - 5.9	>0.05

Chapter III. Studies of immunological parameters

1. Studies of immunological parameters in patients with HIV (N_1 = 57)

Viral load – all 57 HIV patients currently have an undetectable viral load and no virological failure; 13/57 had evidence of poor adherence but no evidence of resistance to current ART on ART resistance testing; 5/57 had non-significant blips <200 c/mL and very rare transient elevations <1000 c/mL but had optimal viral suppression on follow-up.

T-helper lymphocytes ($CD4^+$) – at the beginning the number of $CD4^+$ was from 2 to 1043 cells/μL (average 346 ± 278), at the end – from 257 to 1755 cells/μL (average 719 ± 331) ($p<0.0005$); with characteristics of late onset ($CD4^+$ <350 cells/μL) are 54.55%, with advanced immune deficiency ($CD4^+$ <200 cells/μL) are 30.91%. At the end, relative share of patients with $CD4^+$ >500 cells/μL – 68.42%; with $CD4^+$ <350 cells/μL – 5/57 patients (8.77%) ($p < 0.005$); there are no persons with <200 cells/μL (***Table 4***) (***Fig.28, Fig.29, Fig.30***).

Table 4. Dynamics in the number of $CD4^+$ T-lymphocytes in PLWH (N_1=57)

Start mean ± sd min-max	6 months mean ± sd min-max	12 months mean ± sd min-max	24 months mean ± sd min-max	60 months mean ± sd min-max	End mean ± sd min-max
346 ± 278 2 - 1043	498 ± 284 97 - 1362	551 ± 360 78 - 1653	615 ± 374 173 - 2240	752 ± 344 234 - 1452	719 ± 331 257 - 1755

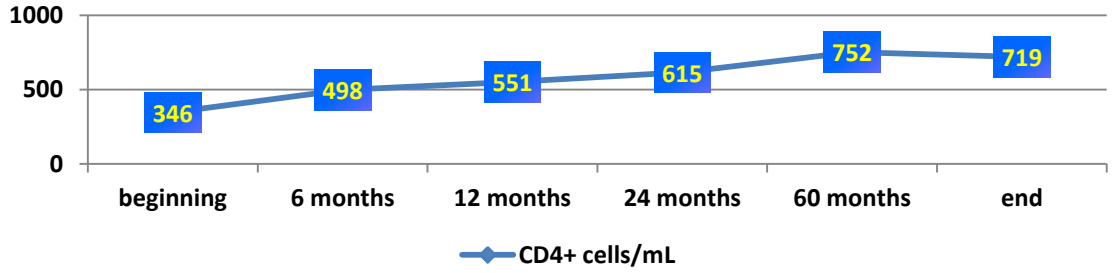


Fig. 28. Dynamics of average CD4+ T-lymphocyte count values in PLWH (N_1=57)

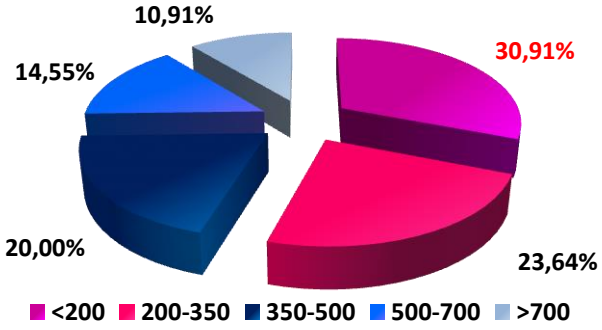


Fig. 29. Structure of PLWH (N_1=57) with different initial $CD4^+$ counts

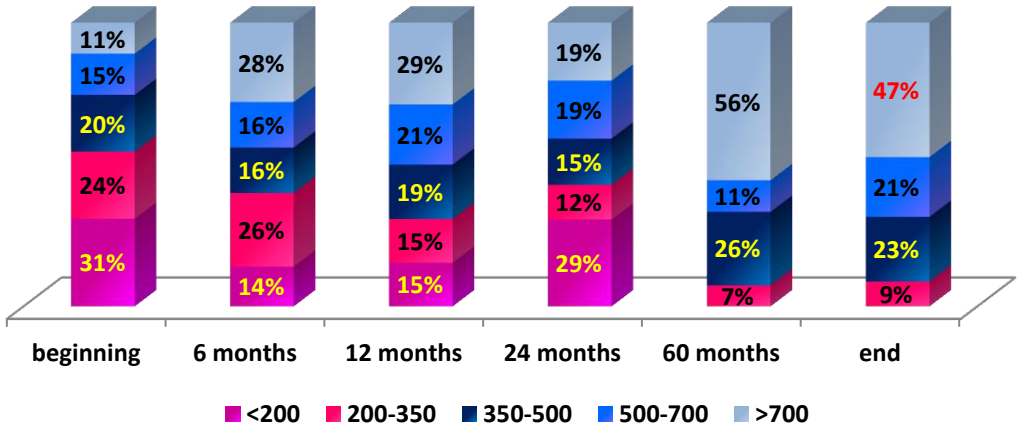


Fig. 30. Dynamics of prevalence of PLWH (N_1=57) with different $CD4^+$ counts at different stages of the study

At the beginning of the study, 6/55 patients (10.91%) had T-helper $CD4^+$ lymphocytes >700 cells/μL, and at the end of the study – 27/57 (47.37%) (***Fig. 31***); 5/57 patients 8.77%) did not reach $CD4^+$ 350 cells/μL, but **there were no patients below 200 cells/μL.**

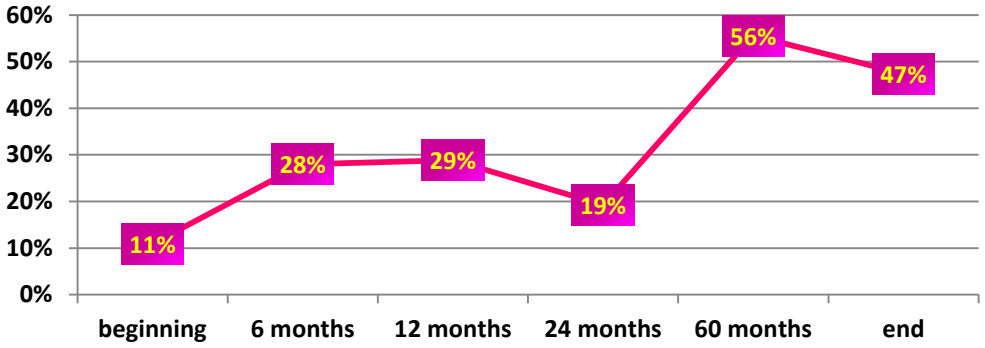


Fig. 31. Dynamics of the prevalence of PLWH with $CD4^+$ over 700 cells/μL

***AIDS phase**

When presenting to the clinic, a total of 33% (19/57 patients) simultaneously meet the clinical-immunological criteria of the AIDS phase (HIV – phase 3), of which 30.91% have a CD4+ count < 200 cells/μL, and the remaining 2 .09% presented with radiographically and microbiologically proven pulmonary opportunistic infection (Mycobacterium tuberculosis and mycotic pneumonia (Obs. Pneumocystis jirovecii), despite having a higher initial $CD4^+$ count > 200 cells/μL.

T-suppressor lymphocytes ($CD8^+$) – at the beginning the number of $CD8^+$ was from 127 to 6589 cells/μL (mean 981 ± 921), at the end – from 174 to 2333 cells/μL (mean 947 ± 436) (p>0.05); 23/54 patients (42.59%) started with an abnormal $CD8^+$ count (over 900 cells/μL), with 7/54 (12.96%) having $CD8^+$ >1500 cells/μL. At the end with $CD8^+$ >900 cells/μL were 26/57 patients (45.61%), with 5/57 (8.77%) having $CD8^+$ >1500 cells/μL (***Table 5***) (***Fig. 32, Fig. 33, Fig. 34***).

Table 5. Dynamics in the number of $CD8^+$ T-lymphocytes in PLWH (N_1=57)

Start mean ± sd min-max	6 months mean ± sd min-max	12 months mean ± sd min-max	24 months mean ± sd min-max	60 months mean ± sd min-max	End mean ± sd min-max
981 ± 920 127 - 6589	1027 ± 508 92 - 2665	956 ± 466 115 - 2421	912 ± 449 163 - 2149	932 ± 442 223 - 1999	947 ± 436 174 - 2333

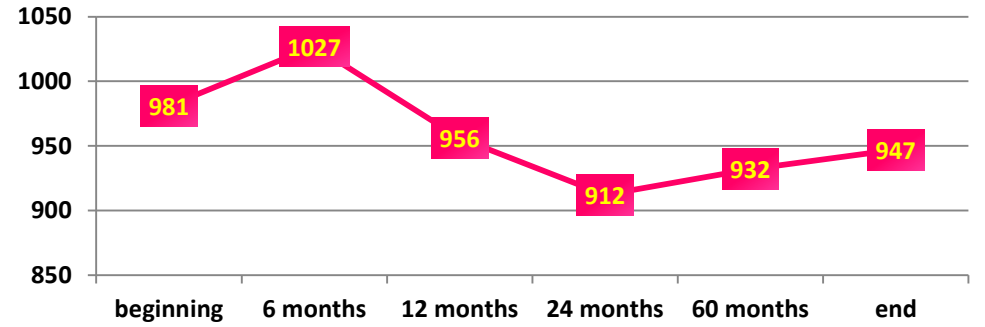


Fig. 32. Dynamics of average $CD8^+$ T-lymphocyte count values in PLWH (N_1=57)

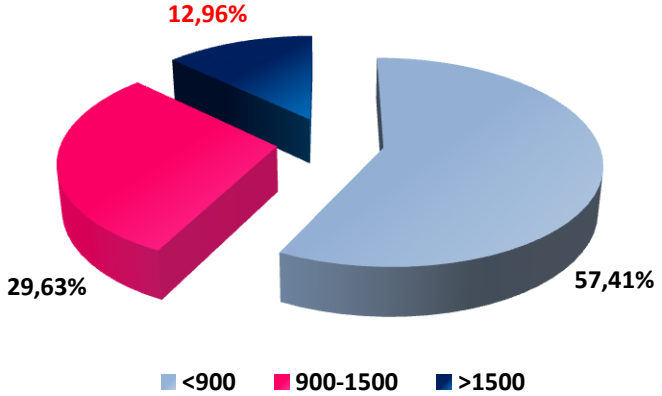


Fig. 33. Relative proportions of PLWH (N_1=57) with different baseline $CD8^+$ counts

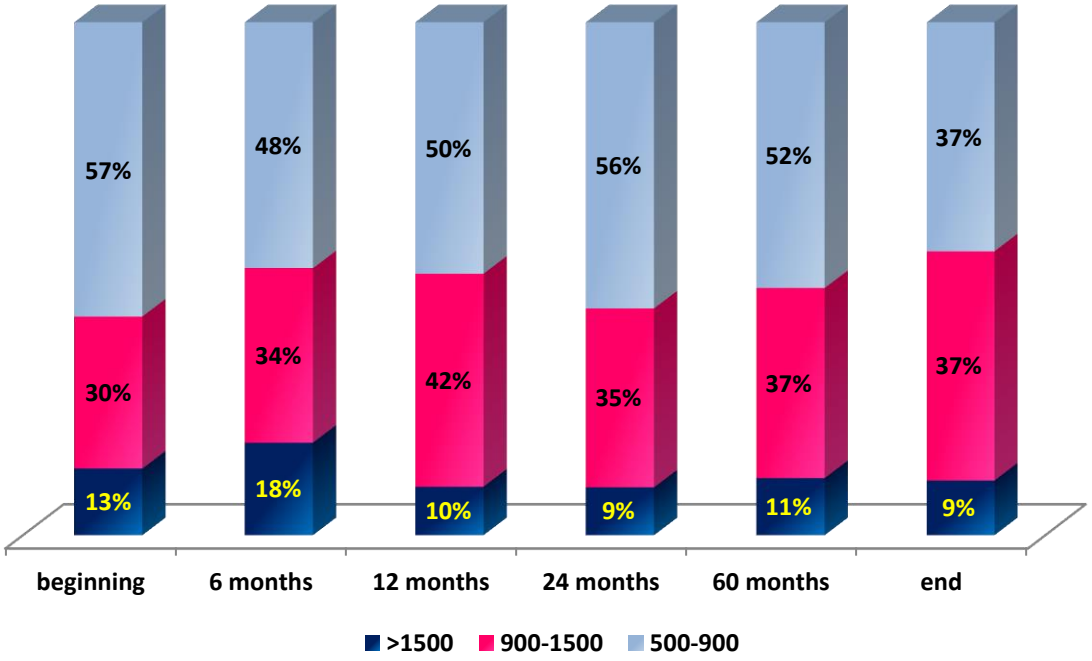


Fig. 34. Dynamics of the relative shares of PLWH (N_1=57) with different CD8+ counts at different stages of the study

CD4$^+$: CD8$^+$ ratio – 45 patients (83.33%) started with a CD4$^+$: CD^8+ ratio ≤ 0.8, with 32 (59.26%) having an index < 0.4. With an optimal CD4$^+$:CD^8+ ratio (> 0.8) there were 9 (16.67%) at the beginning and 29 (50.88%) at the end. With a normal ratio (>1.0) at the beginning there were only 11.11%, at the end 40.35% (p<0.05) (***Table 6***) (***Fig. 35***).

