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DISSERTATION

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Study programme: Hygiene, Preventive Medicine a Epidemiology

A model for optimizing comprehensive care for people with HIV infection

Dissertation

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A model for optimizing comprehensive care for people with HIV infection

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Abstract

Introduction: Today, the disease caused by HIV is considered to be chronic, treatable, with a length and quality of life comparable to that of the general population. Comprehensive care for people living with HIV (PLWH) consists of the correct indication of antiretroviral drugs (ART), optimal motivation, regular use of drugs, passing check-ups and submitting to all the recommendations of the attending physician, which is collectively referred to as adherence.

Aims and objectives: Assessment of adherence to ART treatment in a population of PLWH. Improving the awareness of PLWH, drawing attention to the risk of developing HIV resistance and subsequent treatment failure. **Patient group and follow-up methods:** The basic group consisted of PLWH, long-term follow-up at the HIV center of the Faculty Hospital Plzeň. Adherence was assessed by ART levels, determined in urine by high pressure liquid chromatography (HPLC), inrelation to clinical data, viral load (HIV RNA, i.e. VL) and absolute CD4+ and CD8+ T cell counts. To assess mental and physical status, the modified SF 36 questionnaire was used to measure social ties, education and ability to relax. Statistical evaluation was performed using SAS, V. 9.4 and Statistics software.

Research results: From a group of 151 PLWH, 18 (11.9%) subjects with zero levels and 20 (13.2%) subjects with ART levels up to 10 mg/L were selected. They were followed for 6-12 months. A statistically significantly lower viral load was demonstrated in adherent persons at the time of the test for the presence of ART in the urine. CD4+ values in adherent persons were, as expected, higher, but similar to CD8+ T lymphocyte values, statistical significance was not demonstrated. A questionnaire survey assessed subjective factors influencing the degree of adherence. PLWH consider important: quality care with instilling trust, low risk of developing opportunistic infections, self-sufficiency, quality of sleep, managing leisure activities and good family relationships. Quality of life and satisfaction in the monitored areas were higher in adherent PLWH. The development of resistance against some groups of ART, due to non-adherence, cannot be completely excluded in 11 persons.

Conclusions: Non-adherence may be the cause of the development of HIV resistence and treatment failure on the part of the patient. PLWH with zero and low urinary nucleoside levels were repeatedly instructed about the need for regular, sustained medication use. Regular checks with a laboratory examination serve to detect early the emergence of resistance and some side effects of the treatment, which are initially only detectable in the laboratory.

Keywords: adherence; antiretroviral; HIV; psychosocial; treatment; viral load

Model optimalizace komplexní péče o osoby s infekcí HIV

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Abstrakt

Úvod: Onemocnění vyvolané HIV se dnes považuje za chronické, léčitelné, délkou a kvalitou života srovnatelné s běžnou populací. Komplexní péče o osoby žijící s HIV (PLWH) spočívá ve správné indikaci antiretrovirotik (ART), optimální motivaci, pravidelném užívání léků, absolvování kontrol a podrobení se všem doporučením ošetřujícího lékaře, což se souhrně označuje jako adherence.

Cíle a záměry: Hodnocení adherence léčby ART u souboru PLWH. Zlepšení informovanosti PLWH, upozornit na riziko vzniku rezistence HIV a následné selhání léčby.

Soubor nemocných a metody sledování: Základní soubor tvořily PLWH, dlouhodobě sledované v HIV centru FN Plzeň. Adherence se hodnotila podle hladin ART, stanovených v moči pomocí vysokotlaké kapalinové chromatografie (HPLC), ve vztahu ke klinickým údajům, virové nálože (HIV RNA, tj. VL) a absolutním počtům CD4+ a CD8+ lymfocytů T. K hodnocení psychického a fyzického stavu, sociálních vazeb, vzdělání a schopnosti relaxace sloužil modifikovaný dotazník SF 36. Statistické vyhodnocení se provedlo pomocí software SAS, V. 9.4 a Statistika.

Výsledky výzkumu: Ze souboru 151 PLWH se vybralo 18 (11,9 %) osob s nulovými hladinami a 20 (13,2 %) subjektů s hladinami ART do 10 mg/l. Jejich sledování probíhalo 6-12 měsíců. Prokázala se statisticky významně nižší virová nálož u osob adherentních v době provedení testu na přítomnost ART v moči. Hodnoty CD4+ u adherentních osob byly dle očekávání vyšší, avšak podobně jako u hodnot CD8+ lymfocytů T se statistická významnost neprokázala. Dotazníkové šetření posoudilo subjektivní faktory ovlivňující stupeň adherence. PLWH považují za důležité: kvalitní péči s navozením důvěry, nízké riziko vzniku oportunních infekcí, soběstačnost, kvalitu spánku, zvládání volnočasových aktivit a dobré rodinné vztahy. Kvalita života a spokojenost ve sledovaných oblastech byla u adherentních PLWH vyšší. Vvývoj rezistence proti některým skupinám ART, vlivem non-adherence nelze zcela vyloučit u 11 osob.

Závěry: Non-adherence může být příčinou vývoje rezistence HIV a selhání léčby ze strany pacienta. PLWH s nulovými a nízkými hladinami nukleosidů v moči se opakovaně instruovaly o nutnosti pravidelného, trvalého užívání léčiv. Pravidelné kontroly s laboratorním vyšetřením slouží k včasnému odhalení vzniku rezistence i některých vedlejších účinků léčby, které jsou zprvu detekovatelné pouze laboratorně.

Klíčová slova: adherence; antiretrovirové; HIV; psychosociální; léčba; virová nálož

Declaration

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Content

List of symbols and abbreviations used

Abbreviation	Meaning
ABC	abacavir
AC	AIDS Center, newly HIV center
AIDS	Acquired Immunodeficiency Syndrome
ARV sy	acute retroviral syndrome
ART	antiretroviral therapy (formerly HAART - Highly Active Antiretroviral Therapy, cART - combination ART), the current standard of antiretroviral combination therapy
AZT	azidothymidine (zidovudine)
BIC	biktegravir
CCR5, CXCR4	chemokine coreceptors on the surface of target CD_4^+ cells
CDC	Centers for Disease Control
CD_4^+, CD_8^+	lymphocyte T subpopulations
CMV	cytomegalovirus
CNS	central nervous system
CT	computed tomography
ČSAP	Czech AIDS Aid Society
DRV	darunavir
DTG	dolutegravir
d4T	stavudine
EI	entry inhibitor
ELISA	immunoenzymatic determination of the level of antibodies or antigens
EVG	elvitegravir
EVG/cobi	elvitegravir potentiated with cobicistat
FI	fusion inhibitor
Env V1,V2,V3	components of HIV glycoprotein packaging
FTC	emtricitabine
G-CSF	filgrastim - colony stimulating factor
GCV	ganciclovir
GIT	gastrointestinal tract
HIV-1	Human Immunodeficiency Virus - type 1
HIV-1 RNA	number of copies of HIV-1 RNA, plasma viraemia, viral load
IL-2	interleukin 2
INSTI	HIV integrase inhibitor
KINCM	Department of Infectious Diseases and Travel Medicine
LPV/r	lopinavir potentiatzed with ritonavir
MRI	magnetic resonance imaging
MVC	maraviroc
NNRTI	reverse transcriptase non-nucleoside inhibitor
NRL AIDS SZÚ	National Reference Laboratory for AIDS at the National Institute of Public Health in Prague

N(t)RTI	reverse transcriptase nucleoside (nucleotide) inhibitor
NRTTI	reverse transcriptase translocation inhibitor
NVR	nevirapine
OI	opportunistic infection
PCR	polymerase chain reaction
PEP	post-exposure prophylaxis
PGL	Persistent Generalized Lymphadenopathy
PHI	Primary HIV infection
PI	HIV protese inhibitor
PI/r	HIV protease inhibitor potentiated with ritonavir
PL	general practitioner
PLWH	people living with HIV
PrEP	pre-exposure prophylaxis
QoL	Quality of Life
RAL	raltegravir
RAMs	mutations associated with the development of resistance
RNA	ribonucleic acid
RPV	rilpivirine
RTV, r	ritonavir
TAF	tenofovir alafenamide
TBC	tuberculosis
TDF	tenofovir disoproxyl fumarate
T-20	enfuvirtide
UGT	urogenital tract
UNAIDS	The Joint United Nations Programme on HIV/AIDS
VL	„viral load“ - HIV RNA, detectable by PCR
WB	Western Blot - confirmatory test
WHO	World Health Organization
3TC	lamivudine

1. Introduction

Infectious diseases have been going on throughout human history. Wars, and the accompanying famines and epidemics have fundamentally affected the development of the population on individual continents. At the end of the second millennium, one of the last pandemics appeared, which gradually affected all continents and thus significantly affected not only the lives of many people, but also required large investments in health care and research in many countries. Although the pandemic has lasted for more than 40 years, HIV-related diseases are still spreading across all continents. The AIDS pandemic has claimed almost 40 million lives and the number of people living with HIV (PLWH) at various stages of the disease is similar.

The first two cases of a fully developed disease occurred in the spring of 1981 in New York in two young, until then healthy homosexuals. The attending dermatologist diagnosed Kaposi's sarcoma, rare in this age category [Friedman-Kien 1981]. At the same time, M.S. Gottlieb in Los Angeles, described pneumocystis pneumonia, candidiasis of the oral mucosa and cytomegalovirus infection in five homosexuals [Gottlieb 1981]. Examination of all these patients showed a deep disruption of cellular immunity. In addition, in 1981, another 26 members of the gay community appeared in various parts of the United States with severe immunodeficiency and some opportunistic infection. The new disease was therefore named GRID (Gay-Related Immune Deficiency). It was later found that the disease affects not only men having sex with men, but also women, immigrants from Haiti, hemophiliacs and intravenous drug users (designation 4H). After processing epidemiological data in 1982, it was almost certain that the new disease was caused by an infectious agent. It was therefore named AIDS (Acquired Immunodeficiency Syndrome). [CDC 1982]. This marked the beginning of an era of large-scale research projects in both the USA and European countries.

The most successful were scientific groups led by professors Luc Montagnier (Paris), Robert Gallo (Bethesda) and J. A. Levy (San Francisco), which independently discovered the causative agents of AIDS [Barre-Sinoussi 1983, Gallo 1984, Levy 1984].

In 1986, the causative agent of AIDS was named HIV (Human Immunodeficiency Virus). In 1985, Montagnier and Clavel isolated a genetically related virus called HIV-2 in an AIDS patient living in West Africa [Clavel 1986]. Currently, about 95% of AIDS cases are caused by HIV-1. The infection was found to occur horizontally (most often during sexual intercourse, less blood-infected people) and vertically (perinatally, less often intrauterinely or by breastfeeding).

Discovery of the epidemiology and pathogenesis of the disease has made it possible to launch prevention programs which, together with antiretroviral therapy (ART), have gradually led to a significant decrease in the number of new cases of HIV infection.

2. Aim of the work

- Monitoring adherence to antiretroviral (ART) treatment in a cohort of people living with HIV (PLWH).
- Elaboration of questionnaires focused on ART treatment tolerance, adherence and QoL.
- Determination of levels of selected antiretrovirals (ART) in urine in order to objectify the control of adherence.
- Comparison of the results of a questionnaire survey and objective measurement of urinary levels ART.
- Use of the obtained data to improve demonstrably reduced adherence by introducing effective mechanisms for its control within short-term and long-term monitoring.

Hypothesis H01: Patients regardless of the demonstrable level of ART used in the urine have good adherence to treatment.

Hypothesis H02: HIV RNA (VL) levels do not differ between adherent and non-adherent PLWH.

Hypothesis H03: Absolute numbers of CD4+ T lymphocytes do not differ between adherent and non-adherent PLWH.

Hypothesis H04: Absolute numbers of CD8+ T lymphocytes do not differ between adherent and non-adherent PLWH.

3. Current state of knowledge

3.1 Epidemiology

HIV occurs in high concentrations in blood, semen and cervicovaginal secretions. Therefore, the main modes of infection include risky sexual intercourse, intravenous drug use of devices contaminated with HIV-positive blood, and vertical transmission from an HIV-positive mother to her offspring. The epidemiological situation in the field of HIV/AIDS is still not good and the number of HIV-positive persons and persons in the stage of AIDS is high. The worst situation is in Sub-Saharan Africa, also due to poverty and minimal prevention. The problem is also in the Southeast Asian countries and the countries of the former Soviet Union [Podlekareva 2008].

As of the end of 2020 worldwide, an estimated 38 million people live with HIV according the Common programme of UN for HIV/AIDS (UNAIDS).

It is good news that about 25.4 million PLWH is treated with modern antiretroviral drugs (ART). As of the same date, approximately 40 million people have died from AIDS since the beginning of the pandemic. In just one year alone, it is currently estimated that about 1.5 million people become infected and 0.7 million die from AIDS. [UNAIDS 2020, UNAIDS 2021]. Slightly lower numbers can be expected in the future. Years ago, the UNAIDS 90-90-90 program was announced, according to which 90% of PLWH should be known by 2020, of which 90% should be treated with ART, of which 90% should have undetectable levels of HIV RNA. This program has not been, however, fully implemented yet.

Number of HIV/AIDS positive patients in Czech Republic

In the Czech Republic (CR), the situation is relatively favorable compared to the surrounding countries, even though it has slightly deteriorated during the last years, especially among men who have sex with men (MSM) [NRL AIDS 2021]**.** By the end of 2021, there were 4074 PLWH (Czech nationality and residents) registered in Czech Republic, of which 773 were in the stage of AIDS (Fig. 1). So far 353 persons died of AIDS in the Czech Republic. The most frequent way of transmission of HIV is sexual transmission, especially among MSM. Vertical transmission has so far been confirmed in nine cases in the Czech Republic. In addition to Czech citizens and foreign residents, 507 PLWH foreigners also lived here. The numbers of PLWHs with permanent residence in the Czech Republic as of 31 December 2021 are shown in Fig. 2. Most PLWHs live in Prague, North Moravia, North and West Bohemia. The highest prevalence expressed in relative numbers is still in Prague and the Karlovy Vary region [NRL AIDS 2022].

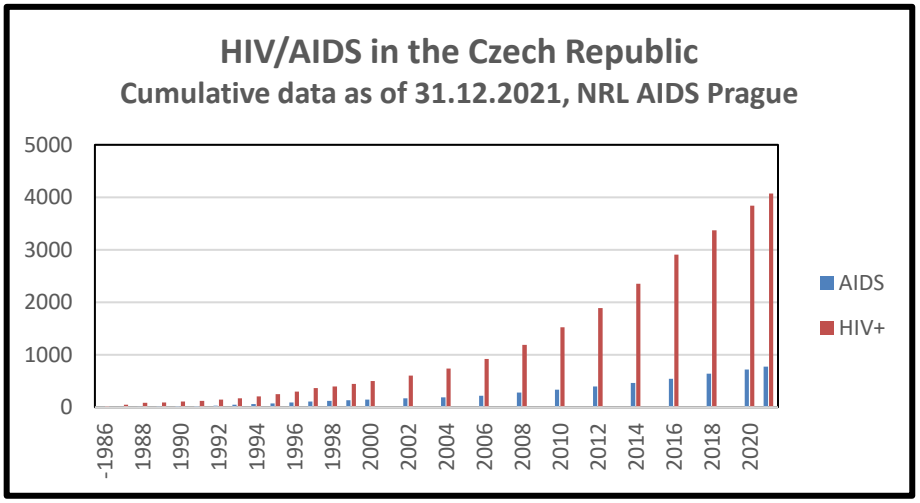

Figure 1. Cumulative data on the incidence of HIV + from 1986 to 2021 in the Czech Republic (source: NRL for AIDS Prague).

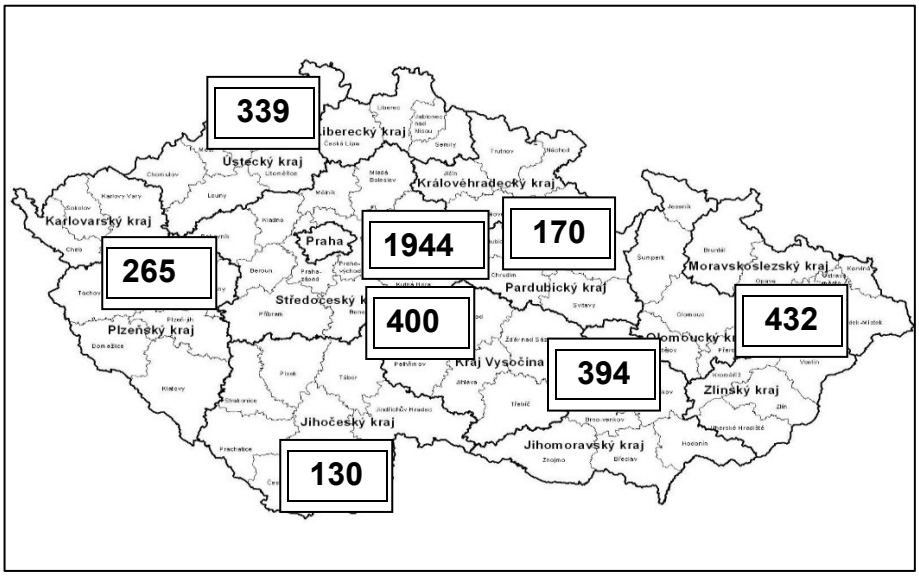

Figure 2. Numbers of PLWH according to the individual HIV centers in the Czech Republic as of 31 December 2021. (source: NRL for AIDS Prague).

Pathogenesis

HIV is one of the retroviruses, i.e. RNA viruses equipped with reverse transcriptase. Error transcripts often occur during the replication cycle, so the genetic information of newly produced HIV may vary. This fact can lead to a change in some biological properties, such as the development of resistance to ART, or higher infectivity [Hofman 2022]. The basic functions and replication cycle of HIV are shown in Fig. 3.

After entry into the host, HIV infects helper CD4+ T cells (regulating cellular and antibody immunity) and macrophages (microglia in the CNS, dendritic cells in the skin and lymph nodes, intestinal mucosa cells, pulmonary macrophages, and many others) [Maartens 2014]. The interaction between the viral particle and the host CD4 + cell occurs through the binding of the viral surface glycoprotein gp120 to the CD4 receptor. In addition to CD4 receptor expression, the chemokine co-receptors CCR5 (molecular weight 40.6 kDa, predominant on macrophages) or CXCR4 (found on CD4 + T cells) are also required [Deng

1996, Dragic 1996, Bleul 1997]. The CCR5 protein is encoded by the ChemR13 gene. People with mutations or deletions of this gene may naturally be less susceptible or completely resistant to HIV-1 infection [Samson 1996, Liu 1996, Huang 1996, Zagury 1998, Kupfer 1999].

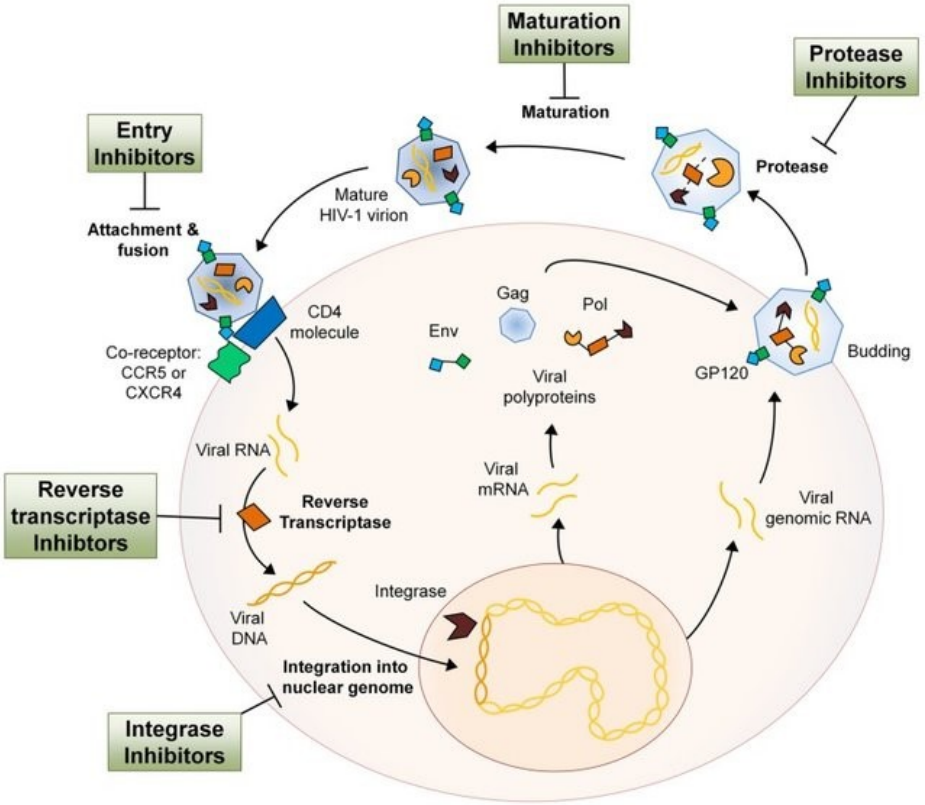

Figure 3. Basic functions, HIV replication cycle and sites of potential antiretroviral interventions [Smith 2013].

In the host cell, HIV replicates in a relatively complex way and is released in large quantities in as little as 2.5 days. In the acute phase, the daily turnover of HIV is estimated at 10^{10} virions. At that time, the infected individual is also highly infectious. Within a few days after HIV infection, memory CD4+ T cells become latent. After the integration of proviral DNA into their genome, HIV is stored in them for a long time. These cells are found in the so - called reservoir organs and tissues of the human body (lymph nodes, brain, intestinal lymphatic tissue, etc.) [Fauci 1999, Schacker 2000].

HIV production is associated with high energy demands, so depleted CD4 + cells decrease during the course of the disease (Fig. 4).