Table 6. Dynamics of the CD4$^+$: CD8$^+$ ratio (mean ± sd; range – min÷max) in PLWH (N_1=57)

Start mean ± sd min-max	6 months mean ± sd min-max	12 months mean ± sd min-max	24 months mean ± sd min-max	60 months mean ± sd min-max	End mean ± sd min-max
0.44 ± 0.39 0.01 - 1.9	0.57 ± 0.41 0.08 - 1.86	0.66 ± 0.52 0.08 - 2.3	0.78 ± 0.45 0.2 - 1.99	0.93 ± 0.46 0.36 - 1.8	0.89 ± 0.49 0.18 – 2.2

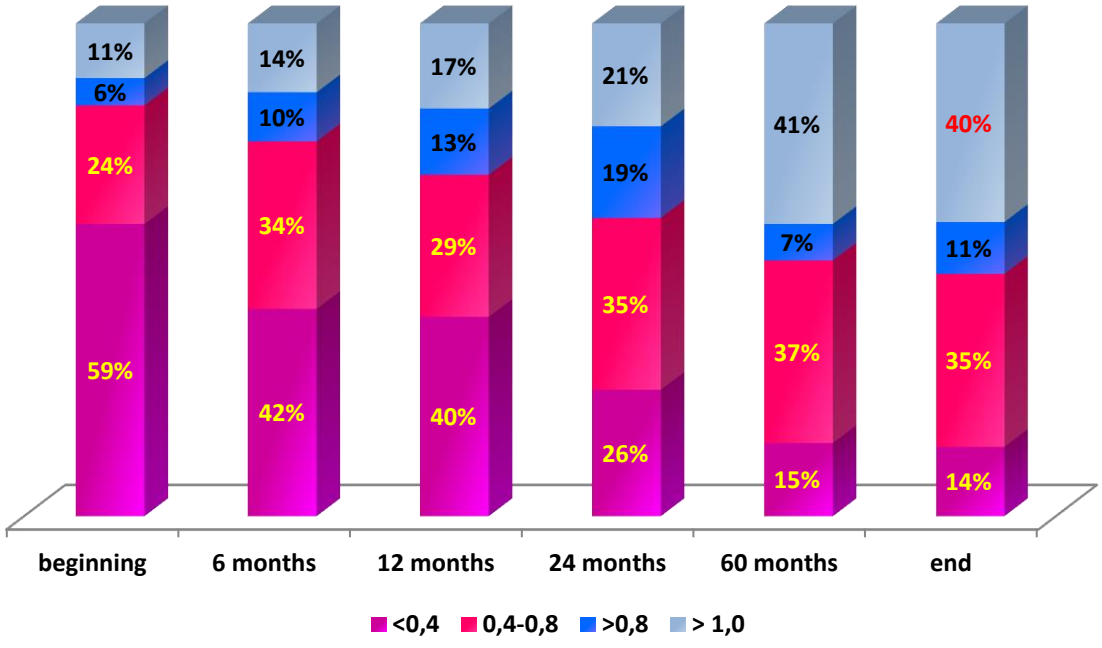


Fig. 35. Dynamics of prevalence of PLWH (N_1=57) with different CD4$^+$: CD8$^+$ ratio at different stages of the study

2. Comparative study of immunological indicators in patients with HIV infection aged 20 to 40 years (n_1 = 27) and over 40 years (n_2 = 30) (*Table 7*) (*Fig. 36*)

Viral load – the mean values of viral load in the two age groups and the relative proportions of patients with an abnormal viral load showed no differences between the two groups.

T-helper lymphocytes ($CD4^+$) – at the beginning a significantly lower average value in the group over 40 years old – 276 ± 238 cells/μL, against 420 ± 301 cells/μL in the younger group ($p<0.05$); the relative share of patients over 40 years of age with $CD4^+$ below 350 cells/μL is higher compared to the 20-40 year group ($p<0.05$); mean values at the end were not significantly different between the two groups, and single patients in the groups had $CD4^+$ below 350 cells/μL. In both groups, the relative proportion of patients with $CD4^+$ below 350 /μL at the end was significantly lower ($p<0.005$).

T-suppressor lymphocytes ($CD8^+$) – initially insignificantly higher average value in the group 20-40 years – 1166 ± 1209 cells/μL, against 796 ± 443 cells/μL in the group over 40 years; no significant difference in the relative proportions with $CD8^+$ above 900 cells/μL between the groups at baseline and at the end.

CD4+ : CD8+ ratio – at begining, the mean values are almost the same in the groups; the relative shares of patients with an index below 0.8 – also; at the end, there are no reliable differences in the average values and the relative proportions of patients with an index below 0.8; in both groups, the average value was significantly improved and the patients with a low index were reduced, the differences being significant ($p<0.005$).

Table 7. Comparative study of initial and final values of $CD4^+$, $CD8^+$, $CD4^+$: $CD8^+$ in PLWH aged 20-40 (n_1 = 27) and over 40 (n_2 = 30)

Groups / Parameter	PLWH 20-40 y. (n_1 = 27)			PLWH >40 y. (n_2 = 30)			OR	p
	≠N n %	mean ± sd (min-max)	95% CI	≠N n %	mean ± sd (min-max)	95% CI		
$CD4^+$ start	↓ 12 44.44	420 ± 301 (2-1043)	301-539	↓ 18 64.29	276±238 (12-1031)	188-367	0.44	<0.05 >0.05
$CD4^+$ end	↓ 3 11.11	673 ± 193 (257-1614)	438-907	↓ 2 6.67	761 ± 338 (267-1755)	635-887	**1.75**	>0.05 >0.05
p	**<0.005**	**>0.05**		**<0.0005**	**<0.005**			
$CD8^+$ start	↑ 13 48.15	1166±1209 (179-6589)	687-1644	↑ 10 37.04	796±443 (127-1562)	620-971	**1.58**	>0.05 >0.05
$CD8^+$ end	↑ 11 40.74	962±489 (343-2264)	990-1342	↑ 15 50.00	933±390 (174-2333)	787-1079	0.69	>0.05 >0.05
p	**>0.05**	**>0,05**		**>0.05**	**>0.05**			
$CD4^+$:$CD8^+$ start	↑ 22 81.48	0.44±0.33 (0.01-1.12)	0.305-0.568	↑ 22 81.48	0.44±0.38 (0.01-1.12)	0.263-0.618	0.77	>0.05 >0.05
$CD4^+$:$CD8^+$ end	↑ 16 59.26	0.85±0.53 (0.18-2.2)	0.638-1.06	↑ 16 59.26	0.85±0.53 (0.18-2.2)	0.758-1.100	**2.18**	>0.05 >0.05
p	**<0.05**	**<0.001**		**<0.0005**	**<0.0005**			

*** ↑ n – number of patients with elevated value; ↓ n – number of patients with decreased value**

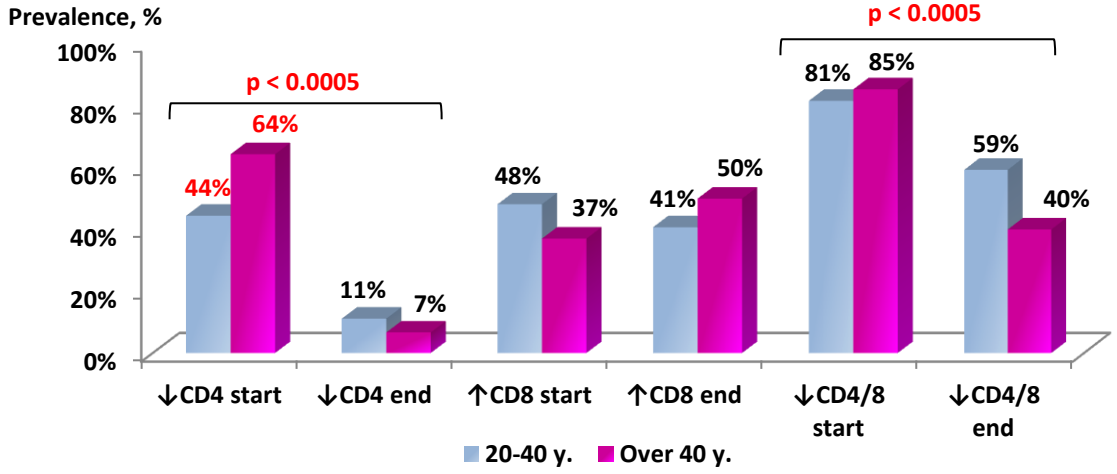


Fig. 36. Relative proportions of PLWH aged 20-40 years (n_1=27) and over 40 years (n_2=30) with abnormal immunological parameters ($CD4^+$, $CD8^+$, $CD4^+$: $CD8^+$) at start and at the end of the study

3. Comparative study of immunological indicators in patients with HIV infection with different initial immune status ($CD4^+$<200 cells/μL – n_1 = 17, $CD4^+$<350 cells/μL – n_2 = 28 and $CD4^+$>350 cells/μL – n_3 = 29) (*Table 8*) (*Fig. 37*)

Viral load – the mean values of the viral load in the two groups and the relative proportions of patients with an abnormal viral load showed no differences between the two groups.

T-helper lymphocytes ($CD4^+$) – at baseline significantly lower mean value in the group with $CD4^+$ <350 cells/μL – 143 ± 110 cells/μL, versus 573 ± 228 in the group with $CD4^+$ >350 cells/μL ($p<0.0005$); mean values at the end were also significantly lower in the group with $CD4^+$ <350 cells/μL ($p<0.005$); three patients in the $CD4^+$ <350 cells/μL group did not progress above this value.

T-suppressor lymphocytes ($CD8^+$) – baseline significantly higher average value in the group with $CD4^+$ >350 cells/μL – 1275 ± 1209 cells/μL, against 707 ± 386 cells/μL in the group with $CD4^+$ <350 cells. /μL ($p<0.025$); with a significant difference in the relative proportions of patients with $CD8^+$ >900 cells/μL between groups at the end.

CD4+ : CD8+ ratio – at the beginning, the average value of the index was lower in the group with $CD4^+$ < 350 cells/μL ($p<0.0005$); the relative share of patients with an index below 0.8 is higher in the same group ($p<0.005$); at the end, significant differences in the mean values ($p<0.0005$) and the relative shares of patients with an index below 0.8 ($p<0.005$); in both groups, the mean value was significantly improved and patients with a low index were reduced, the differences being significant ($p<0.0005$).

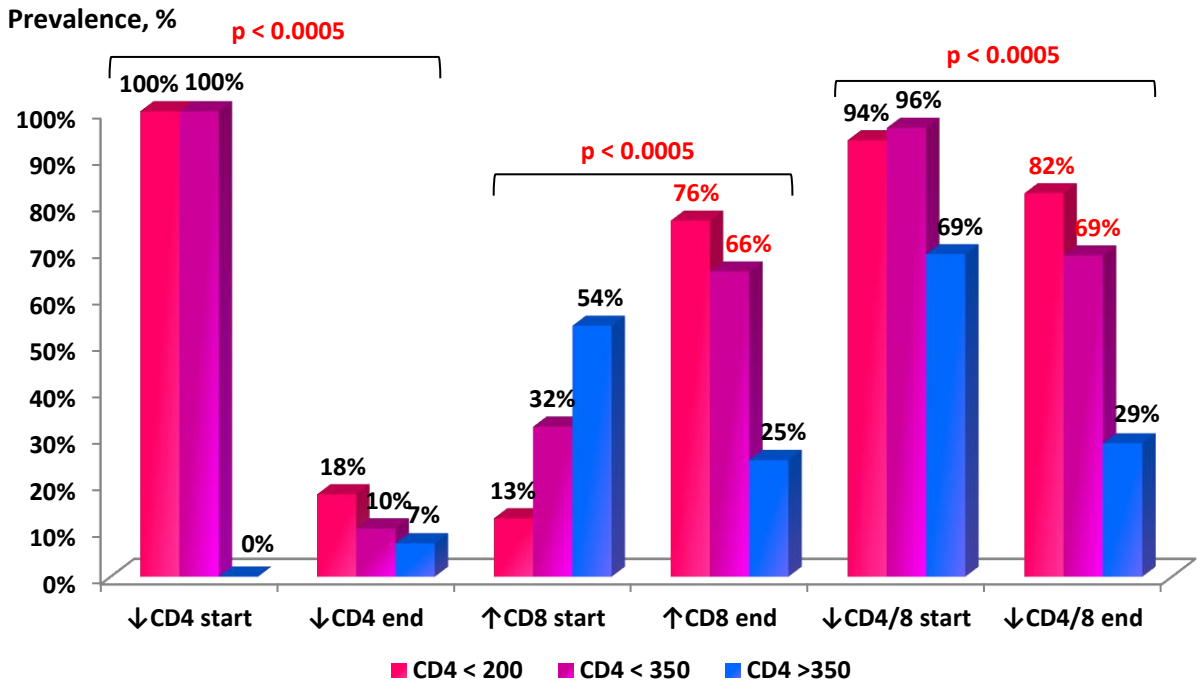


Fig. 37. Relative proportions of PLWH with baseline $CD4^+$ <200 (n_1 = 17), <350 (n_2 = 28), and >350 cells/μL (n_3 = 29) with abnormal immunological indicators at the beginning and at the end of the study

Table 8. Comparative study of initial and final values of $CD4^+$, $CD8^+$, $CD4^+$: $CD8^+$ in PLWH with starting $CD4^+$<200 (n_1 = 17), <350 (n_2 = 28) and >350 cells/μL (n_3 = 29)