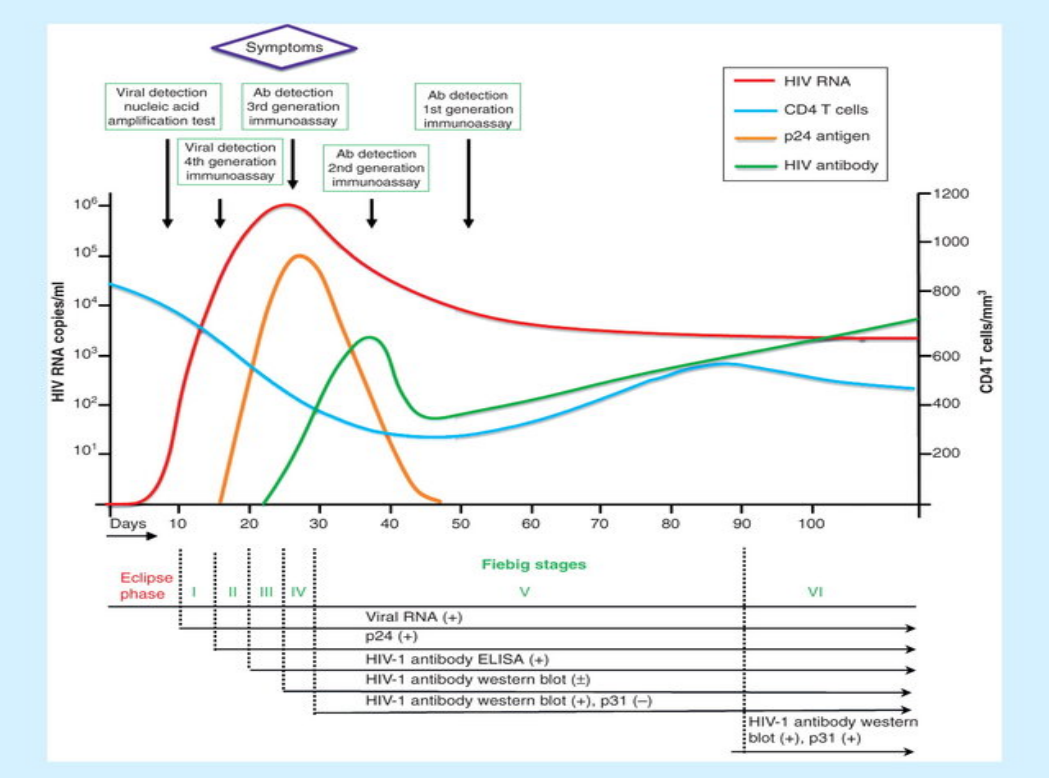

Figure 4. Trajectories of HIV-RNA viremia, CD4 T cells, p24 antigen and HIV antibody over the early phase of HIV infection. Sequence of appearance of different generations of HIV diagnostic assays is presented. Fiebig staging which represents a mean estimation of time from viral acquisition, divided into six phases, has also been superimposed. Eclipse phase is defined by the absence of any marker including p24 and viral RNA [Raoulty 2015].

In addition to the cytopathogenic effect, the CD8 + cytotoxic cells themselves soon become involved, killing the infected CD4 + cells as part of a specific immune response. The decrease in CD4 + T cell counts during infection correlates with the progression of infection. Deep deficit (absolute CD4 + counts below 100 or even below 50/μl) is a condition for the development of opportunistic infections, often multiple. Therefore, the absolute number of CD4 + lymphocytes (determined by flow cytometry, FACS) is also considered a basic immunological indicator evaluating the current performance of the immune system.

The second important variable is the absolute amount of viral RNA in one ml of blood, which in the first examination indicates the phase of untreated disease and after the start of treatment on its success. The CD4 / CD8 index is considered an expression of the depth of immunodeficiency [Hofman 2022]. Part of the proviral DNA is stored in reservoirs (with a maximum in the lymphatic tissue) in the so-called resting CD4 + lymphocytes with a long half-life. Therapeutic intervention in the reservoirs is very difficult, so at present we cannot completely cure HIV infection. However, some modern approaches suggest that even reservoirs may not be the definitive barrier [Churchill 2016].

3.2 Clinical findings, classification and treatment of HIV infection

Primary infection, clinical category A

Two to six weeks after the entry of HIV into the host, up to 90% of those infected may develop acute retroviral syndrome (ARV sy), or primary HIV infection (PHI). It usually lasts one to two weeks. Clinically, ARV sy most often manifests as a febrile condition with tonsillopharyngitis and lymphadenitis (referred to as "mononucleosis-like" syndrome), or as a febrile illness with irritating dry cough, muscle and joint pain, flu-like syndrome (therefore "flu-like" syndrome). As many as 70% of those infected have a volatile rash, often of a morbilliform nature, other times ulcerations and soils may occur in the oral cavity and other mucous membranes, sometimes neurological symptoms are present, and HIV enteropathy is less frequently observed. An asymptomatic or very mild course in the acute phase usually means a more favorable course in the future and vice versa.

Laboratory examination reveals leukopenia with lymphopenia with very low numbers of CD4 + CD45RA T lymphocytes. From the third week onwards, neutropenia, thrombocytopenia and lymphocytosis (activated CD8 + / DR + / CD38 +, CD11a / CD18 +) tend to inverse the CD4 + / CD8 + index, which persists of life. Sedimentation of erythrocytes, sometimes even liver aminotransferases, is usually increased in half of people and CRP. Immunological examination shows a reduced response to antigens and mitogens, and a disorder of B lymphocyte function. At this time, the high plasma concentration of HIV RNA (PCR), the so-called viral load, or load, is the rule, and the p24 antigen is usually present in the peripheral blood. Seroconversion occurs within 12 weeks. At that time, the

p24 antigen disappears and specific anti-HIV antibodies appear first in the IgM class, later in the IgG class. These can be demonstrated, for example, by immunochromatographic rapid test or ELISA. The reactive sample must be confirmed in the National Reference Laboratory (NRL), e.g. by Western Blot (WB).

ARVs resolve spontaneously within three weeks and an so-called asymptomatic period begins, which can last for several years. An untreated person is infectious to the environment, especially through the blood. HIV infection in pregnancy can lead to vertical transmission of the infection to the fetus.

At the end of the asymptomatic phase, the nodular syndrome develops, so-called persistent generalized lymphadenopathy (PGL). Acute infections, the asymptomatic phase and PGL are now classified in clinical category A (Tab. 1).

Table 1. Classification of HIV infection according to the CDC, valid from 1.1.1993

Theoretically, there are nine combinations (A1,A2,A3,B1,B2,B3,C1,C2,C3)

Clinical category **CD4+**	**A:** Acute, asymptomatic PGL….	**B:** candidiasis, leukoplakia….	**C:** pneumocystosis, TBC, CMV, Toxoplasmosis, Kaposi's sarcoma
>=500/µl (>=29 %) (1)	A1	B1	C1
200-499/µl (29-14 %) (2)	A2	B2	C2
<200/µl (<=14 %) (3)	A3	B3	C3

Clinical category B

In untreated people, the decline in immunity is accompanied by weight loss, night sweats, febrile illness, loss of appetite and fatigue, and so-called 'small' opportunistic infections. This phase is called early symptomatic. "Small" opportunistic infections include tonsilopharyngitis and vulvovaginitis (C. albicans), hairy leukoplakia of the tongue (coinfection with EB virus), recurrent herpes zoster (VZV), bacillary angiomatosis (Bartonella hensellae), listeriosis (L. monocytogenes). Lymphoid interstitial pneumonitis (caused by HIV) is described in children, and papillomavirus cervical dysplasia or carcinoma in situ (CIN) occurs in women. Category B also includes febrile conditions (> 38.5 ° C), diarrheal diseases lasting more than one month, some manifestations of autoimmunity and

peripheral neuropathy. Immune performance in untreated patients is still declining (CD4 + T cells, Th1 subpopulation). Impaired regulation of immunoglobulin synthesis is associated with high IgG levels without a neutralizing effect on HIV. Anemia and thrombocytopenia are the rule. Serum beta2-microglobulin and neopterin levels increase. Recurrences of infections are becoming more common [Malstan 2020].

Clinical category C (AIDS)

Severe immune deficiency, which usually occurs over the years in untreated patients, is characterized by the presence of so-called "large" opportunistic infections and tumors associated with HIV. Typical opportunistic infections include pneumocystis pneumonia, toxoplasmic encephalitis, oesophageal, tracheal, bronchial or pulmonary candidiasis, chronic anal herpes simplex or herpetic bronchitis, CMV pneumonia, oesophagitis, retinitis or generalized infection, TBC and mycobacteria. Severe immunodeficiency can be accompanied by HIV-associated tumors such as Kaposi's sarcoma, systemic non-Hodgkin's lymphoma, primary brain lymphoma, invasive cervical cancer, other tumors, but also other types of malignancies PLWHs tend to be more common, depending on the severity of immunodeficiency.

Nosological units associated with direct HIV action include HIV-encephalopathy, which may progress to AIDS dementia syndrome (ADC), and wasting syndrome (significant weight loss accompanied by fever and diarrhea, referred to as wasting syndrome). Haematological changes have also been reported at this stage: anemia, leukopenia, lymphopenia and CD4 + T cells fall below 200/µl. The so-called AIDS-indicative diseases are listed in **Tab. 2**.

Table 2. AIDS – indicative diseases. Classification CDC (1.1.1993).

Aspergilosis
CMV retinitis, generalized infection (except the liver, spleen and nodes)
herpes simplex - chronic anal or nebo herpetic bronchitis, pneumonia or esophagitis
histoplasmosis disseminated or extrapulmonary
HIV encephalopathy
izosporiosis chronic intestinal (>1 month)
esophageal, tracheal, bronchial or pulmonary candidiasis
Kaposi's sarcoma
Cervical cancer invasive
coccidioidomycosis disseminated
extrapulmonary cryptococcal infection
cryptosporidiosis chronic intestinal (>1 month)
lymphoma primary cerebral
lymphomas malignant (Burkitt, immunoblastic)
mycobacteriosis disseminated or extrapulmonary
pneumocystic pneumonia
recurrent pneumonia (> two over the course of one year)
progresive multifocal leukoencephalopathy
recurrent salmonella bacteremia
toxoplasmic encephalitis
tuberculosis
wasting syndrom

Current antiretroviral therapy

The first effective, commercially available drug to treat HIV infection has been available since 1987. Azidothymidine (AZT, also known as zidovudine), originally developed for tumor chemotherapy, was the first nucleoside inhibitor of viral reverse transcriptase (NRTI) and is still used today in specialty indications. Since 1995, the era of combination therapy

with new groups of drugs has begun. The first were viral protease inhibitors (PIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs), later inhibitors of gene fusion and HIV entry. At present, HIV integrase inhibitors (INSTIs), introduced in 2007, are preferred in combination. Combination therapy using two NRTIs, or one NRTI and one NtRTI together with one INSTI is the current treatment standard, leading to a rapid reduction in HIV RNA in the blood [EACS 2021]. Within one to two months, it is possible to reduce blood levels of HIV RNA (so-called viral load, VL - viral load) below a detectable amount (i.e. 20 copies / ml).

Currently, HIV/AIDS treatment is at a very good level and it is possible to achieve a long-term decrease in viremia in an infected organism up to undetectable level. However, it is always essential to start treatment early. In the past, the treatment preparations had often been used after a while and according to the absolute number of CD4+ T-lymphocytes in the patient's blood. The strategy of recent years is to initiate treatment for HIV-positive individuals as soon as possible, regardless of the number of CD4+ T-lymphocytes in peripheral blood [EACS 2021].

New antiretroviral therapy (ART) drugs substantially increase the quality of life of patients, since their side effects have declined significantly [Price 2016]. Preparations are usually available either as single-component tablets or as a fixed dose combination of several medicines, or as a single tablet regimen. The number of drugs that HIV-positive patients have to use in connection with their diagnosis has been significantly reduced compared to the past.