Groups / Parameter	PLWH with $CD4^+$ <200 кл./μL (n_1 = 17)			PLWH with $CD4^+$ <350 кл./μL (n_2 = 29)			PLWH with $CD4^+$ >350 кл./μL (n_3 = 28)			OR	p
	≠N n %	**mean ± sd (min-max)**	**95% CI**	**≠N n %**	**mean ± sd (min-max)**	**95% CI**	**≠N n %**	**mean ± sd (min-max)**	**95% CI**		
$CD4^+$ start	↓ 17 100.00	58 ± 43 (2-135)	**36.1-80.2**	↓ 29 100.0	143 ± 110 (2-304)	**102-185**	↓ 1 3.85	573 ± 228 345-1043)	**481-665**	**700**	**<0.0005 <0.0005**
$CD4^+$ end	↓ 0 0.00	561 ± 221 (267-1021)	**448-675**	↓ 3 10.34	608 ± 239 (267-1327)	**517-699**	↓ 2 7.14	835 ± 375 (257-1755)	**689-980**	**1.5**	**<0.005** >0.05
p	**<0.0005**	**<0.0005**		**<0.0005**	**<0.0005**		**>0.05**	**<0.0025**			
$CD8^+$ start	↑ 2 12.50	536 ± 315 (127-1216)	**368-704**	↑ 9 32.14	707 ± 386 (127-1538)	**557-857**	↑ 14 53.85	1275 ± 1209 (325-7589)	**787-1764**	0.41	**<0.025** >0.05
$CD8^+$ end	↑ 13 76.47	1180 ± 499 (174-2264)	**923-1437**	↑ 19 65.52	1114 ± 484 (174-2333)	**929-1297**	↑ 7 25.00	775 ± 302 (343-1514)	**658-892**	**5.70**	**<0.0025 <0.001**
p	**<0.0005**	**<0.0005**		**<0.01**	**<0.001**		**<0.025**	**<0.025**			
$CD4^+$:$CD8^+$ start	↓ 15 93.75	0.16 ± 0.14 (0.01-1.0)	**0.04-0.29**	↓ 27 96.43	0.23 ± 0.21 (0.01-1.0)	**0.15-0.316**	↓ 18 69.23	0.66 ± 0.42 (0.13-1.9)	**0.49-0.83**	**12**	**<0.0005 <0.005**
$CD4^+$:$CD8^+$ end	↓ 14 82.35	0.57 ± 0.33 (0.18-1.5)	**0.39-0.74**	↓ 20 68.97	0.66 ± 0.42 (0.18-2.2)	**0.50-0.83**	↓ 8 28.57	1.12 ± 0.45 (0.48-1.9)	**0.95-1.30**	**5.56**	**<0.0005 <0.001**
p	**<0.0005**	**<0.0005**		**<0.005**	**<0.0005**		**<0.0025**	**<0.0005**			

* ↑ n* - number of patients with elevated value; ↓ n** - number of patients with decreased value

Chapter IV. Results of studies of biomarkers of inflammation - IL-6, hsCRP and D-dimer

1. 1. Studies of biomarkers of inflammation (IL-6, hscrp, d-dimer) in HIV patients ($N_1 = 57$) compared to healthy individuals ($N_2 = 28$) (*Table 9*) (*Fig. 38*)

IL-6 – at the start – with no significant difference in the mean values of the biomarker in the two groups and in the relative proportions of individuals with an elevated biomarker in the groups. After 6 months – significantly higher mean value and higher relative proportion of patients with elevated IL-6 in the second study compared to the first in the target group; significantly higher mean biomarker values in the second study of the target group versus the control group and significantly higher relative proportion of target group subjects with elevated IL-6 in the second study relative to the relative proportion of control group subjects with elevated IL-6.

hsCRP – at the start – significantly higher mean hsCRP in the target group compared to controls and significantly higher relative proportion of individuals with elevated hsCRP in the target group. After 6 months – significantly higher mean hsCRP value and higher relative proportion of individuals with elevated hsCRP in the target group.

D-dimer – at the start – significantly higher mean D-dimer in the target group, with no significant difference in the relative proportions of individuals with elevated D-dimer in the two groups. After 6 months – no significant difference in the mean values and in the relative shares of individuals with an elevated biomarker in the second study compared to the beginning in both groups; with no significant difference in mean values and relative proportions of individuals with elevated D-dimer in the target versus control group.

Table 9. Biomarkers of inflammation (IL-6, hsCRP and D-dimer) in target and control groups

Groups / Bio markers	Target group ($N_1 = 57$)			Conrol group ($N_2 = 28$)			OR	ϕ	p
	↑ n* %	mean ± sd (min-max)	95% CI	↑ n* %	mean ± sd (min-max)	95% CI			
IL-6	4 7.14	3.88 ± 3.17 (1.5-23.34)	3.03- 4.741	1 3.57	3.75 ± 2.57 (1.5-14.03)	2.76- 4.75	**2.08**	**0.230**	>0.05 >0.05
2-nd invest.	14 27.45	5.90 ± 3.48 (1.5-19.93)	4.91- 6.88	2 7.69	4.70 ± 2.38 (2.21-14.0)	3.74- 5.67	**4.54**		**<0.0025** **<0.001**
p	**<0.025**	**<0.0005**		**>0.05**	**>0.05**				
hsCRP	9 16.07	2.44±2.40 (0.09-10.9)	1.76- 3.12	0 0.00	1.37±1.29 (0.12-4.59)	0.86- 1.88	**4.98**	**0.236**	**<0.01** **<0.0005**
2-nd invest.	11 21.57	4.24±3.77 (0.6-29.65)	1.50- 2.61	1 3.85	1.87±1.81 (0.6-8.77)	0.51- 1.97	**6.88**		**<0.001** **<0.01**
p	**>0.05**	**<0.025**		**>0.05**	**>0.05**				
D-dimer	7 12.50	0.38±0.26 (0.21-1.96)	0.31- 0.45	2 7.41	0.29±0.14 (0.09-0.8)	0.23- 0.34	**1.79**	**0.058**	**<0.0025** >0.05
2-nd invest.	2 4.00	0.33±0.09 (0.21-0.6)	0.31- 0.360	2 7.69	0.31±0.08 (0.2-0.51)	0.28- 0.341	0.5		>0.05 >0.05
p	**>0.05**	**>0.05**		**>0,05**	**>0.05**				

*** ↑ n*** - number of patients with elevated value *****

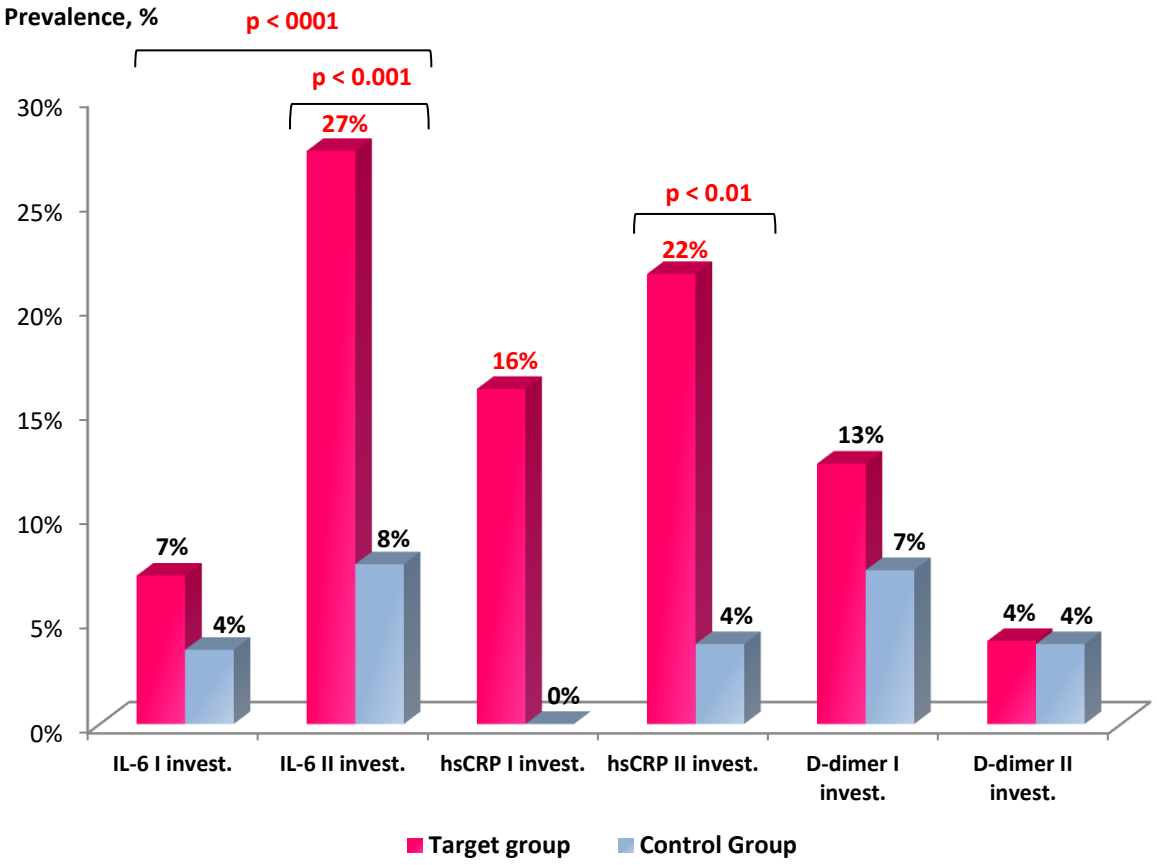


Fig. 38. Relative proportions of patients with elevated inflammatory biomarkers in targeted HIV (N_1=57) and the control (N_2=28) groups

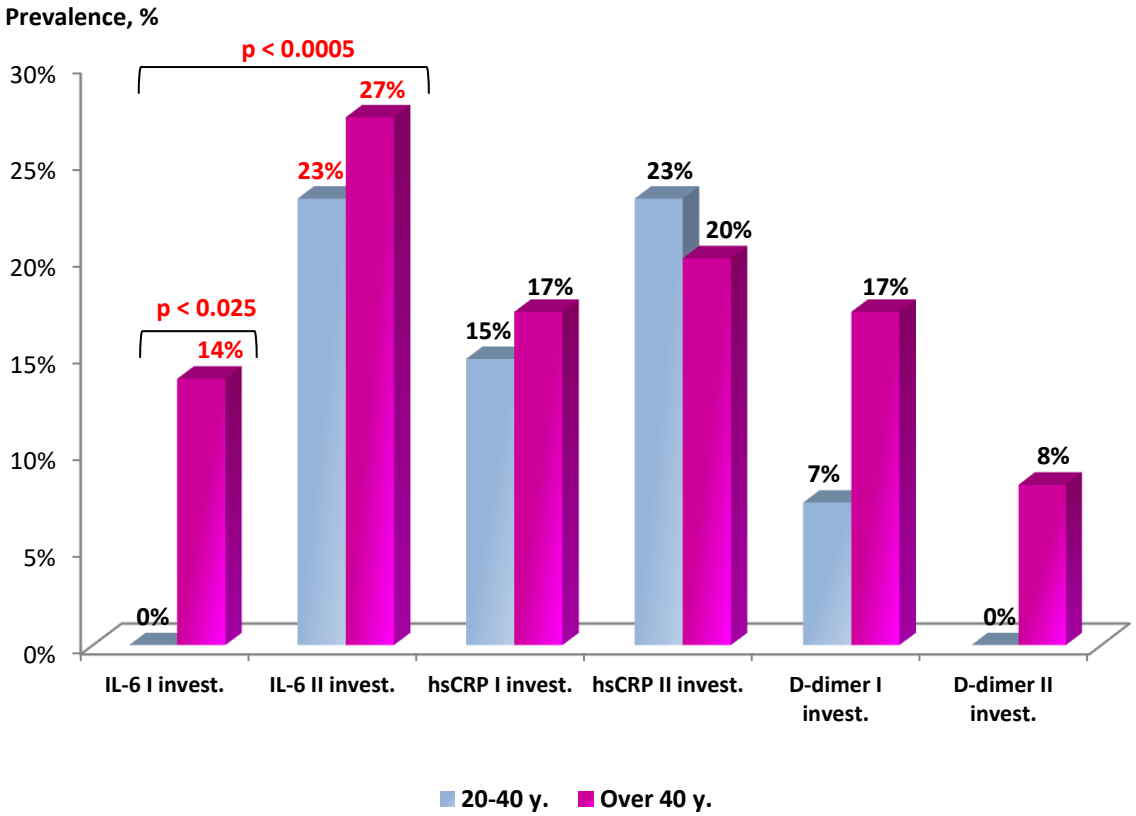


Fig. 39. Relative proportions of PLWH aged 20-40 (n_1=27) and over 40 (n_2=30) with elevated biomarkers of inflammation

2. Comparative study of inflammatory biomarkers (IL-6, hsCRP and D-dimer) in PLWH aged 20-40 years (n_1=27) and over 40 years (n_2=30) (*Table 10*) (*Fig. 39*)

IL-6 – a significantly higher relative proportion of patients over 40 years of age with increased biomarker compared to those under 40 years ($p<0.025$); significantly higher average values of the biomarker in the second study compared to the first in both groups ($p<0.005$); significantly higher relative proportion of patients with increased biomarker in the second study compared to the first only in the 20-40 year group ($p<0.005$).

hsCRP – significantly higher average value of the biomarker in the second study compared to the first only in the group up to 40 years ($p<0.05$) In other aspects, there are no significant differences.

D-dimer – significantly higher average value of the biomarker in the younger age group only in the second examination ($p<0.05$).

Table 10. Comparative study of biomarkers of inflammation (IL-6, hsCRP and D-dimer) in PLWH aged 20-40 (n_1=27) and over 40 (n_2=30)

Groups / Bio markers	PLWH 20-40 y. (n_1 = 27)			PLWH >40 y. (n_2 = 30)			OR	p
	↑ n* %	mean ± sd (min-max)	95% CI	↑ n* %	mean ± sd (min-max)	95% CI		
IL-6	0 0.00	3.26 ± 1.50 (1.5-6.81)	2.663- 3.853	4 13.79	4.47 ± 4.12 (1.5-23.24)	2.902- 6.033	0.00	>0.05 **<0.025**
2-nd invest.	6 23.08	5.22 ± 3.15 (1.5-12.81)	3.945- 6.490	6 27.27	6.57 ± 3.89 (2.61-19.93)	4.843- 8.293	0.8	>0.05 >0.05
p	**<0.005**	**<0.005**		**>0.05**	**<0.05**			
hsCRP	4 14.81	2.29±2.12 (0.19-9.77)	1.294- 3.292	5 17.24	2.57±2.52 (0.09-10.87)	1.613- 3.533	0.835	>0.05 >0.05
2-nd invest.	6 23.08	5.06±4.79 (0.6-29.65)	1.911- 8.210	5 20.00	3.40±2.13 (0.66-8.32)	2.515- 4.275	**1.2**	>0.05 >0.05
p	**>0.05**	**<0.05**		**>0.05**	**>0.05**			
D-dimer	2 7.41	0.34±0.18 (0.21-1.07)	0.269- 0.409	5 17.24	0.29±0.14 (0.42-0.32)	0.298- 0.541	0.384	>0.05 >0.05
2-nd invest.	0 0.00	0.31±0.07 (0.21-0.45)	0.284- 0.34	2 8.33	0.35±0.11 (0.23-0.6)	0.309- 0.399	0.00	**<0.05** >0.05
p	**>0,05**	**>0.05**		**>0.05**	**>0.05**			

*** ↑ n*** - number of patients with elevated value *****

3. Comparative study of biomarkers of inflammation in patients with HIV infection with initial CD4+ counts <350 cells/μL (n_2 = 29) and CD4+ >350 cells/μL (n_3 = 28) (*Table 11*) (*Fig. 40*)

IL-6 – significantly higher relative proportion of patients with $CD4^+$ <350 cells/μL with elevated biomarker compared to those with $CD4^+$ >350 cells/μL at the second examination ($p<0.05$); significantly higher average value of the biomarker in the second study compared to the first in the group with $CD4^+$ <350 cells/μL ($p<0.0005$); significantly higher relative proportions of patients with an elevated biomarker in the second study compared to the first in both groups ($p<0.05$).

hsCRP – significantly higher relative proportion of patients with an elevated biomarker at the first examination in the group with $CD4^+$ <350 cells/μL ($p<0.05$). There are no significant differences in the other aspects of comparison.

D-dimer – significantly higher mean value of the biomarker in the group with $CD4^+$ <350 cells/μL only in the second examination ($p<0.05$).

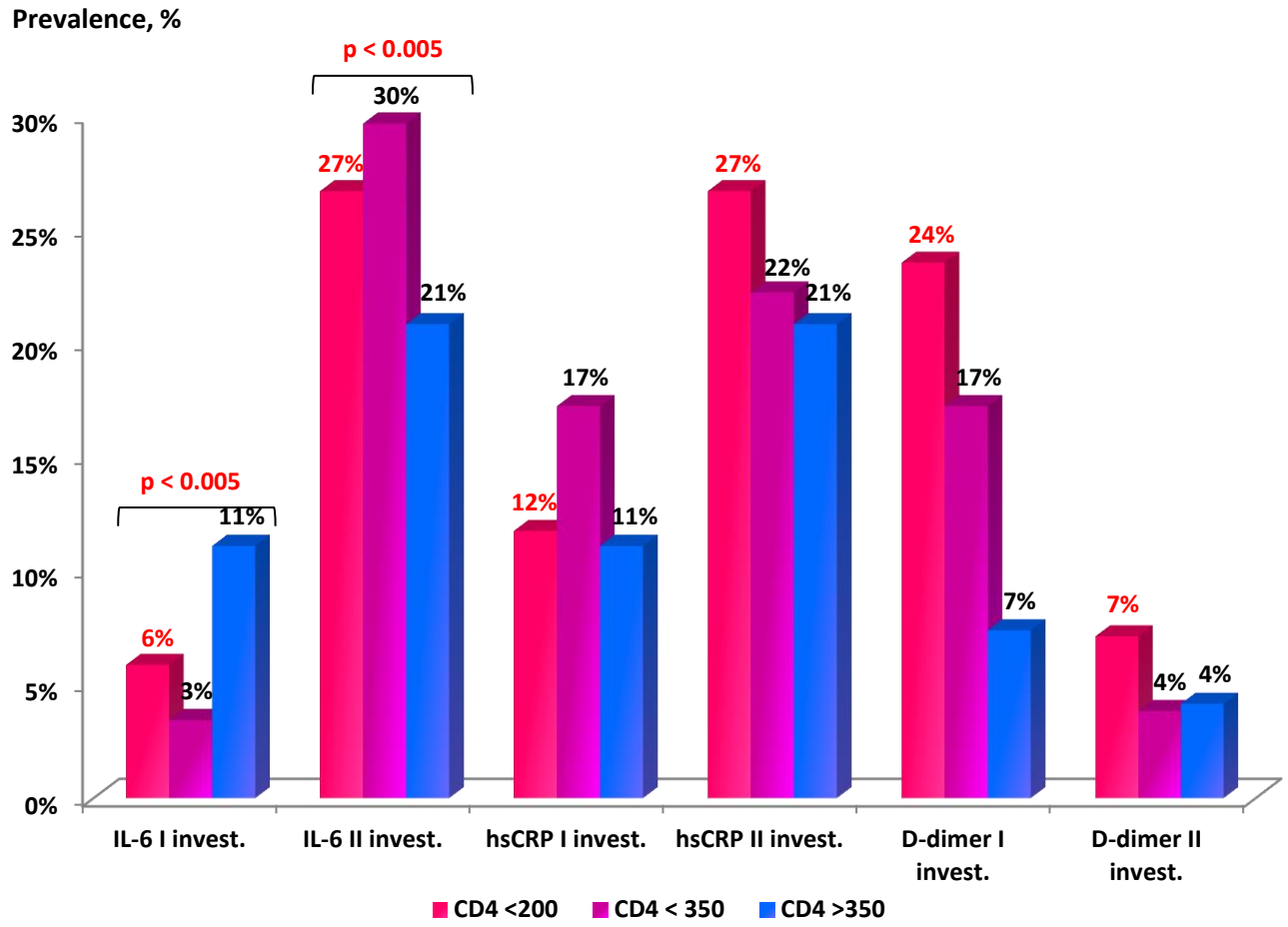


Fig. 40. Relative proportions of PLWH with $CD4^+$ <200 cells/μL (n_1 = 17), $CD4^+$ <350 cells/μL (n_2 = 29) and $CD4^+$ >350 cells/μL (n_3 = 28) with elevated biomarkers of inflammation

Table 11. Comparative study of biomarkers of inflammation in PLWH with baseline CD4$^+$ <200 cells/μL (n_1 = 17), CD4$^+$ <350 cells/μL (n_2 = 29) and CD4+ >350 cells/μL (n_3 = 28)

Groups / Bio markers	PLWH with CD4$^+$ <200 кл./μL (n_1 = 17)			PLWH with CD4$^+$ <350 кл./μL (n_2 = 29)			PLWH with CD4$^+$ >350 кл./μL (n_3 = 28)			OR	p
	↑ n* %	mean ± sd (min-max)	95% CI	↑ n* %	mean ± sd (min-max)	95% CI	↑ n* %	mean ± sd (min-max)	95% CI		
IL-6	1 5.88	3.42 ± 2.03 (1.5-8.62)	2.374- 4.459	1 3.45	3.43 ± 1.69 (1.5-8.62)	2.791- 4.078	3 11.11	4.37 ± 4.21 (1.5-23.24)	2.701- 6.035	**3.5**	>0.05 >0.05
2-nd invest.	4 26.67	7.35 ± 4.92 (2.61-19.93)	4.629- 10.08	8 29.63	6.59 ± 4.09 (1.5-19.93)	4.972- 8.205	5 20.83	5.12 ± 2.50 (1.5-10.4)	4.065- 6.178	0.625	>0.05 **<0.05**
p	>0.05	**<0.005**		**<0.005**	**<0.0005**		**<0.005**	>0.05			
hsCRP	2 11.76	2.52±2.47 (0.09-10.87)	1.050- 4.000	5 17.24	2.29-2.55 (0.09-10.87)	1.318- 3.256	3 11.11	2.60±2.50 (0.31-9.77)	1.613- 3.588	0.6	>0.05 **<0.05**
2-nd invest.	4 26.67	7.26±6.55 (0.6-29.65)	1.971- 12.55	6 22.22	5.36±4.48 (0.6-29.65)	2.398- 8.318	5 20.83	2.99±2.44 (0.6-9.02)	1.959- 4.024	0.921	>0.05 >0.05
p	>0.05	**<0.05**		>0.05	>0.05		>0.05	>0.05			
D-dimer	4 23.53	0.44±0.41 (0.22-1.96)	0.231- 0.652	5 17.24	0.40±0.32 (0.22-1.96)	0.277- 0.521	2 7.41	0.36±0.18 (0.21-1.07)	0.292- 0.431	0.384	>0.05 >0.05
2-nd invest.	1 7.14	0.35±0.11 (0.23-0.60)	0.289- 0.420	1 3.85	0.36±0.10 (0.21-0.60)	0.316- 0.397	1 4.17	0.31±0.08 (0.21-0.59)	0.278- 0.343	1.087	**<0.05** >0.05
p	>0.05	>0.05		>0.05	>0.05		>0.05	>0.05			

*** ↑ n* - number of patients with elevated value**

Chapter V. Studies of correlations of specific risk factors and clinical laboratory indicators with biomarkers (IL-6, hsCRP and D-dimer) and their role in chronic inflammation

1. Study of correlation between BMI and biomarkers of chronic inflammation

Biomarkers of chronic inflammation were compared in two groups of patients – with normal BMI (≤ 25) (n_1 = 26 patients) and with excess BMI (> 25) (n_2 = 25 patients). Biomarker mean values and relative proportions of patients with elevated biomarkers were compared between groups. In each group, the indicators at the beginning of the study were compared with those at the end (***Table 12) (Fig. 41***).

1.1. IL-6 – at baseline significantly higher mean IL-6 ($p<0.025$) and higher relative proportion of patients with elevated IL-6 ($p<0.01$) in the group with excess BMI. At the end, there was no reliable difference between the groups; significantly higher mean value ($p<0.001$) and higher relative proportion of patients with elevated IL-6 ($p<0.025$) at the end of the study in the normal BMI group.

1.2. High-sensitivity CRP (hsCRP) – at the beginning, a significantly higher mean value of hsCRP in the group with an excess BMI ($p<0.025$), without significant differences in the relative proportions of patients with elevated hsCRP; after 6 months no significantly different mean values, but a higher relative proportion of patients with elevated hsCRP in the group with excess BMI ($p<0.025$).

1.3. D-dimer study – at baseline higher mean value ($p<0.05$) and higher relative proportion of patients with elevated D-dimer in the normal BMI group ($p<0.025$). In dynamics – in the group with normal BMI at the beginning significantly higher average value ($p < 0.05$) and relative share of patients with elevated D-dimer ($p<0.005$), in the group with abnormal BMI – insignificantly higher at the end.

In summary, there was a weak correlation of IL-6 ($\phi = 0.146$), a weak correlation of hsCRP ($\phi = 0.190$) and a very weak correlation of D-dimer ($\phi = 0.094$) with excess BMI.

Table 12. Biomarkers of inflammation in PLWH with normal and excess BMI

Groups / Bio markers	PLWH with BMI ≤ 25			PLWH with BMI > 25			OR	ϕ	p
	↑ n %	mean ± sd (min-max)	95% CI	↑ n %	mean ± sd (min-max)	95% CI			
IL-6	0 0.00	2.94 ± 1.50 (1.5-6.81)	2.33- 3.55	8 32.00	4.97 ± 4.27 (1.5-23.24)	3.23- 6.72	0.85	**0.146**	**<0.025** **<0.01**
2-nd invest.	4 18.18	5.33 ± 2.98 (1.5-12.81)	4.01- 6.65	9 39.13	6.64 ± 4.00 (2.02-19.9)	4.91- 8.37	0.49		>0.05 >0.05
p	**<0.025**	**<0.001**		>0.05	>0.05				
hsCRP	3 11.54	1.83 ± 1.35 (0.09-9.77)	0.88- 2.78	6 24.00	3.31 ± 2.62 (0.34=10.87)	2.23- 4.34	0.41	**0.190**	**<0.025** >0.05
2-nd invest.	2 9.09	3.30 ± 2.85 (0.6-28.45)	0.71- 5.90	8 34.78	5.54 ± 5.11 (0.6-29.65)	2.90- 8.18	0.375		>0.05 **<0.025**
p	>0.05	>0.05		>0.05	>0.05				
D-dimer	6 23.08	0.44 ± 0.37 (0.22-1.96)	0.30 - 0.59	1 4.00	0.33 ± 0.07 (0.21-0.57)	0.30- 0.37	**7.2**	**0.094**	>0.05 **<0.025**
2-nd invest.	0 0.00	0.32 ± 0.08 (0.23-0.49)	0.28- 0.35	2 9.09	0.36 ± 0.11 (0.21-0.6)	0.31- 0.41	0.48		>0.05 >0.05
p	**<0.005**	**<0.05**		>0,05	>0.05				