Thanks to the simplifying of treatment and the use of medication for patients, their optimal adherence can be more easily facilitated, thus making the whole process more efficient, which ultimately results in a better patient's health and a more cost-effective

treatment. Good adherence to treatment brings another very positive and vital benefit. It has been proven that adherent patients less likely developed HIV resistance to established treatment [Low 2016]**.** It is assumed that new antiretroviral drugs will be soon active even in the next stages of the HIV replication cycle, and thanks to their new properties, it will be possible to extend dosing intervals to weeks or up to months [Lundgren 2015, Snopková 2019]**.**

Antiretrovirals that are currently used in combination ART are divided into several groups:

1-Reverse transcriptase inhibitors

These substances are further divided into nucleoside/nucleotide (azidothymidine, tenofovir disoproxil fumarate or alafenamide, abacavir) and non-nucleoside reverse transcriptase inhibitors (etravirine, rilpivirine, doravirine) and they are now widely available.

2-Integrase inhibitors

These are HIV-blocking substances with a very rapid onset of action and are currently considered as one of the first-choice drugs. These include raltegravir, elvitegravir, dolutegravir, biktegravir and cabotegravir.

3-Protease inhibitors

These medications can block the HIV-protease (e.g. ritonavir, darunavir, atazanavir) and inhibit the maturation of the virion.

In addition, there are some other groups, mostly used as a salvage therapy in case of failing the older treatment (fusion, entry and other receptor inhibitors). The use of antiretrovirals within preferred drug combinations leads to appropriate combination of the individual substances into one tablet so that they do not adversely affect each other but allow the patient to use only one pill a day. Normally are available double combinations or triple

combinations of individual drugs [Günthard 2016, Seyler 2018]**.** The combination of some drugs is shown in Tab. 4 [EACS 2021, Ryom 2022].

Development of other antiretrovirals continues, and other drug combinations are already planned. Unfortunately, despite the ongoing research on an appropriate vaccine in this area, significant advances have not been achieved yet, and the existing vaccines have been failing [Cohen 2016].

The ART treatment does not target HIV virus in latently infected CD4+ cells in reservoirs including lymphatic tissue of gastrointestinal tract, lymph nodes, spleen, bone marrow, prostate, brain, therefore we are not able to cure the HIV infection yet. However, it has become a chronic well treatable disease provided the patient is highly adherent to treatment.

Since 2015, antiretroviral therapy has been initiated as soon as possible, immediately after the diagnosis of HIV positivity, regardless of peripheral blood CD4+ T-lymphocyte counts. All necessary biological material is collected prior to initiation [Lundgren 2015]. ART is considered to be successful if the HIV RNA copy number is <20 copies/ml six months after ART initiation.

The current trend in the implementation of new ART is to simplify and optimize treatment modalities. This dwell mainly in simple dosing (usually once per day), a small number of tablers already containing a complete combination of two or three active substances. Good tolerability of new drugs is the prerequisite. The new products influence well even the resistant HIV because they have a high biological threshold (they require many so-called resistance-associated mutations), a high inhibitory quotient (even small amounts of these substances reach high inhibitory concentrations), and a minimum of adverse effects, both clinical and laboratoř [Sedláček 2020].

The preparations are supplied either as monocomponent tablets (**Tab. 3)** or as fixed combinations of several drugs (**Tab. 4**). The development of new ARTs is focused on new targets in the HIV replication cycle in addition to existing ones (**Tab. 5**).

Table 3. Monocomponent preparations

Drug group	**Drug / Medical product**
NRTI/NtRTI Nucleosid/nukleotid reverese transcriptase inhibitors	zidovudine (ZDV), Retrovir 250 mg lamivudine (3TC), Epivir 300 mg emtricitabine (FTC), Emtriva 200 mg abacavir (ABC), Ziagen 600 mg tenofovir (TDF, TAF), Viread 300 mg (TDF)
NNRTI Non-nucleoside reverse transcriptase inhibitors	efavirenz (EFV), Stocrin/Sustiva 200 mg, 600 mg etravirine (ETV), Intelence 200 mg rilpivirine (RPV), Edurant 25 mg; Rekambys 900 mg (3 ml), nanoparticular suspension, i.m.
PI Protease inhibitors	darunavir (DRV), Prezista 300 mg, 600 mg, 800 mg ritonavir (RTV), Norvir 100 mg (inhibitor of CYP 450, currently only a substance potentiating the effect of other PIs)
INSTI Integrase inhibitors	raltegravir (RAL), Isentress 400, 600 mg dolutegravir (DTG), Tivicay 50 mg cabotegravir (CAB), Vocabria 30 mg tbl; 600 mg (3 ml) nanoparticular suspension, i.m.
FI Fusion inhibitors	enfuvirtid (T-20), Fuzeon 90 mg inj. s.c.
EI Entrance inhibitors (antagonists of CCR5 receptor) Antagonist of CD4+(mAb IgG4) Antagonist of gp 120	maravirok (MVC), Celsentri, Selsentry 150 mg, 300 mg ibalizumab (IBA), Trogarzo 200 mg inj. i.v. fostemsavir (Rukobia) 600 mg tbl.

Table 4. Preferred combinations. Fixed-dose (FDR) and Single tablet regimens (STR)

Double combination	**Trade name, dosage**	**Triple Combination**	**Obchodní Trade name, dosage**
zidovudin (300 mg) + lamivudin (150 mg)	např. Combivir 2×1 tbl.	tenofovir DF (300 mg) + emtricitabin (200 mg) + rilpivirin (25 mg)	Eviplera / Complera 1×1 tbl.
abakavir (600 mg) + lamivudin (300 mg)	Kivexa 1×1 tbl.	emtricitabin (200 mg) + rilpivirin (25 mg) + tenofovir AF (25 mg)	Odefsey 1×1 tbl.
tenofovir DF (300 mg) + emtricitabin (200 mg)	Truvada 1×1 tbl.	doravirin (100 mg) + lamivudin (300 mg) + tenofovir DF (300 mg)	Delstrigo 1x1 tbl.
lopinavir (200 mg) + ritonavir (50 mg)	Kaletra 2×2 tbl. (1×4 tbl.)	darunavir (800 mg) + cobicistat (150 mg) + emtricitabin (200 mg) + tenofovir AF (10 mg)	Symtuza 1x1 tbl.
darunavir (800 mg) + cobicistat (150 mg)	Rezolsta/Prezcobix 1×1 tbl.	abacavir (600 mg) + dolutegravir (50 mg) + lamivudin (300 mg)	Triumeq 1×1 tbl.
dolutegravir (50 mg) + rilpivirin (25 mg)	Juluca 1x1 tbl.	elvitegravir (150 mg) + cobicistat (150 mg) + emtricitabin (200 mg) + tenofovir DF (300 mg)	Stribild 1×1 tbl.
emtricitabine (200 mg) + tenofovir AF (10 mg, 25 mg)	Descovy 1×1 tbl.	elvitegravir (150 mg) + cobicistat (150 mg) + emtricitabin (200 mg) + tenofovir AF (10 mg)	Genvoya 1×1 tbl.
dolutegravir (50 mg) + lamivudin (300 mg)	Dovato 1x1 tbl.	bictegravir (50 mg) + emtricitabin (200 mg) + tenofovir AF (25 mg)	Biktarvy 1x1 tbl.

AF – alafenamid fumarate; DF – disoproxil of fumarate

Table 5. New targets, new substances studied (Available online: http://i-base.info/htb/37221)

Substance	Target of action / characteristics
Islatravir (MK-8591, EFDA)	NRTTI, has T/2 120 hours., low dosage once per week, good tolerance
MK-8504 a 8583	NRTI, proléky tenofoviru
bNAbs	broad-acting neutralizing monoclonal antibodies, for prevention and treatment, long T/2, application every 2-6 months, used in combinations
UB-421	CD4, infusion 1-2 weeks, alternative to ART when discontinuing treatment
VRC01 a VRC01LS	CD4, 1 infusion per 1-2 weeks, for treatment even as PrEP. S.c. for newborns
PGT-121	against V3 Env epitope, IgG1
GS-9722 (elipovimab)	against V3 Env, derived from PGT-121
3BNC117	CD4a against V3 Env, long T/2
PGDM1400	CD4, outer area Env, against V1/V2 and other targets
10-1074	CD4 a against V3 Env, long T/2
N6 a N6-LS	gp 120
leronlimab, PRO-140	EI, for CCR5, humanized IgG4, s.c. 1x per week, maintenance monotherapy
GS-6207	capsid inhibitor, acts at two sites of the cycle, s.c. per 6 months
GSK3640254	Maturation inhibitor, T/2 24 hours. p.o. It does not have cross-resistances.
elsulfavirine	NNRTI, prodrug, similar to EFV, Russian license, p.o. 1x1, i.m, sc. per 1 month
Combinectin	FI, acts on gp41 and CD4, sc. 1 x per month
ABX464	rev inhibitor, blocks HIV assembly, even as monotherapy
BIT255	targets are macrophages, adds to ART to reduce reservoirs
GS-9131	NRTI, in combination with other ART

Despite definite successes of ART, PLWHs with advanced disease (CD4+ T cells below 350//μl) [Malstan 2020] are still present and need to be helped to fight against opportunistic pathogens by prophylactic/therapeutic administration of other agents (ATB, antivirals, antifungals, antiparasitic agents, nutritional support, certain growth factors substitution,

hormones or cytokines, etc.). This comprehensive approach significantly improves the quality of life of HIV-positive people and reduces morbidity and mortality [Wandeler 2016].

3.3 Post-exposure and pre-exposure prophylaxis

However, the movement further is the finding that antiretroviral therapy can be used not only to treat HIV infection but also prophylactically. This can be especially used for people at risk, such as healthcare professionals who are providing nursing care. In other words, in case of injury caused by an object that can be contaminated by infected blood, antiretroviral drugs can be given as a preventative treatment for 30 days [Snopková 2019]. This approach is called post-exposure prophylaxis (PEP). Similarly, the method can be used in other high-risk situations such as assault with injury, rape or prevention of vertical transmission. In France, Switzerland, England, and also in Czech Republic PEP is widely available [Henderson 1999, Sedláček 2000, Sultan 2014, Snopková 2019].

Antiretroviral agents can be used for so-called pre-exposure prophylaxis (PrEP) [Eakle 2018, Rodger 2019]. The combination of tenofovir disoproxil fumarate with emtricitabine has reported very high protectiveness in studies with HIV discordant pairs. The preparations are administered either continuously (one tablet daily) or "on demand", as needed (two tablets 2-24 hours before risk intercourse, then one tablet at 24 and 48 hours). If the risk situation persists, it can be continued continuously [Molina 2017]. People administred by PrEP are pr prescribed medication for a maximum of three months. They are regularly screened for anti-HIV, anti-VHC and RPR antibodies, tested for sexually transmitted diseases, check blood count, and liver and kidney functions.

3.4 Nonadherence and selection of resistant HIV-1 strains

Resistance may appear when ART is used incorrectly. Inadequate patient cooperation (treatment non-adherence) is a major source Tab. 6, Fig. 4).