*** ↑ n* - number of patients with elevated value**

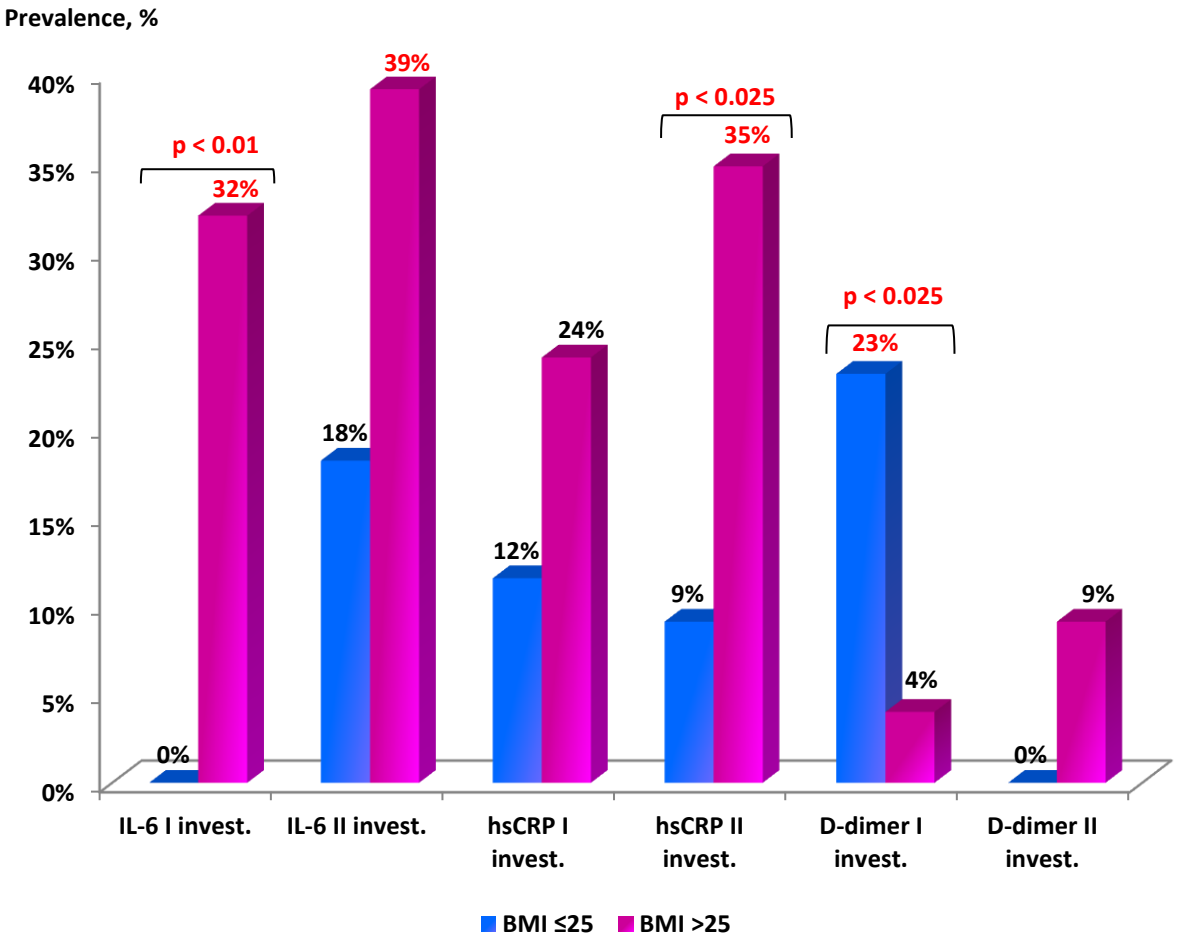


Fig. 41. Relative proportions of PLWH with normal (n_1=26) and abnormal (n_2=25) BMI with elevated biomarkers of inflammation

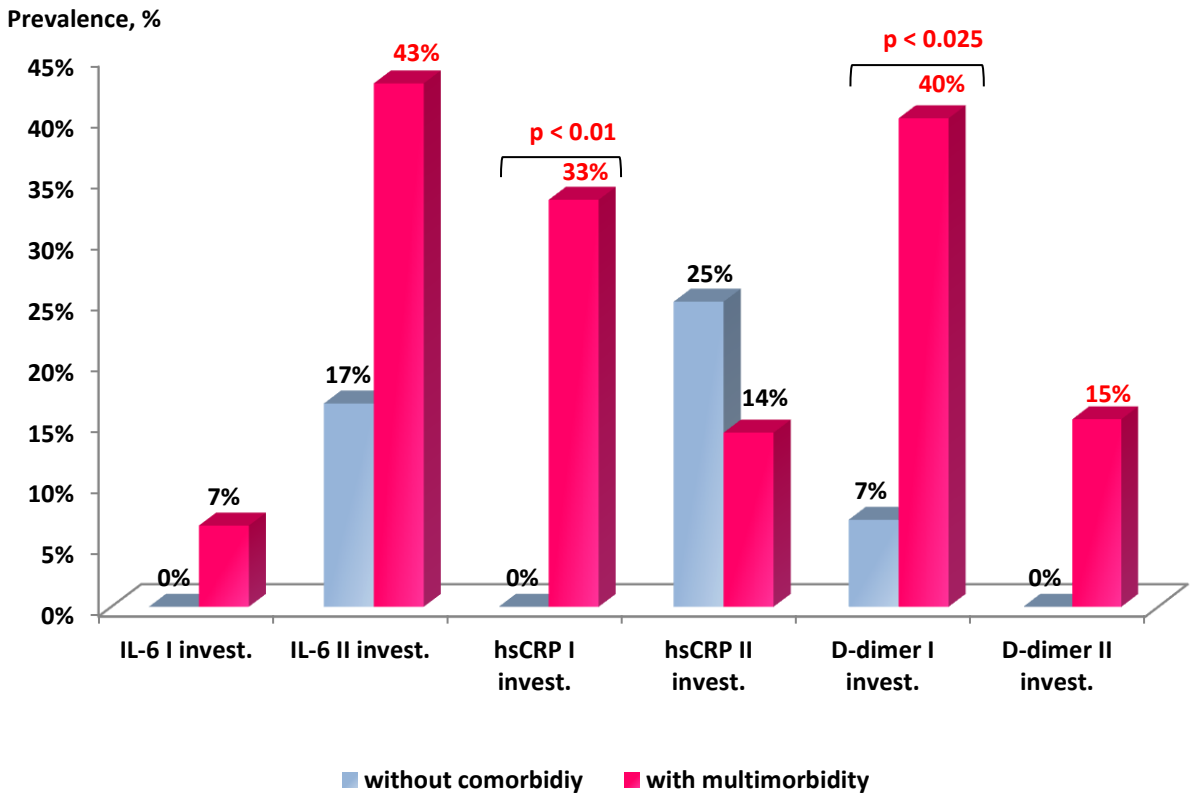


Fig. 42. Relative proportions of PLWH without comorbidity (n_1=14) and with multimorbidity (n_2=15) with elevated biomarkers of inflammation

2. Study of correlation between multimorbidity and biomarkers of chronic inflammation

Biomarkers of chronic inflammation were compared in two groups – without comorbidity (n_1 = 14 patients) and with multimorbidity (n_2 = 15 patients). Biomarker mean values and relative proportions of patients with elevated biomarkers were compared between groups. In each group, the indicators at the beginning were compared with those at the end (***Table 13***) ***(Fig. 42***).

2.1. IL-6 – at the beginning a significantly higher average value of the biomarker in the group with multimorbidity ($p<0.025$), in the group without comorbidity – no patients with elevated IL-6, in the other group – one patient. At the end – no significant difference between the groups. In dynamics - a significantly higher mean value of IL-6 at the end of the study compared to the beginning in both groups, but only in the group with multimorbidity a higher relative proportion of patients with an elevated biomarker ($p<0.025$).

2.2. High-sensitivity CRP (hsCRP) – at the start, a significantly higher mean value and a higher relative proportion of patients with an elevated biomarker in the group with multimorbid patients ($p<0.01$). At the end, there is no reliable difference between the groups. In dynamics - no significant difference in the mean values and in the relative proportions of patients with elevated hsCRP in both groups.

2.3. D-dimer study – at the start, higher mean value and relative proportion of patients with elevated d-dimer in the multimorbidity group ($p<0.025$), at the end higher mean value in the multimorbidity group ($p<0.05$), with no significant difference in the relative proportions of patients with elevated d-dimer between groups. In dynamics - no significant difference in the average values and in the relative proportions of patients with elevated d-dimer in both groups.

In summary, there was a weak correlation of IL-6 (ϕ = 0.283), a weak correlation of hsCRP (ϕ = 0.136) and a very weak correlation of D-dimer (ϕ = 0.042) with the presence of multimorbidity.

Table 13. Biomarkers of inflammation in PLWH without comorbidity and with multimorbidity

Groups / Bio markers	PLWH without comorbidity			PLWH with comorbidity			OR	ϕ	p
	↑ n %	mean ± sd (min-max)	95% CI	↑ n %	mean ± sd (min-max)	95% CI			
IL-6	0 0.00	2.88 ± 1.05 (1.5-4.68)	2.28- 3.49	1 6.67	4.41 ± 2.16 (1.5-8.62)	3.21- 5.60	0.93	**0.283**	**<0.025** >0.05
2-nd invest.	2 16.67	5.25 ± 2.84 (2.02-12.81)	3.45- 7.06	6 42.86	6.93 ± 5.06 (1.5-19.93)	4.00- 9.85	0.27		>0.05 >0,05
p	>0.05	**<0.025**		**<0.025**	**<0.05**				
hsCRP	0 0.00	1.41 ± 1.38 (0.19-4.77)	0.62- 2.21	5 33.33	3.84 ± 3.46 (0.34-10.9)	1.93- 5.76	0.15	**0.136**	**<0.01** **<0.01**
2-nd invest.	3 25.00	4.37 ± 3.89 (0.6-28.45)	0.65- 9.38	2 14.29	3.37 ± 3.26 (0.6-8.32)	1.37- 5.37	**2.00**		>0.05 >0.05
p	>0.05	>0.05		>0.05	>0.05				
D-dimer	1 7.14	0.32 ± 0.10 (0.22-0.6)	0.26 - 0.38	6 40.00	0.58 ± 0.44 (0.23-1.96)	0.32- 0.84	0.12	**0.042**	**<0.025** **<0.025**
2-nd invest.	0 0.00	0.32 ± 0.07 (0.23-0.45)	0.28- 0.36	2 15.38	0.39 ± 0.12 (0.25-0.6)	0.32- 0.46	0.5		**<0.05** >0.05
p	>0.05	>0.05		>0,05	>0.05				

*** ↑ n* - number of patients with elevated value**

3. Study of correlation between cholesterol and biomarkers of chronic inflammation

Biomarkers of chronic inflammation were compared in two groups - with hypercholesterolemia (n_1 = 33 patients) and with normal cholesterol (n_2 = 24 patients). Biomarker mean values and relative proportions of patients with elevated biomarkers were compared between groups. In each group, the indicators at the beginning were compared with those at the end (***Table 14***) ***(Fig. 43***).

3.1. IL-6 – no significant difference in mean and relative proportions of patients with elevated IL-6 between groups at baseline and at baseline. In dynamics, a significantly higher mean value of IL-6 at the end compared to the beginning in both groups, but only in the group with elevated cholesterol the relative proportion of patients with an increased biomarker at the end was higher ($p<0.05$).

3.2. High-sensitivity CRP (hsCRP) – no significant difference in mean value and relative proportions of patients with elevated IL-6 between groups at baseline and at baseline. In dynamics - only in the group with normal cholesterol a significantly higher average value of hsCRP at the end ($p<0.05$), without a significant difference in the relative shares of patients with elevated in both groups.

3.3. D-dimer study – at baseline higher mean value ($p<0.025$) and higher relative proportion of patients with elevated D-dimer in the elevated cholesterol group ($p<0.05$). After 6 months, only the mean value was significantly higher in the group with elevated cholesterol ($p<0.05$). In dynamics in both groups without a significant difference in the mean values and relative proportions of patients with elevated D-dimer.

In summary, there was a weak correlation of IL-6 ($\phi = 0.124$), a weak correlation of hsCRP ($\phi = 0.135$) and a very weak correlation of D-dimer ($\phi = 0.174$) with excess cholesterol.

Table 14. Biomarkers of inflammation in PLWH with elevated and normal cholesterol

Groups / Bio markers	PLWH with elevated cholesterol			PLWH with normal cholesterol			OR	ϕ	p
	↑ n %	mean ± sd (min-max)	95% CI	↑ n %	mean ± sd (min-max)	95% CI			
IL-6	3 9.38	4.08 ± 1.98 (1.5-8.62)	3.37- 4.80	1 4.17	3.62 ± 4.32 (1.5-23.24)	1.80- 5.45	**2.379**	**0.124**	>0.05 >0.05
2-nd invest.	9 30.00	6.03 ± 3.91 (1.5-19.93)	4.57- 7.49	4 19.05	5.72 ± 2.83 (1.5-12.81)	4.43- 7.00	**1.821**		>0.05 >0,05
p	**<0.025**	**<0.01**		>0.05	**<0.05**				
hsCRP	3 9.38	2.84 ± 2.81 (0.09-10.9)	1.82- 3.85	2 8.33	0.19-7.28 (0.09-10.87)	1.08- 2.74	**1.138**	**0.135**	>0.05 >0.05
. 2-nd invest.	4 13.33	3.56 ± 3.06 (0.6-14.86)	2.42- 4.70	5 23.81	5.22±4.24 (0.6-29.65)	1.47- 8.97	0.492		>0.05 >0.05
p	>0.05	>0.05		>0.05	**<0.05**				
D-dimer	3 9.38	0.44±0.33 (0.21-1.96)	0.32- 0.55	0 0.00	0.31±0.06 (0.23-0.45)	0.28- 0.33	**2.379**	**0.174**	**<0.025** **<0.05**
2-nd invest.	2 6.90	0.35 ± 0.11 (0.21-0.60)	0.31- 0.39	0 0.00	0.31±0.06 (0.21-0.41)	0.28- 0.34	**1.481**		**<0.05** >0.05
p	>0.05	>0.05		>0,05	>0.05				

*** ↑ n* - number of patients with elevated value**

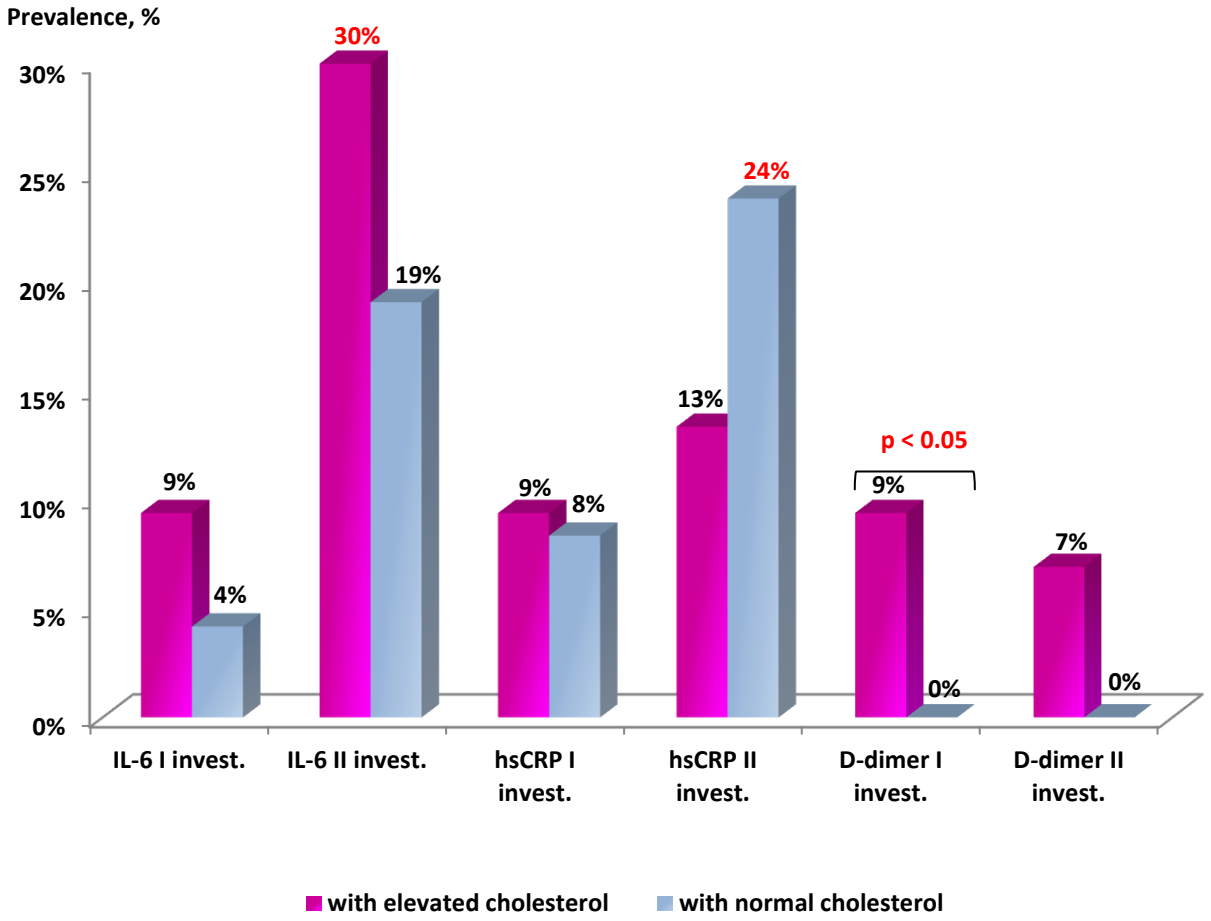


Fig. 43. Relative proportions of PLWH with elevated (n_1=33) and normal (n_2=24) cholesterol with elevated biomarkers of inflammation

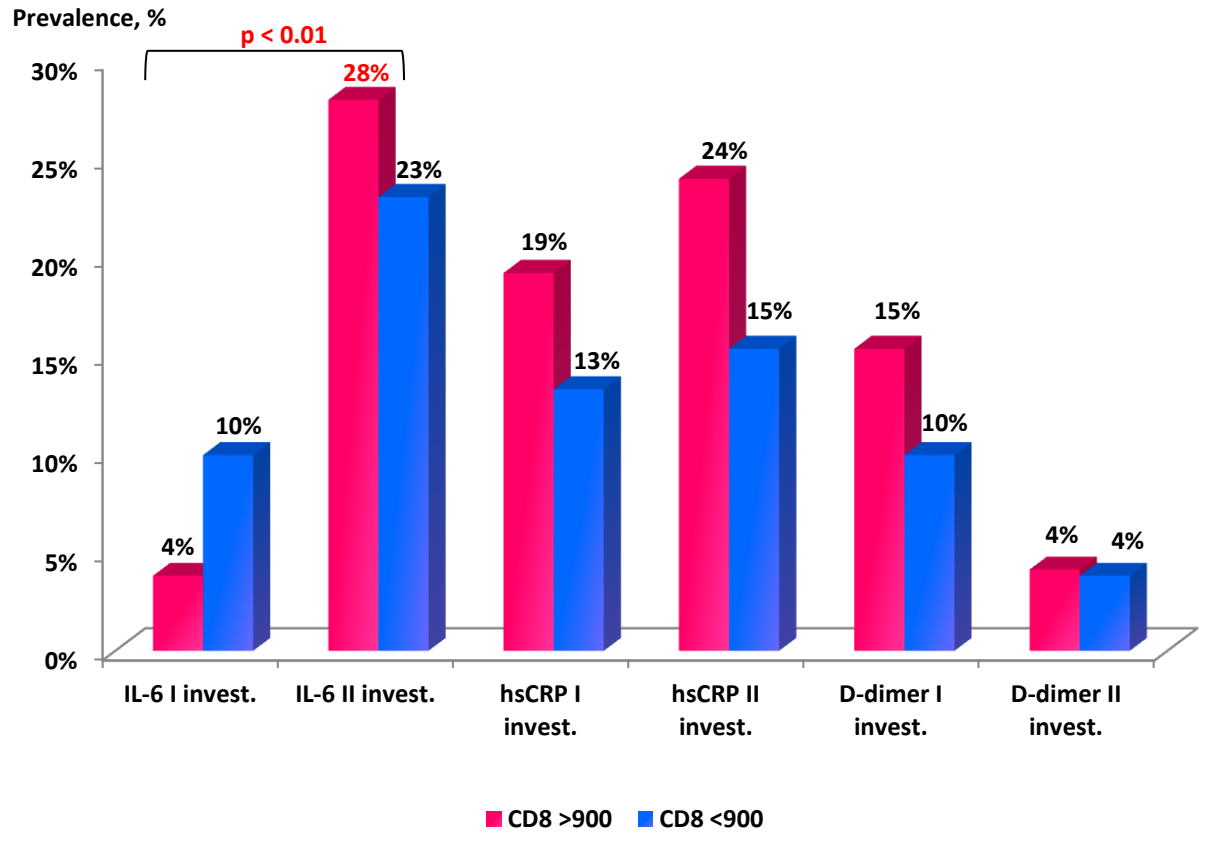


Fig. 44. Relative proportions of plwh with elevated (n_1=26) and normal (n_2=31) $CD8^+$ counts with elevated biomarkers of inflammation

4. Study of correlation between CD8+ and biomarkers of chronic inflammation

Biomarkers of chronic inflammation were compared in two groups – with increased $CD8^+$ counts (>900 cells/μL) (n_1 = 26 patients) and with normal $CD8^+$ counts (<900 cells/μL) (n_2 = 31 patients). Biomarker mean values and relative proportions of patients with elevated biomarkers were compared between groups. In each group, the indicators at the beginning were compared with those at the end (***Table 15***) (***Fig. 44***).

4.1. IL-6 – no significant difference in mean and relative proportions of patients with elevated IL-6 between groups at baseline and at baseline. In dynamics, a significantly higher mean value of IL-6 at the end compared to the beginning in both groups ($p<0.01$, $p<0.015$, respectively), but only in the group with an increased $CD8^+$ count, the relative proportion of patients with an increased biomarker in end is higher ($p<0.025$).

4.2. High-sensitivity CRP (hsCRP) – no significant difference in mean biomarker value and relative proportions of patients with elevated hsCRP between groups at baseline, but at end-point mean value was significantly higher in the elevated CD8+ count group ($p<0.0005$). No significant dynamics in the mean values and relative proportions of patients with elevated hsCRP in both groups.

4.3. D-dimer – no significant difference in mean and relative proportions of patients with elevated biomarker between groups at baseline and end. There was no significant difference in the mean values and relative proportions of patients with elevated d-dimer at baseline and at baseline across groups.

In summary, there was a very weak correlation of IL-6 (ϕ = 0.056), a weak correlation of hsCRP (ϕ = 0.108) and a very weak correlation of D-dimer (ϕ = 0.008) with an abnormal $CD8^+$ count.

Table 15. Biomarkers of inflammation in PLWH with elevated and normal $CD8^+$ counts

Groups / Bio markers	PLWHwith elevated $CD8^+$ count			PLWH with normal $CD8^+$ count			OR	ϕ	p
	↑ n %	mean ± sd (min-max)	95% CI	↑ n %	mean ± sd (min-max)	95% CI			
IL-6	1 3.85	3.74 ± 1.88 (1.5-8.62)	2.98- 4.50	3 10.00	4.01 ± 4.00 (1.5-23.24)	2.52- 5.50	0.36	**0.056**	>0.05 >0.05
2-nd invest.	7 28.00	6.12 ± 4.19 (1.5-19.93)	4.39- 7.85	6 23.08	5.69 ± 2.69 (2.02-13.71)	4.60- 6.78	**1.296**		>0.05 >0,05
p	**<0.025**	**<0.01**		>0.05	**<0.05**				
hsCRP	5 19.23	2.82 ± 3.01 (0.09-10.87)	1.60- 4.03	4 13.33	2.11-1.96 (0.19-6.57)	1.38- 2.84	**1.548**	**0.108**	>0.05 >0.05
2-nd invest.	6 24.00	5.51 ± 4.78 (0.6-29.65)	2.30- 8.72	4 15.38	3.03 ± 2.31 (0.6-9.02)	2.09- 3.96	**1.737**		**<0.0005** >0.05
p	>0.05	>0.05		>0.05	>0.05				
D-dimer	4 15.38	0.43±0.36 (0.22-1.96)	0.29- 0.58	3 10.00	0.34 ± 0.10 (0.21-0.6)	0.30- 0.37	**1.636**	**0.008**	>0.05 >0.05
2-nd invest.	1 4.17	0.34 ± 0.10 (0.21-0.60)	0.30- 0.38	1 3.85	0.33 ± 0.09 (0.21-0.59)	0.29- 0.36	**1.087**		>0.05 >0.05
p	>0.05	>0.05		>0,05	>0.05				

*** ↑ n* - number of patients with elevated value**

5. Study of correlation between $CD4^+$: $CD8^+$ ratio and biomarkers of chronic inflammation

Biomarkers of chronic inflammation were compared in two groups - with a CD4+ : CD8+ ratio <0.8 (n_1 = 28 patients) and with a $CD4^+$: $CD8^+$ ratio >0.8 (n_2 = 29) considered optimal for the status. Biomarker mean values and relative proportions of patients with excesses were compared between groups. In each group, the indicators at the beginning were compared with those at the end (***Table 16***) ***(Fig. 45***).

The biomarkers of chronic inflammation were compared in two groups – **with a $CD4^+$: $CD8^+$ ratio <0.8 (n_1 = 28 patients) and with a $CD4^+$: $CD8^+$ ratio >0.8 (n_2 = 29)** considered optimal for the status. Biomarker mean values and relative proportions of patients with excesses were compared between groups. In each group, the indicators at the beginning were compared with those at the end ***(Table 16) (Fig. 45).***

5.1. IL-6 – no significant difference in mean and relative proportions of patients with elevated IL-6 between groups at baseline and at baseline. Significantly higher mean (p<0.0005) and higher relative proportion of patients with elevated IL-6 at end compared to baseline only in group with elevated CD8+ : CD8+ ratio (p<0.025).

5.2. High-sensitivity CRP (hsCRP) – no significant difference in the mean value and in the relative proportions of patients with elevated hsCRP between groups at baseline, but at the end the mean value of the biomarker was significantly higher in the group with an increased CD8+ : CD8+ ratio (p<0.05). Only in the group with CD4+ : CD8+ <0.8 was there a significantly higher mean value at the end compared to the beginning (p<0.05).

5.3. D-dimer – no significant difference in mean and relative proportions of patients with elevated biomarker between groups at baseline and end. In both groups, there was no significant difference in the mean values and relative proportions of patients with elevated D-dimer between baseline and endpoint.

In summary, there was a weak correlation of IL-6 (ϕ = 0.116), a weak correlation of hsCRP (ϕ = 0.106) and a very weak correlation of D-dimer (ϕ = 0.016) with an abnormal $CD4^+$: $CD8^+$ ratio.