Table 6. Factors influencing patients' adherence to treatment

Causes of impaired adherence	**Reformation pathways**
Complicated medication regimen	Combinations of drugs are administered at the same time
Many medications in multiple daily doses	Single-dose regimens in one daily dose
Alimentary restrictions	Selection of suitable ART components
Impaired tolerance of certain medications	Substitution of intolerable medicines
Frequent travel, time zone changes	Calculation of optimal dosage time, mobile app
Chronic diarrheal diseases, diseases wit impaired drug resorption	Treatment of underlying disease

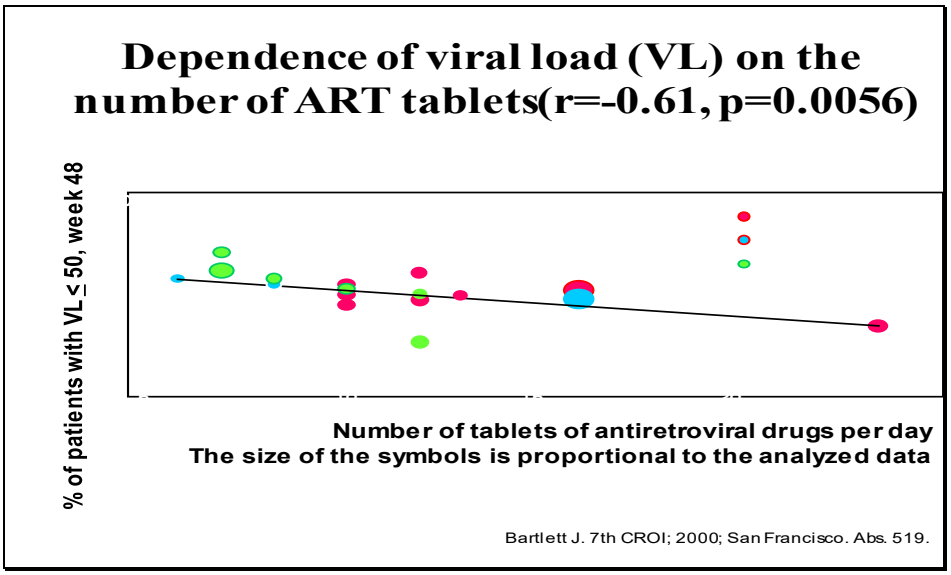

Figure 5. Relationship between viral load and number of ART tablets.

From Fig. 5 is obvious, that the more ART tablets a patient takes, the worse his adherence tends to be. Occasionally, single doses are individually forgotten to take. If the adherence decreases below 95%, resistant strains of HIV may be selected. The emergence of resistance to individual antiretrovirals depends on the number of so-called resistance-associated mutations (RAMs). The development of resistance to different classes of antiretrovirals can be of different paces. The typical manifestation is a worsening of the response to ART, which is initially detected in the laboratory (viremia increase, CD4+ T-cell count decrease) [Lazarini 1999, Bartlett 2000].

3.5 HIV-associated opportunistic infections and tumors, prophylaxis and treatment

In this chapter, we will briefly mention the opportunistic infections (OIs), that led to the discovery of the new AIDS disease in the early 1980s. Nowadays, they are rarely encountered. Early initiation of ART is sufficient to prevent their occurrence. Nevertheless, PLWH is occasionally present in an aggravated state caused by one or more OIs. In general, the more profound the immunodeficiency found in PLWH, the higher the risk of OIs is. However, some cancers associated with HIV infection (Kaposi's sarcoma – HHV-8, primary brain lymphoma - EBV, invasive cervical cancer - papillomavirus) also have a viral etiology.

Primary prophylaxis is indicated in the late stages of HIV infection (CD4+ < 200/μl) when there is a risk of developing opportunistic infections [Machala 1995]. If properly performed, it reduces the risk of their development. After the first attack of opportunistic infection, secondary prophylaxis is indicated in some cases, sometimes for life. This reduces the occurrence of recurrences.

At present, we rarely meet recurrences. The timely initiation of ART is a sufficient prevention of their occurrence. Nevertheless, sometimes PLWH comes in a worsened state, caused by just one or more OIs. In general, the deeper the immunodeficiency found in PLWH, the higher the risk of developing OI. The overview of OI sis depicted in **Tab. 7**.

Table 7. Classification of opportunistic infections according to etiology.

Viral infections
Chronic perianal and genital herpes simplex
Herpetic bronchitis, pneumonia or oesophagitis
CMV retinitis, esophagitis, colitis, encephalitis
Progressive lumbosacral polyradiculopathy
Lymphoid interstitial pneumonitis
Progressive multifocal leukoencephalopathy
Bacterial infections
Recurrent bacterial pneumonia (>2 per year)
Pulmonary and extrapulmonary tuberculosis
Disseminated or extrapulmonary mycobacteriosis
Recurrent salmonella bacteraemia
Protozoal infections
Toxoplasmic encephalitis
Cryptosporidium enteritis
Intestinal microsporidiosis and isosporidiosis
Fungal infections
Pneumocystis pneumonia

Oropharyngeal and vulvovaginal candidiasis, candida esophagitis, bronchitis and pneumonia
Cryptococcal meningitis and other extrapulmonary infections
Disseminated histoplasmosis
Progressive coccidioidomycosis
Aspergillosis
North American blastomycosis
Sporotrichosis, phaeohyphomycosis, zygomycosis, talaromycosis

The risk of OIs development is reduced if primary prophylaxis is properly implemented. After the first attack of opportunistic infection, secondary prophylaxis is indicated in some instances, sometimes lifelong. This limits the occurrence of recurrences **(Tab. 8**).

Table 8. Recommended prophylaxis for selected OIs.

Primary	Secondary
P. jirovecii (CD4+<200/mm^3)	*P. jíroveci*
T. gondii (CD4+<100/mm^3 + specif. IgG)	*T. gondii* – lifelong
M. tuberculosis (PPD>5mm or exposition)	-
M. avium complex (CD4+<50/mm^3)	*M. avium complex* – lifelong
	Cytomegalovirus
	C. neoformans, H. capsulatum, C. immitis

Medications for OI prophylaxis may be discontinued if CD4+ T cell counts are above 200/μl for 3-6 months [Machala 1995, Kovacs 2000].

3.6 PLWH care organizations in the Czech Republic, HIV centers, non-government organizations. Optimizing PLWH care.

Prevention as the foundation of the fight against HIV

Prevention activities together with ART are the most important components of the fight against HIV/AIDS. In the Czech Republic, workplaces caring for the first AIDS patients began to be built in the 1980s, following foreign models. The first AIDS center was established at the Prague Bulovka Hospital in 1986. In the next years, identical centers were gradually established in six regional cities to provide comprehensive care for PLWH and to address prevention issues in the broadest sense of the meaning. Currently, eight HIV centers are functional (there are two centers in Prague) and another center in Liberec is almost ready. In addition to these facilities, there exists a several number of low-threshold centres and other NGOs that have become involved in the care of PLWH. An important role plays the Czech AIDS Help Society, established in Prague at the Light House, where, in addition to general care, accommodation and other social assistance can be provided for PLWH who find themselves in life distress. At present, emphasis is also placed on the importance of ART in prevention. International studies have shown a significant reduction in the risk of HIV infection in so-called serodiskordant couples (one partner from the pair is HIV positive, the other is not). The shift in treatment effectiveness is currently so significant that PLHFs with undetected viral loads are considered non-infectious. This is expressed by the symbols „U=U“ (Undetectable= Untransmittable) [Bor 2021].

PLWH care optimalisation model

1. Positive screening test for anti-HIV antibodies and confirmed HIV+ result is communicated to the patient.
2. Epidemiological investigation with a focus on contact deprivation.
3. Taking care in one of 8 HIV centers (often catchment area based on the place of residence for easier access)
4. Laboratory, imaging and clinical tests for optimal selection of ART
5. Early therapy initiation
6. Regular clinical and laboratory follow-ups (initially after three months, after achieving stable laboratory parameters after six months)
7. The patient's adherence to treatment control, determination of the next follow-up (retention), and answering any questions. Emphasis on healthy lifestyle, sport, need for psychosocial or economic support
8. Primary and secondary prophylaxis, treatment of opportunistic infections and tumors, co-infections, comorbidities, autoimmune, HIV-associated diseases in PLWH with advanced immunodeficiency
9. Cooperation with non-government organizations when optimizing and providing comprehensive care of PLWH [Hofman 2022].

4. Cohort of monitored persons, methodologies and statistical analysis.

The study was carried out in the years 2010-2020 in the HIV center (formerly the AIDS center) of the Clinic of Infectious Diseases and Travel Medicine of the Faculty of Medicine of the University of the Czech Republic and the Faculty of Medicine in Pilsen (KINCM), which was formally established in December 1993. Since 1995, it has performed the function of a workplace providing comprehensive care of PLWH in the Pilsen and Karlovy Vary regions. For this purpose, an ever-updating team of counselors and other collaborators from other workplaces of the FN Plzeň was established in the past.

Cohort of PLWH monitored in the HIV center of the FN Plzeň

By December, 31st 2021, 265 HIV-positive persons were registered in the Pilsen and Karlovy Vary Regions, of which 66 have already met the criteria of AIDS classification. Approximately 200 people are included in the long-term dispensary care program. By the end of 2021, 142 people were treated with ART in the HIV center of the FN Plzeň.

A total of 151 PLWH were selected for the purposes of our study. Inquiries on ART adherence status are conducted continuously for all PLWH at each follow-up. Subjects were continuously selected for our study when insufficient ART adherence was suspected, manifesting either clinically or laboratoryally. Those who either self-admitted to insufficient adherence, or had repeatedly demonstrated a decrease in CD4+ T lymphocytes, or a worsening of virological findings compared to the previous check-up, were selected primarily.

Study design

The study was designed as prospective, controlled, and open. PLWH were enrolled continuously according to detected clinical and laboratory signs of nonadherence, or when it was suspected (with an undetected level of monitored NRTIs in the urine).

The control group consisted of adherent subjects with detectable but low urinary NRTI levels. The data was anonymized from the beginning by removing the final part of the social security number. Consultation with the Ethics Committee of the FN and LF UK in Pilsen revealed that the informed consent of the probands is not necessary in this case.

ART adherence control methods used in other studies

1. Direct control of the number of unused tablets at every three-month check-up. There are also electronic closures of medicine bottles, recording every meeting [Nachega 2011]. This method is very time-consuming and rarely applicable in practice. Differences in patient data are often very different from reality. This method of adherence control was used in our study.

2. Regular monitoring of clinical and laboratory response to ART treatment (numbers of CD4+ T lymphocytes, HIV-viremia, markers of inflammation, elevation of selected

laboratory markers, considered a side effect of the respective treatment). This is an indirect method, confirming the long-term trend of treatment with ART, which is used most often. However, it cannot capture the temporary/transient deterioration of laboratory parameters, and in addition, ART of higher generations usually have very little expressed side effects of treatment, both clinical and laboratory.

3. Objective measurement of drug concentrations in blood or urine. PLWH are instructed since initiation on ART about the need to regularly take medications at the same time to maintain effective blood levels of medications. Blood concentrations of antiretrovirals should therefore be constant in the long term. This prevents the multiplication of more resistant variants of HIV. Drug concentrations can be determined in various biological materials, but blood plasma or serum is most often used. For laboratory processing, we chose the determination of ART concentrations in urine (cm) as a safer alternative.

For the needs of our workplace, an original methodology for the determination of NRTIs in urine using HPLC was developed more than 20 years ago at the Department of Clinical Pharmacology of the FN Plzeň [Stehlík 2000, Sedláček 2001]. We focused on determining substances that are a regular part of antiretroviral combinations, i.e. AZT, 3TC and FTC.

Properties of analytes suitable for determination by HPLC

Zidovudine (AZT) is resorbed in the digestive tract by 60-70% after oral administration. The highest concentrations are detected in the blood in 0.5-1.5 hours. In the liver, up to 80% of AZT binds with glucuronic acid to form an inactive metabolite, glucuronide (5gAZT), and 15-25% of AZT is excreted unchanged in the urine by tubular excretion [Retrovir SUKL 2022].