Table 16. Biomarkers of inflammation in PLWH with $CD4^+$: $CD8^+$ ratio < 0.8 and > 0.8

Groups / Bio markers	PLWH with $CD4^+$: $CD8^+$ < 0.8			PLWH with $CD4^+$: $CD8^+$ > 0.8			OR	ϕ	p
	↑ n %	mean ± sd (min-max)	95% CI	↑ n %	mean ± sd (min-max)	95% CI			
IL-6	1 3.57	3.35 ± 1.80 (1.5-8.62)	2.65- 4.05	3 10.71	4.42 ± 4.08 (1.5-23.24)	2.84- 6.00	0.309	**0.116**	>0.05 >0.05
2-nd invest.	9 32.14	6.60 ± 4.15 (1.5-19.93)	2.54- 8.21	5 21.74	5.04 ± 2.23 (1.5-8.71)	4.08- 6.01	**1.705**		>0.05 >0,05
p	**<0.025**	**<0.0005**		>0.05	>0.05				
hsCRP	5 17.86	2.68 ± 2.57 (0.09-10.87)	1.56- 3.79	4 14.29	2.20-2.10 (0.19-7.28)	1.38- 3.01	**1.304**	**0.106**	>0.05 >0.05
2-nd invest.	7 25.00	5.42 ± 5.39 (0.6-29.65)	2.55- 8.29	4 17.39	2.81 ± 2.16 (0.6-7.7)	1.88- 3.75	**1.583**		**<0.05** >0.05
p	>0.05	**<0.05**		>0.05	>0.05				
D-dimer	3 10.71	0.37 ± 0.18 (0.22-1.07)	0.30- 0.44	4 17.39	0.40 ± 0.32 (0.21-1.96)	0.27- 0.52	0.72	**0.016**	>0.05 >0.05
2-nd invest.	1 3.70	0.35 ± 0.09 (0.23-0.60)	0.31- 0.38	1 4.35	0.32 ± 0.09 (0.21-0.59)	0.28- 0.36	0.846		>0.05 >0.05
p	>0.05	>0.05		>0,05	>0.05				

*** ↑ n* - number of patients with elevated value**

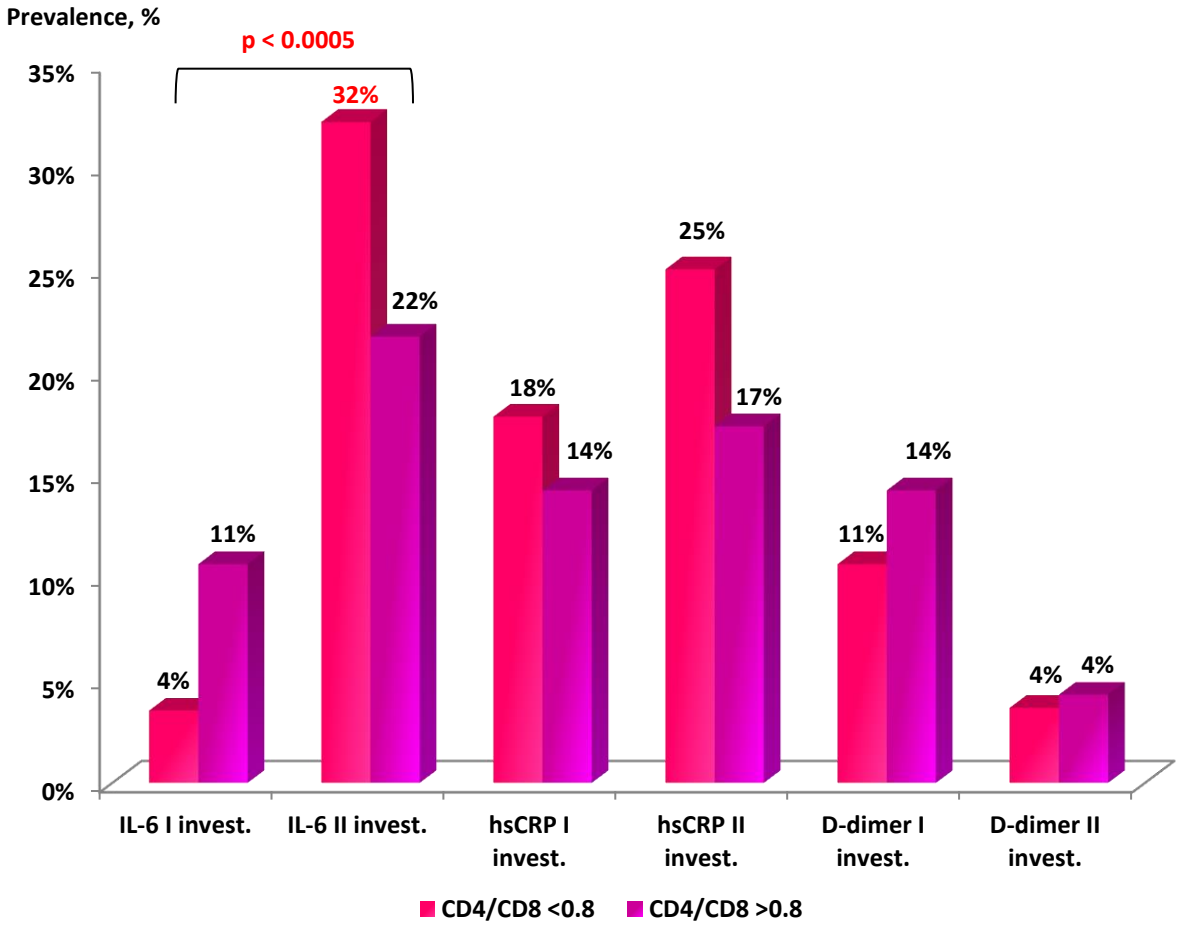


Fig. 45. Relative proportions of PLWH with $CD4^+$:$CD8^+$ ratio < 0.8 (n_1=28) and > 0.8 (n_2=29) with elevated inflammatory biomarkers

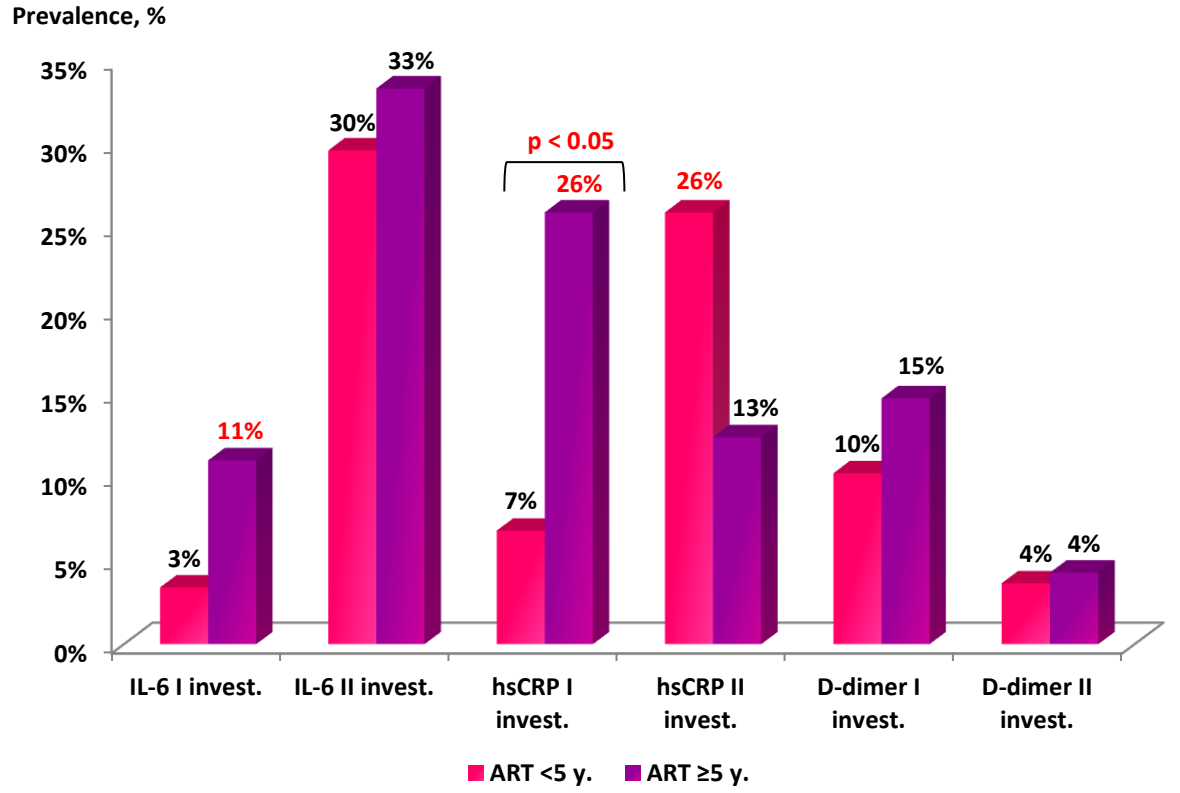


Fig. 46. Relative proportions of PLWH with elevated inflammatory biomarkers taking ART up to 5 years (n_1=29) and over 5 years (n_2=28)

6. Study of correlation between duration of ART and biomarkers of inflammation

Biomarkers of chronic inflammation were compared in two groups - in HIV patients on ART for up to 5 years (n_1 = 29) and on ART for more than 5 years (n_2 = 28). The mean values of the biomarkers and the relative proportions of patients with excess ones were compared between the groups. In each group, the indicators at the beginning were compared with those at the end (***Table 17***) (***Fig. 46***).

6.1. IL-6 – significantly higher mean value of the biomarker in the second study in the ART group up to 5 years compared to the first study ($p<0.0005$); significantly higher relative proportions of patients with an elevated biomarker in the second study compared to the first in each of the two groups ($p<0.005$).

6.2. hsCRP – significantly higher mean value of the biomarker at the second examination compared to the first in the ART group up to 5 years ($p<0.01$); a significantly higher relative proportion of patients with an elevated biomarker in the second compared to the first examination only in the group with ART up to 5 years ($p<0.05$); significantly higher mean value of the biomarker at the second examination in the ART group up to 5 years compared to the other group ($p<0.05$); significantly higher relative proportion of patients with an elevated biomarker at the first examination in the group with ART over 5 years compared to the other group ($p<0.05$).

6.3. D-dimer – no significant differences between the two groups in all aspects of comparison.

Table 17. Comparative Study of Biomarkers of Inflammation in PLWH, performed ART up to 5 years and over 5 years

Groups / Bio markers	PLWH with ART to 5 years			PLWH with ART > 5 years			OR	p
	↑ n* %	mean ± sd (min-max)	95% CI	↑ n* %	mean ± sd (min-max)	95% CI		
IL-6	1 3.45	3.51 ± 1.74 (1.5-8.62)	2.851- 4.172	3 11.11	4.28 ± 4.21 (1.5-23.24)	2.619- 5.951	0.286	>0.05 >0.05
2-nd invest.	8 29.63	6.48 ± 3.68 (1.5-19.93)	5.020- 7.932	8 33.33	5.25 ± 3.19 (1.5-13.71)	3.900- 6.595	0.842	>0.05 >0,05
p	**<0.005**	**<0.0005**		**<0.05**	**>0.05**			
hsCRP	2 6.90	1.92±1.79 (0.19-7.1)	1.238- 2.603	7 25.93	2.99-2.03 (0.09-10.87)	1.795- 4.194	0.212	>0.05 **<0.05**
2-nd invest.	7 25.93	5.55±4.49 (0.6-29.65)	2.588- 8.515	3 12.50	2.77±2.16 (0.6-9.02)	1.862- 3.685	0.700	**<0.05** >0.05
p	**<0.05**	**<0.01**		**>0.05**	**>0.05**			
D-dimer	3 10.34	0.40±0.34 (0.22-1.96)	0.275- 0.533	4 14.81	0.36±0.13 (0.21-0.75)	0.305- 0.406	0.663	>0.05 >0.05
2-nd invest.	1 3.70	0.33±0.09 (0.23-0.60)	0.291- 0.360	1 4.35	0.34±0.10 (0.21-0.59)	0.302- 0.387	0.846	>0.05 >0.05
p	**>0.05**	**>0.05**		**>0.05**	**>0.05**			

*** ↑ n* - number of patients with elevated value**

A generalized multifactorial model of aging in PLWH

Aging, developing into senescence, emerges as an extremely complex multifactorial process, further stimulated and complicated in combination with a chronic disease such as HIV infection. The life cycle scenario of PLWH has changed dramatically and in a positive direction – from survival to extended life, with a gradual shift in focus from youth to the complexities of adulthood and old age. Achieving optimal viral suppression and increasing $CD4^+$ counts through increasingly innovative ART are key, but really only first steps in subsequent long-term patient care. Immune recovery is an individual process that depends on the time of initiation of therapy, initial immune status and persistent residual viremia, immune activation, chronic inflammation and impaired intestinal integrity. Dynamic monitoring of PLWH should include a thorough analysis of immunological and clinical indicators with a view to prevention and adequate control of increasing multimorbidity and subsequent polypharmacy. All these emerging cases are directly linked to a number of individual risk factors from the lifestyle of each patient, such as unbalanced nutrition, lack of adequate physical activity, smoking, abuse of psychoactive substances, which gradually develop into metabolic syndrome, addictions and mental disorders in the conditions of increased social isolation and stigma. The impact of the different generation antiretroviral therapy carried out to date, which has both a pronounced protective and health-supporting effect, but also a somewhat negative impact due to its associated drug interactions, unwanted side effects and metabolic disorders, must also be taken into account. The introduction over the past 5 years of the latest generation single-tablet regimens such as BIC/FTC/TAF and DTG/3TC provide convenient administration, markedly effective infection control, more favorable effects on metabolic health, and ease of implementation in almost any comorbid and pharmaceutical profile. the patient, facilitating the daily work of medical specialists. The next step is likely to be the gradual introduction on a global scale of the innovative long-acting injection regimens, which will continue all the positive therapeutic effects, but provide better adherence to therapy and a lower level of personal stigma. It is the combination of all these factors, related or unrelated to HIV infection, under the control of long-term ART that are summarized in the figure below (***Fig. 47***).

The use of a complex multidisciplinary approach in the simultaneous care of physical, psychological and social health is the most ambitious goal of all specialists involved in the field. Disease prevention, by modifying risk factors, regular preventive examinations, vaccinations, as well as early diagnosis, should be prioritized over the active treatment of an already apparent health problem. An annual review of the standard diagnostic panel for the treatment of PLWH with a view to defining key clinical-laboratory parameters and, if necessary, supplementing with more sensitive and hitherto unused biomarkers of immune recovery and chronic inflammation would help this. Involvement in the process and other medical specialties will contribute to the more detailed approach in achieving a better quality of life for PLWH and reduced stigma. The updating of established therapeutic regimens for the treatment of accompanying non-infectious diseases is necessary in the changing dynamic comorbid patient profile. The results of one of the most promising studies "REPRIEVE" (N = 7,769 PLWH) showed, for example, that the inclusion of the lipid-lowering drug pitavastatin for 24 months reduced the possibility of progressive accumulation of atherosclerotic plaques by more than 30%. These changes would contribute to reducing the risk of serious cardiovascular events by nearly 35%.