2. Lamivudine (3TC) is well absorbed from the gastrointestinal tract. Its bioavailability after oral administration is 80-85% in adults. The average time (tmax) to reach maximum blood serum concentrations (cmax) after oral administration is around one hour. The mean volume of distribution after intravenous administration of 3TC is 1.3 L/kg. The plasma half-life of 3TC after oral administration is 18 to 19 hours. 3TC is eliminated mainly by renal excretion in unchanged form [Epivir SUKL 2021].

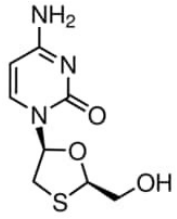

Figure 5. Lamivudine (Epivir, 3TC, Zeffix),
$C_8H_{11}N_3O_3S$, **Molecular Weight**: 229.26 g·mol^{-1}

3. **Emtricitabine** (FTC) (Fig. 6) is rapidly and extensively absorbed after oral administration. Peak plasma concentrations are reached approximately 1 to 2 hours after dosing. After a dose of 200 mg of FTC in the form of hard capsules once daily, the steady-state peak plasma concentration (cmax) was 1.8 ± 0.7 μg/ml, the trough concentration (cmin) was 0.09 ± 0.07 μg/ml. Absolute bioavailability p.o. of the FTC form is estimated at 93%. The volume of distribution after intravenous administration of FTC was 1.4 ± 0.3 L/kg, thus FTC is widely distributed in both cellular and extracellular fluids. The metabolism of emtricitabine is limited. Biotransformation produces the 3'-sulfoxide diastereoisomer (approximately 9% of the dose). Conjugation with glucuronic acid produces 2'-O-glucuronide (approximately 4% of the dose). FTC is primarily excreted by the kidneys (approximately 86%) and feces (approximately 14%). Thirteen percent of the FTC dose appeared in the urine as three metabolites. After oral administration, the elimination half-life of FTC is approximately 10 hours [Emtriva EMA 2008].

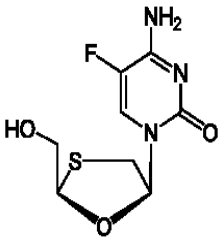

Figure 6. Emtricitabine, FTC (Emtriva)
$C_8H_{10}FN_3O_3S$. **Molecular Weight:** 247.24 g·mol^{-1}

4.1 Determining drugs from the NRTI group seems to be the easiest, but working out a setting up the method for the determination of these substances took quite a lot of time. Pure standards were obtained as a gift from the manufacturers or purchased from Sigma.

Since single-tablet regimens (STRs) have been in use for nearly a decade, investigations have become much simpler. The determination of one analyte from the relevant STR combination provides us with a complete picture of drug use. The concentrations of the above-mentioned substances in urine vary in mg/l, therefore we determined the levels of the above-mentioned analytes chromatographically using HPLC. The principles of HPLC had been described elsewhere [Hedaya 1988, Lacroix 1990, Molema 1992, Schrive 1994]. Determination of AZT, d4T, 3TC and FTC by high performance liquid chromatography - analytical method is reversed-phase HPLC [column Supelco (Bellefonte, PA, USA) LC-ABZ 250x4.6 mm; 5 um particle size] with UV detection (274 nm). The analyses were performed on a component system consisting of a Module SP 8770 pump, UV 2000 detector, SP 8875 autosampler and SP 4100 integrator (Spectra Physics, San Jose, CA, USA) (**Fig. 7).** All models were connected with system LabNet.

Figure 7. HPLC device, company assembly **Spectra Physics**. San Jose, CA, USA

During the implementation of the methodology, a large number of urine samples were tested regardless of CD4+ T cell count results and viremia. This made it possible to optimize it and find out the range of urinary concentrations during the correct use of ART. These findings allowed us to estimate the possible duration of ART withdrawal. The range of NRTI concentration values for the purposes of our determination was set to 0-7693 mg/l. For the purposes of the study, urine samples were collected in the morning, no later than five hours after taking the morning dose of medication. Individual patients were examined 1 to 11 times as needed. Urine samples were stored frozen at -80°C and processed in batches.

4.2 The HIV RNA PCR investigation takes place at the NRL for AIDS Prague.

Tests and an instrument system from the company **Roche Cobas 4800 (Fig. 8**) are used to examine the viral load. These are two devices connected by a computer, labeled x480 and z480. The X480 provides automatic extraction of nucleic acids and mixing of PCR reaction mixtures. The result is a plate that is manually transferred to the z480, which is a real-time PCR thermocycler. **An Applied Biosystem 3500 Genetic Analyzer Sequencer** (Fig. 9) is used for RNA sequencing to determine HIV subtypes and resistance-associated RNA mutations (RAMs).

Viral load (VL) test results may be as follows:

a. HIV-1 RNA not detected, i.e. simply 0 c/ml

b. < 20 c/ml, i.e. HIV-1 RNA was captured, but the value is out of linearity dependence and cannot be precisely determined

c. 20 to 10e7 c/ml, i.e. a specific VL value is determined

d. above 10e7 c/ml is again outside the linear dependence and the exact value cannot be determined.

Figure 8. Roche: Cobas 4800

Figure 9. Sequencer **Applied Biosystem 3500 Genetic Analyzer**

4.3 Determination of lymphocyte subpopulations using a flow cytometer (FACS) Navios (Beckmann Coulter, USA), (Fig. 10). The examination is carried out at the Institute of Immunology and Allergology of the Faculty of Medicine in Pilsen. Kits for the

determination of lymphocyte surface features TBNK +HLA-DR 5T Pack are a product of Exbio Praha a.s.

Figure 10. Flow cytometer **Navios (Beckmann Coulter, USA).**

Routine hematological and biochemical examinations. These examinations may in some cases, due to certain side effects of drugs, indirectly point to the long-term use of ART. However, we did not use them for the purposes of our study.

4.4 Standardized questionnaire survey. In this case was used the modified RAND 36 - Item Health Survey (SF - 36) questionnaire (Tab. 9). Similar processes can objectively evaluate the level of the ART adherence in PLWH, and thus the efficiency of the whole curative process.

Table 9. Items of modified Health Survey (SF - 36) questionnaire

1) Gender: MALE - FEMALE

2) Year of birth: ……

3) **You live in the:**

Municipality up to 10,000 inhabitants ……1

City over 10,000 to 50,000 inhabitants ……...2

City over 50,000 to 100,000 inhabitants……. 3

Large city with over 100,000 inhabitants ……4

4) **Your completed education:**

Unfinished primary ……. 1

Finished primary.…….…….2

High school without high school diploma ……...3

High school with high school diploma ……4

Higher education at a college or university. ……5

5) Do you sufficiently understand the information given to you during treatment (examination results, information on health status, etc.) in the medical facility: YES - NO

6) Did finding out your diagnosis have any effect on the normal course of your life?

YES - NO If YES, which one?

7) Please assess your overall health before determining your diagnosis: *(check the appropriate box)*

1	**2**	**3**	**4**	**5**

8) Please evaluate your current state of health overall

1	**2**	**3**	**4**	**5**

9) Please evaluate your overall mental well-being before determining your diagnosis

1	**2**	**3**	**4**	**5**

10) Please evaluate your current mental state overall

1	2	3	4	5

1 significantly below average
2 slightly below average
3 average
4 slightly above average
5 significantly above average (excellent)

11) Does your current treatment limit or complicate your daily activities or normal life in any way? YES NO If yes, how?

12) THE IMPORTANCE you attach to the areas listed here in your life:

	absolutely necessary	very important	Moderately important	Not important	insignificant
be physically self-sufficient					
sleep well					
family relationships					
to love and be loved					
have hobbies in free time					

13) SATISFACTION in the following areas of your life:

	completely satisfied	very satisfied	satisfied	dissatisfied	very disappointed
physical self-sufficiency					
sleep					
Family relations					
love					
hobby					

Patients´ clinical data were obtained from patients´ files. The data were then blinded.

4.5 Statistics. The data were statistically processed with the methods as followed: SAS, V. 9.4 (SAS Institute Inc., Cary, NC, USA) was used for all statistical analyses. Basic characteristics are expressed as means, medians, minimum and maximum where appropriate. The nonparametric Wilcoxon Two Sample test was used to assess the differencies between two group of patients. The cut-off level was defined as the 95% percentile. These 95% percentiles were calculated by the SAS proc univariate default method and the observation numbered closest to the number of non-missing results multiplied by p (where p = 0.95 in our case). Box and wisker plots were used to the graphical statistical displays. Graphs were performed using Statistica SW.

Ethical Commission approval. The joint Ethical Commission of the Faculty of Medicine of Charles University and the Faculty Hospital in Pilsen was informed in detail about the study during the preparation in 2010, and due to the fact that the initial anonymization of the data was carried out, it was decided that the submission of the project for approval to conduct the study, nor the informed consent of the probands are not mandatory.

4. The results of the work and newly discovered facts

The patient cohort consisted of 151 PLWH, of whom 35 (23.1%) were women (Tab. 10). Nineteen persons (12.6%) had university education. There were 32 foreigners (21.2%), (12 came from Ukraine, 6 from Vietnam, 2 each from Bulgaria, Kenya, Poland and Russia, and one each from Malaysia, Georgia, Romania, Slovakia, Slovenia, and Thailand).

Table 10. Demographic and clinical characteristics (*n = 151*)

Parameter	
Age (years) [median (min, max)]	41 (21– 71)
Female gender [n (%)]	35 (23,1)
HIV history	
Duration of known HIV infection (years) [median (IQR)]	8 (3– 14)
Mode of acquisition [n (%)]	
MSM	92 (61,0)
Heterosexual	39 (25,8)
Injecting drug use	18 (11,9)
Mother- to- child transmission	0
Nosocomial (from the country of the origin)	2 (1,3)
Total samples collected	409
Total samples examined (1-11 of each person)	404
Examined AZT	5
Examined 3TC	127
Examined FTC	272
Median 3TC concentration in urine (mg/l), (min, max)	75,55 (2,2; 1840,6)
Median FTC concentration in urine (mg/l), (min, max)	34,5 (0,2; 397,7)
Range of all NRTI concentrations in urine (mg/l)	0,2 – 1840,6
Comorbidities [n (%)]	
Chronic cardiovascular disease	33 (21,9)
Chronic neuropsychiatric disease	8 (5,3)
Diabetes mellitus	4 (3,3)
Chronic pulmonary disease	3 (2,0)
Active malignancy	4 (3,3)
Systemic autoimmune disease	1 (0,7)
Chronic renal disease	1 (0,7)
Dyslipidaemia	28 (18,5)
Chronic hepatic disease (cirrhosis)	1 (0,7)
Coinfections [n (%)]	
Chronic HCV Infection	11 (7,3)
Chronic HBV Infection	13 (8,6)
Chronic HBV and HCV Infection	4 (3,3)
Tuberculosis	0
Syphilis	2 (1,3)
Death	4 (3,3)

Abbreviations: 3TC, lamivudine; AZT, zidovudine; FTC, emtricitibine; HBV, hepatitis B virus; HCV, hepatitis C virus; IQR, interquartile range; MSM, men who have sex with men

The average age of PLWH of the core group was 41.7 +- 9.6 years, median 41 years. At the time of the examination, the youngest person was 21 years old and the oldest one 71 years old.

An overview of drugs used at the time of examination of NRTIs in urine in non-adherent patients and in the control group is given in **Table 11**.