Long-term success is unattainable without the active involvement of each patient in the treatment process. Good information builds personal opinion and motivation, which grows in the best case into a balanced healthy habit, general satisfaction and hence to a perceived improved quality of life of PLWH.

Undoubtedly, aging and HIV are extremely hot topics, with continuous relevance and need for innovation. Research on HIV is helping to better understand the aging process, and the study of aging is further elucidating how chronic diseases affect and change the body. Their combined analysis arouses increasing interest and opens new horizons in the direction of innovative research and, in the future, improved patient care.

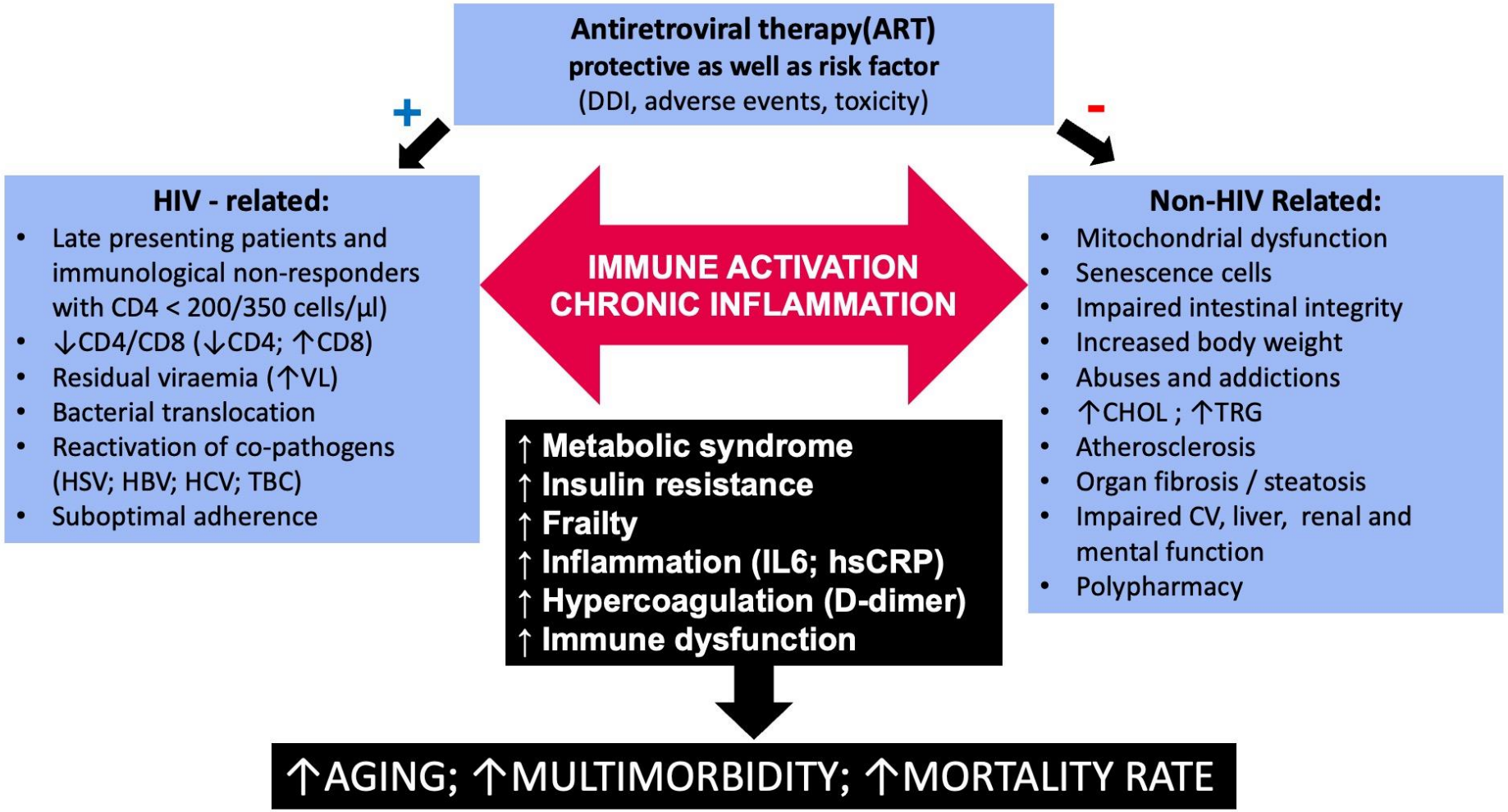


Fig. 47. A multifactorial model of aging with HIV

CONCLUSIONS

1. The demographic and epidemiological characteristics of the HIV group were identical and follow those of the affected general national and European population with a trend towards increasing age, sexual route of viral transmission (mainly MSM) and late presentation for treatment.

• **MSM transmission** mainly among the younger age group and in PLWH with starting $CD4^+$>350 cells/μL; more prevalent heterosexual pathway in adults over 40, with late-onset characteristics ($CD4^+$<350 cells/μL);

• **Passively detected** HIV status reliably more often at age over 40 and/or advanced immune deficiency; **actively detected** mainly patients up to 40 years;

• The **"linkage to care"** indicator is an average of 281 days (from 5 to 2821 days).

2. The behavioral characteristics of the modern HIV population mediate impaired control of chronic conditions, easier infection with other infectious agents and a generally deteriorated quality of life.

• The relative share of **daily smokers** is 2.5 times higher in the PLWH group compared to the control group;

• In the PLWH group, 1/5 are **health uninsured**;

• **Coinfections** – only in the target group, mainly syphilis and recurrent herpes infection, mainly in adults and those started with advanced immune deficiency.

• No correlation between **BMI** and HIV status.

• Significantly more frequent **multimorbidity** in the HIV group.

• Significantly more frequent **hospitalizations** in the last two years in the HIV group.

• Significantly more frequent **subjective complaints** in PLWH.

• PLWH **over 40 years** of age have more frequent comorbidities, worsened metabolic parameters and hospitalizations.

3. There is a reliable dynamic in the values of some basic laboratory parameters during the course of ART.

• The relative proportion of PLWH with **elevated total cholesterol** increased significantly – 31% at ART initiation, 58% at study end. Significantly higher mean values in patients older than 40 years and in those with starting $CD4^+$ <200 cells/μL.

• **Erythrocytes** – significantly lower mean values among PLWH; MCV – increases dynamically and is significantly higher in the infected.

• **Serum creatinine and AST** – increase in dynamics in PLWH, but within the reference range.

4. The dynamics of immune recovery is different for individual immunological indicators ($CD4^+$, $CD8^+$, $CD4^+$:$CD8^+$ ratio) and is influenced by age and initial immune status at ART initiation.

• **$CD4^+$** T-helper lymphocytes increase convincingly during ART, incl. and in elderly patients. At the beginning, the late-presenting ones ($CD4^+$<350 cells/μL) were 55%, at the end – 8.8%, and at the end there were no PLWH with $CD4^+$<200 cells/μL.

• **$CD8^+$** – with a constant average number and no reliable dynamics regarding the relative shares of patients with elevated $CD8^+$.

• **$CD4^+$: $CD8^+$** normalizes more slowly than the $CD4^+$ count. At the end of the study with a restored normal ratio (>1.0) were 40%. 14% remain with a persistently worsened index (< 0.4).

• **At different initial immune status,** high initial $CD4^+$ counts are also associated with high $CD8^+$ counts. At the end of the study, there was a reverse trend, with mean $CD8^+$ counts being significantly higher in PLWH starting with low $CD4^+$ counts.

5. The established correlations between biomarkers of chronic inflammation (IL-6, hsCRP, D-dimer) and clinical-laboratory indicators confirm their potential as a reliable tool for a more precise prognostic assessment of the overall condition of the modern patient with controlled HIV infection.

• **IL-6** is more sensitive, more specific and informative than hsCRP and D-dimer, reliably increasing in dynamics and with a greater relative share in PLWH compared to uninfected. Correlates with advancing age, immunodeficiency, high $CD8^+$ count, excess BMI, multimorbidity, and elevated cholesterol.

• **hsCRP** is less sensitive than IL-6, but an informative, easily performed and financially acceptable screening alternative. It is reliably elevated in dynamics with a greater relative proportion in PLWH compared to uninfected controls. Correlates with age, immunodeficiency, high $CD8^+$ count, excess BMI, and multimorbidity;

• **D-dimer** has a significantly higher average value among PLWH compared to HIV-negative, but without reliable dynamics. Very weak correlation with other clinical-laboratory parameters probably due to long-term performed ART.

CONTRIBUTIONS

ORIGINAL CONTRIBUTIONS

Original scientific contributions for Bulgaria:

1. The standard established diagnostic panel in the monitoring of HIV-infected patients was reviewed, with a view to focus on key clinical and laboratory parameters to possibly include more sensitive biomarkers of immune recovery and chronic inflammation.

2. A correlation between positive HIV status and degree of chronic inflammation was established by studying the dynamics of specific biomarkers and clinical-laboratory indicators in a case-control study.

3. The role of late initiation of antiretroviral therapy and different initial immune status among HIV-infected individuals on the degree of subsequent immune recovery and chronic inflammation was investigated.

4. The importance of age in the process of immune recovery and chronic inflammation among the HIV-infected population was studied

5. Correlations between specific risk factors, clinical-laboratory indicators and biomarkers (IL-6, hsCRP and D-dimer) alongside their role in chronic inflammation were studied.

6. A general multifactorial model was developed and created, considering important HIV-related and non-HIV-related parameters, ongoing ART and the possibility of immune dysfunction, chronic inflammation and risk of complications in the aging process with controlled HIV infection.

CONFIRMATIVE AND SCIENTIFIC-APPLIED CONTRIBUTIONS

1. Global trends of higher HIV prevalence among MSM men and a gradual increase in life expectancy among PLWH are confirmed.

2. A higher relative proportion of MSM transmission was observed among younger PLWH with high baseline CD4+ T-helper lymphocytes, and the heterosexual pathway was more prevalent in older, mostly late-presenting patients.

3. Higher rates of risk behavioral features and impaired primary prophylaxis among PLWH in the context of persistent stigma and social isolation mediate poor control of chronic comorbid conditions and the need for more frequent hospitalizations.

4. The correlation between existing HIV positive status, increased cardiovascular risk and occurrence of dysmetabolic conditions is confirmed.

5. Early HIV confirmation and immediate initiation of ART are key to immune recovery and reduction of chronic inflammation.

PUBLICATIONS RELATED TO THE THEME OF THE DISSERTATION

with the participation of Dr. Ivaylo Nikolaev Pakov

1. **Ivaylo Pakov,** Adelaida Ruseva, Irena Gencheva, Tsetsa Doichinova, Milena Karcheva, Kalina Terzieva, Lyudmila Pakova, Biserka Vasileva, Galya Gancheva. **IL-6, D-dimer and High-Sensitivity C-Reactive Protein in HIV Infection – Preliminary Study.** *Journal of IMAB.* 2023 Jul-Sep;29(3):5099-5102

2. **Ivaylo N. Pakov. Immune reconstitution in late-presenting HIV-positive case with idiopathic liver cirrhosis and ischemic brain stroke.** *J Biomed Clin Res.* 2023;16(1):66-73.

3. **Ivaylo Pakov,** Tsetsa Doychinova. **HIV-associated mortality and causes of death in late presenting patients.** *Medinfo.* 2020;3:58-62.

PARTICIPATIONS IN SCIENTIFIC FORUMS RELATED TO THE DISSERATION TOPIC:

1. **Ivaylo Pakov**, Adelaida Ruseva, Irena Gencheva, Tsetsa Doychinova, Milena Karcheva, Kalina Terzieva, Lyudmila Pakova, Biserka Vasileva, Galya Gancheva. **Biomarkers of Chronic Inflammation in the Aging Process with HIV and Long-term Antiretroviral Therapy.** 7th National Scientific Conference on HIV and Coinfections: "40 Years Since The Discovery of the HIV", 01-02 December 2023, Expo Hotel Sofia, Bulgaria

2. **I. Pakov. Dynamic evaluation of specific and non-specific biomarkers of immune recovery in long term HIV-infection.** 5th National Scientific Conference on HIV and Coinfections, 26-27 November 2021, Sofia, Bulgaria

3. **I. Pakov**, Ts. Doychinova. **Clinical features and challenges in treatment of HIV – positive patients older than 50 years.** 4th National Scientific Conference on HIV and Coinfections, 29-30 November 2019, Sofia, Bulgaria.

PARTICIPATION IN A SCIENTIFIC RESEARCH PROJECT, FUNDED BY MEDICAL UNIVERSITY – PLEVEN

Scientific project (D1/2023) on the topic: ***"Investigating the role of the biomarkers IL-6, D-dimer and hsCRP as indicators of chronic inflammation in the aging process with HIV infection"***

Research team: Dr. Ivaylo Nikolaev Pakov; Prof. Dr. Milena Dimitrova Karcheva, MD, PhD; Prof. Galya Ivanova Gancheva-Boycheva MD, PhD; Prof. Dr. Tsetsa Georgieva Doychinova-Naidenova, MD, PhD; Prof. Dr. Adelaide Lazarova Ruseva, MD, PhD; Assoc. Dr. Irena Ivanova Gencheva-Angelova, MD, PhD; Dr. Lyudmila Ivanova Pakova, MD; Dr. Kalina Dimitrova Terzieva, MD, PhD; Biserka Ilieva Vasileva, MD, PhD