Table 11. Treatment characteristics of analyzed group of PLWH (***n* = 38)**
ART characteristics based on third agent

ART	**Non-andherent (n=18)**	**Controll group (n=20)**
INSTI	8	10
NNRTI	4	5
PI	6	5
Other regimens	0	0
n of patients receiving:		
-NRTIs		
Lamivudine/emtricitabine	18	20
TDF	11	3
TAF	2	14
Either TDF or TAF	13	17
Abacavir	5	3
-INSTIs		
Dolutegravir	2	4
Raltegravir	1	1
Elvitegravir	5	4
Bictegravir	0	1
-NNRTIs		
Efavirenz	1	0
Rilpivirine	2	5
Nevirapine	1	0
-Pis		
Ritonavir-or cobicistat-boosted darunavir	3	4
Ritonavir-boosted lopinavir	3	0
Ritonavir-boosted atazanavir	0	1

NRTIs – nucleoside reverse transcriptase inhibitors, **INSTIs –** integrase strand transfer inhibitors, **NNRTIs** – non-nucleoside reverse transcriptase inhibitors, **PIs -** protease inhibitors

<u>Objective 1:</u> Determination of urine levels of selected antiretroviral drugs (ART) to objectify adherence control. Monitoring ART adherence in a cohort of people living with HIV (PLWH).

Two groups of patients were selected from the basic cohort. The first group included persons with a high suspicion of non-adherence who had zero levels of nucleoside analogues in their urine at the time of the examination. The second group consisted of people with low but not zero levels of NRTIs in their urine. Both groups of probands were analyzed in parallel with regard to clinical status, immunological and virological indicators. (Tab. 12, Tab. 13).

In addition to anamnestic data, the inclusion in the study group was guided by the VL, CD4+, CD8+ values from the control examination, preceding the urine collection for the examination of nucleoside levels (marked VL-1, CD4 -1, CD8 -1). In each patient from both groups, the values of VL, CD4+, CD8+ were also recorded on the day of urine collection for nucleoside examination and the same was done at the next check-up in 3-6 months (marked VL+1, CD4 +1, CD8 +1). The data were processed using statistical methods suitable for sets of small numbers.

The clinical courses of the disease in both monitored groups were also compared. We were interested in whether at least the minimal level of ART in the urine can correlate well with the clinical status and adherence reported by the patient.

Table 12. Non-adherent group (n=18). Data of VL, CD4+ and CD8+ lymphocytes T.

	Mean +- SD	**Median**	**Min; Max**
VL	58851,28 +- 244881,46	19	19; 1040000
VL -1	53157,50 +- 223575,08	38	19; 949000

VL +1	36564,67 +- 108048,37	19	19; 403000
CD4+	484,44 +- 267,94	515	110; 960
CD4+ -1	504,44 +- 299,93	470	50; 1050
CD4+ +1	540 +- 325,32	535	50; 1400
CD8+	704,61 +- 322,85	670	153; 1380
CD8+ -1	818,89 +- 482,50	720	140; 1690
CD8+ +1	832,78 +- 331,39	785	330; 1470

VL viral load (c/ml), **CD4+** lypmphocyte T (/µl), **CD8+** lymphocyte T (/µl) (VL -1, CD4+ -1, CD8+ -1: results of previous exam on 3-6 months ago, VL +1, CD4+ +1, CD8+ +1 results of the next exam 3-6 months after the urine sample testing).

Table 13. Adherent group (n=20). Data of VL, CD4+ and CD8+ lymphocytes T.

	Mean +- SD	**Median**	**Min; Max**
VL	32,05 +- 41,49	19	19; 196
VL -1	381,10 +- 1579,28	19	19; 7090
VL +1	22,11 +- 7,77	19	19; 47
CD4+	767,00 +- 420,80	800	130; 13300
CD4+ -1	717,05 +- 380,52	676	50; 1320
CD4+ +1	844,74 +- 469,21	800	130; 1680
CD8+	807,80 +- 369,18	715	256; 1480
CD8+ -1	770,89 +- 347,50	730	230; 1430
CD8+ +1	840,74 +- 362,77	830	214; 1520

VL viral load (c/ml), **CD4+** lypmphocyte T (/µl), **CD8+** lymphocyte T (/µl) (VL -1, CD4+ -1, CD8+ -1: results of previous exam on 3-6 months ago, VL +1, CD4+ +1, CD8+ +1 results of the next exam 3-6 months after the urine sample testing).

In 2010-2020, a total of 404 urine tests for the presence of AZT, 3TC or FTC (depending on the type used ART) were performed in 151 PLWH of the HIV Center of the University Hospital in Pilsen. Urine was sampled in 2-6 hours after taking the morning dose of medication. Each person underwent 1-11 examinations as needed during the reporting period. In 358 urine samples obtained from 129 patients, the monitored drug was detected, therefore these patients were evaluated as adherent to treatment even with regard to the favorable findings of VL and CD4+ T-lymphocytes (Tab. 14).

Table 14. Evaluation of the entire group of patients (n=151)

	Urine samples	**Patients**	**note**
Total	**404**	**151**	
Positive (TP)	**358**	**129**	
Negative (TN)	**23**	**18**	
Incorrect collection	**23**	**4**	**15 persons of all samples**
Overall adherence	**93,0 %**	**87,8 %**	

In 23 samples (4x AZT, 14x FTC, 3x d4T and 2x 3TC) the expected drug was not demonstrated, as an examination for the wrong drug was requested. This set served as a control to verify the reliability of the methodology. In 23 samples (1x AZT, 15x FTC and 7x 3TC) obtained from 18 PLWH the expected drug was not detected in urine. These patients were evaluated as non-adherent for the day the examination was performed. In these persons, the values of VL, CD4+ and CD8+ lymphocytes T were subsequently compared on the day of the determination of the drug in the urine, and at the previous check-up, i.e. 3-6 months

ago and 3-6 months after. The data are in The average adherence rate in the whole group was 93,0%. Total patients´ adherence was 87,8%.

We found that the majority of our patients (87.8%) had fairly good, but from the point of view of current requirements (i.e. 95%), insufficient treatment adherence during the monitored period. AZT levels in urine 2-6 hours after taking the morning dose of the drug ranged from 0 to 177 mg/l, 3TC and FTC levels ranged from 0-1840.6 mg/l, respectively. 0-397.7 mg/l.

In the first group (18 people with zero urinary NRTI levels), the development of VL, CD4 and CD8 was monitored 3-6 months before and 3-6 months after the nonadherent episode.

In the second group (20 people with low but not zero levels of NRTIs in urine, serving as a control group), the development of VL, CD4 and CD8 was also monitored during regular clinical check-ups 3-6 months before and 3-6 months after urine collection for level examination NRTI.

Comparison of both groups of patients:

1. In the group with zero adherence (antiretroviral levels not measurable), **a statistically significantly higher viral load** was found compared to the group with at least partial adherence (P=0.046) (Fig. 11). In this case, the null hypothesis (H_0) can be rejected at the specified 5% level of significance.
2. The viral load determined 3-6 months before the urine collection for testing the levels of antiretroviral drugs is not statistically significantly higher in the group with zero adherence (P=0.056). Using a set with a larger number of probands, statistical significance could be demonstrated (**Fig. 12**).

3. Viral load determined 3-6 months after urine collection for examination of antiretroviral levels is not statistically significantly higher in the group with zero adherence (P=0.139) (**Fig. 13)**.
4. The absolute numbers of CD4+ T lymphocytes determined on the day of urine collection for testing antiretroviral levels are not statistically significantly lower in the group with zero adherence (P=0.059). Using a set with a larger number of probands, statistical significance could be demonstrated (**Fig. 14**).
5. The absolute numbers of CD4+ T lymphocytes determined 3-6 months before the urine collection for the examination of antiretroviral levels are not statistically significantly lower in the group with zero adherence (P=0.120).
6. The absolute numbers of CD4+ T lymphocytes determined 3-6 months after urine collection for testing antiretroviral levels are not statistically significantly lower in the group with zero adherence (P=0.070). Using a set with a larger number of probands, statistical significance could be demonstrated.
7. The absolute numbers of CD8+ T lymphocytes determined on the day of urine collection for the examination of antiretroviral levels are not statistically significantly lower in the group with zero adherence (P=0.592) (**Fig. 15**).
8. The absolute numbers of CD8+ T lymphocytes determined 3-6 months before urine collection for the examination of antiretroviral drug levels are not statistically significantly lower in the group with zero adherence (P=0.940).
9. The absolute numbers of CD8+ T lymphocytes determined 3-6 months after urine collection for the examination of antiretroviral drug levels are not statistically significantly lower in the group with zero adherence (P=0.916).

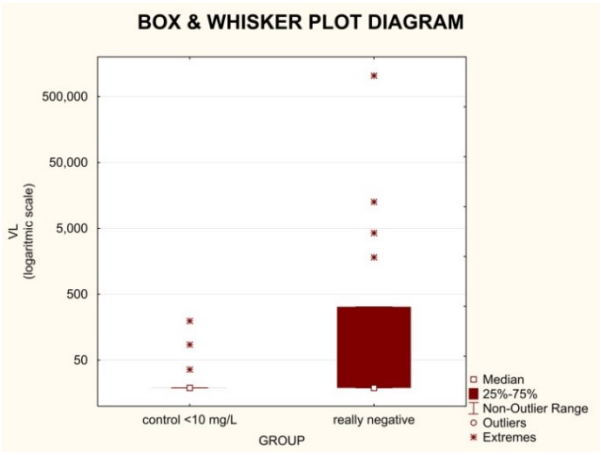

Figure 11. VL (c/ml) at the time of detection of NRTIs in urine samples.

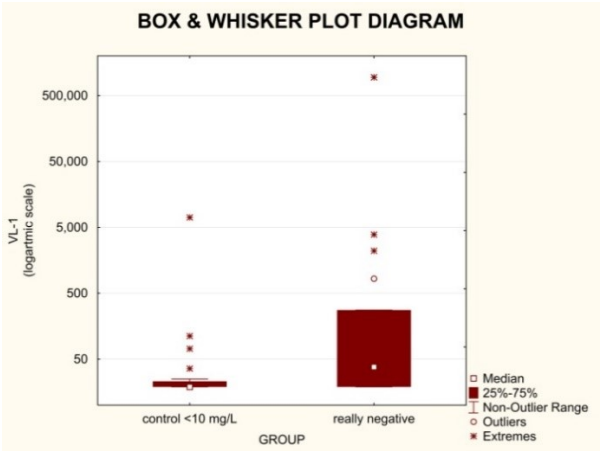

Figure 12. VL (c/ml) at 3-6 months before the detection of NRTIs in urine samples.

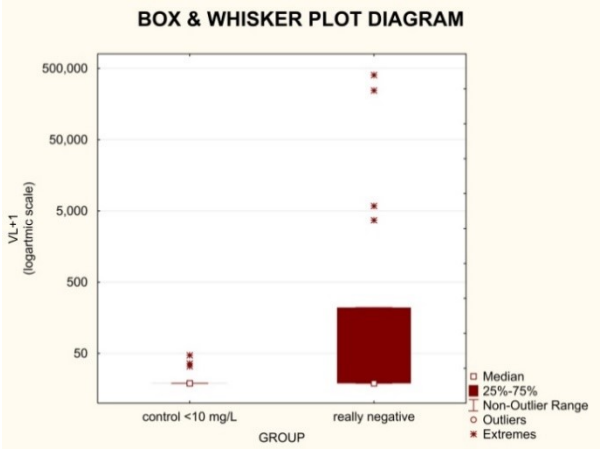

Figure 13. VL (c/ml) at 3-6 months after the detection of NRTIs in urine samples.

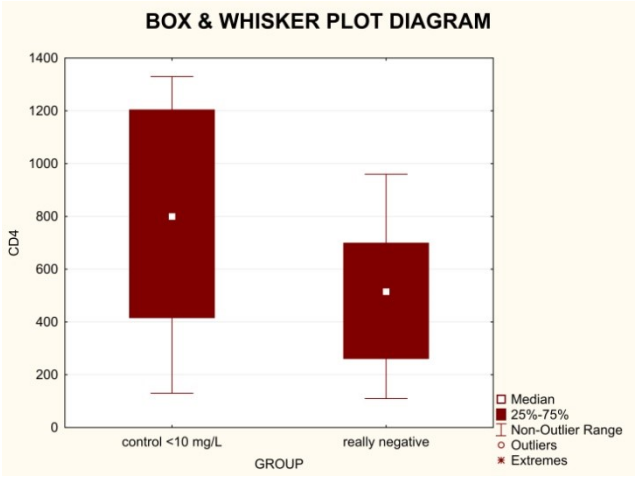

Figure 14. CD4 lymphocytes T (in µl) at the time of detection of NRTIs in urine samples.

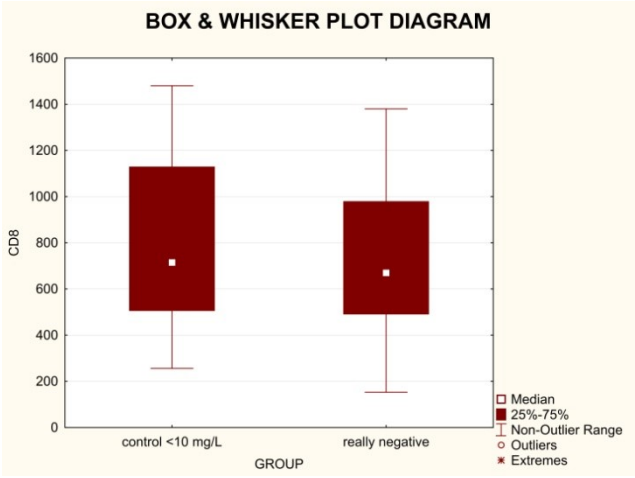

Figure 15. CD8 lymphocytes T (in µl) at the time of detection of NRTIs in urine samples.

<u>Objective 2</u>: Survey evaluation on the adherence to the antiretroviral treatment

For 43 PLWH of the core group, a questionnaire survey was conducted using a partially modified RAND 36 - Item Health Survey (SF-36) questionnaire, and the obtained data were compared with the results of the examination of biological material, taken with the consent of all respondents. It was thus possible to roughly compare treatment adherence in individual

patients and at the same time evaluate it in the context of the perception of quality of life by individual PLWH. Individual respondents had to mark their subjective perception of the importance and importance of selected areas of life on a scale from 1 to 5 (1 = very unimportant/very bad, 5 = very important/very good). Subsequently, using the same point scale (1 – 5), they subjectively evaluated what real results in selected areas of quality of life they actually achieve in their opinion. These data were then analyzed for both adherent and non-adherent patients and compared to each other. The PLWH are represented across the population, even in terms of education [SČÍTÁNÍ 2021]. We were therefore interested in whether our set of PLWH corresponds to national data on educational attainment.

Table 15. The highest achieved education of inhabitants of the Czech Republic compared with PLWH of the studied group.

acquired education	% portion of the population of the Czech Republic	% proportion of PLWH included in the research survey
unknown	5,8 %	-
Without education	0,6 %	0 %
basic education	12,5 %	16,28 %
High school without high school diploma	31 %	39,54 %
High school with high school diploma	32,5 %	30,23 %
Higher education	17,6 %	13,95 %

The distribution of PLWH included in the survey was very close to the percentage distribution of the population of the Czech Republic by education (Tab. 15).

By processing questionnaires evaluating various aspects of the quality of life of PLWH, we were also able to assess the subjective perception of the entire issue by individual patients, including their level of treatment adherence. Quite expected was the finding that high-quality care is of great importance in inducing a high degree of trust and thus adherence, and thus a positive impact on the quality of life of PLWH. Minimizing concerns related to comorbidities, opportunistic infections and their prevention also has a direct effect on

treatment adherence. Adherent patients reported an overall higher quality of life and higher satisfaction in individual monitored areas. The assumption that adherent patients are more balanced (achieving smaller variances) in assessing the importance of selected areas of their lives as well as their subjective achievement or fulfillment was confirmed. E.g. physical self-sufficiency is considered significant by both adherent and non-adherent patients at the level of 4 points. According to subjective perception, adherent patients achieve it on average from ¾, non-adherent from ½ (Fig. 16).

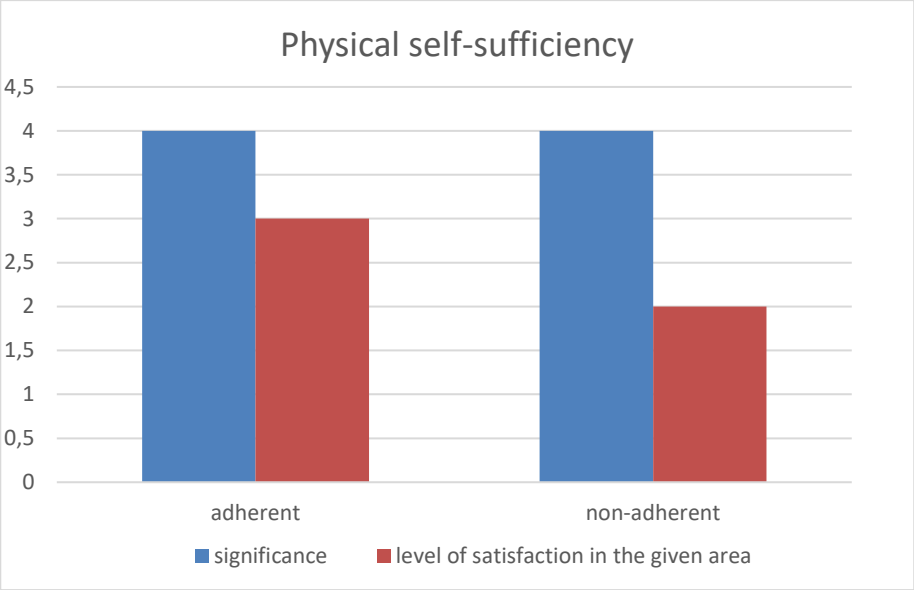

Figure 16. Physical self-sufficiency. Evaluation of the QoL questionnaire.

Adherent patients rate the importance of quality sleep an average of 4 points. It is absolutely essential for non-adherent PLWH (5 points). Adherent patients achieve an average of 3 out of 4 points in his subjective evaluation and filling of the given area, non-adherent patients achieve only 2 out of 5 points (Fig. 17). It can be seen from both previous data that some health parameters are perceived worse by non-adherent patients than by adherent ones.

Figure 17. Quality sleep. Evaluation of the QoL questionnaire.

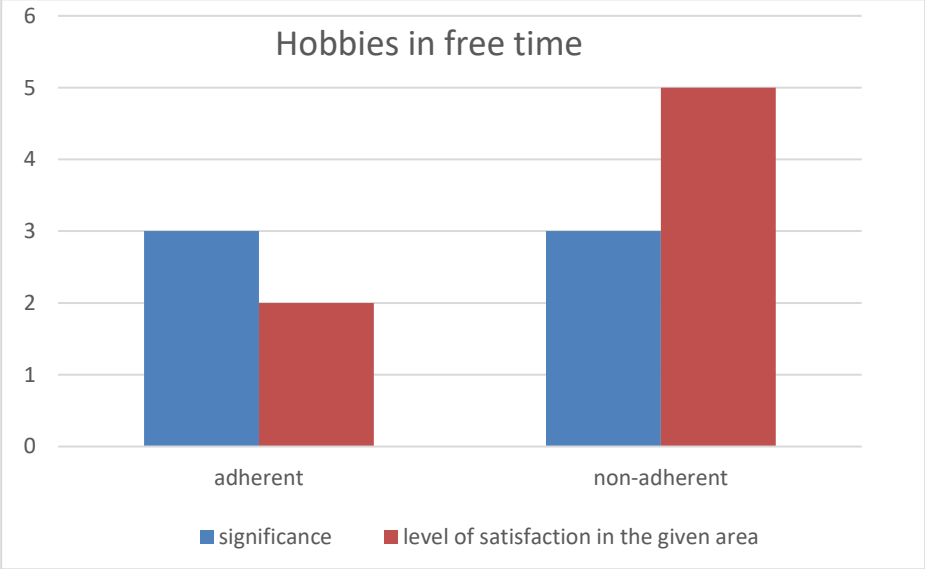

Figure 18. Hobbies in free time. Evaluation of the QoL questionnaire.

Leisure activities and hobbies are significant for both groups of patients at the level of 3 out of 5 possible points. However, the adherent patients of our group were not very satisfied in this area (2 points), in contrast to the non-adherent patients, who were very satisfied (5 points) (Fig. 18). According to their subjective assessment, they find more use in leisure activities than they consider necessary. This phenomenon may be related to specific

personality traits or a more casual approach of some PLWH to their life, and a less formal solution to their real problems.

Both groups of patients consider the area of love to be very important (5 points). Adherent patients then subjectively fulfill this area and are satisfied in it (4 points), compared to non-adherent patients who are dissatisfied in this area (on average they reach 2 points) (Fig. 19).

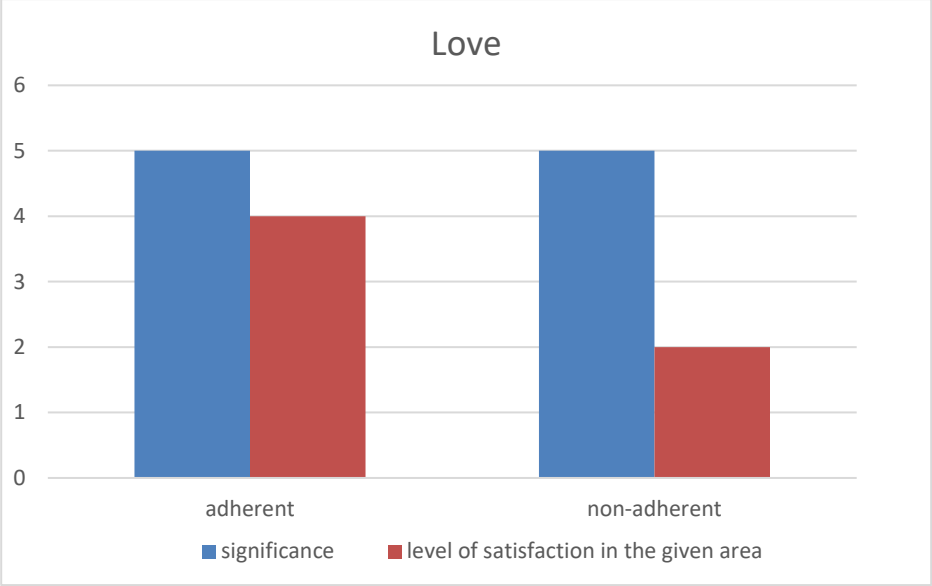

Figure 19. Love. Evaluation of the QoL questionnaire.

Both groups consider good family relationships to be important from the point of view of quality of life (Fig. 20), to which they attach a significance of 4 points. Adherent patients rate fulfillment in this area 3 points, non-adherent patients only 2 points, therefore they are in this area dissatisfied.

Similar differences were demonstrated in both adherent and non-adherent patients in other areas such as work, diet, self-care ability and others.

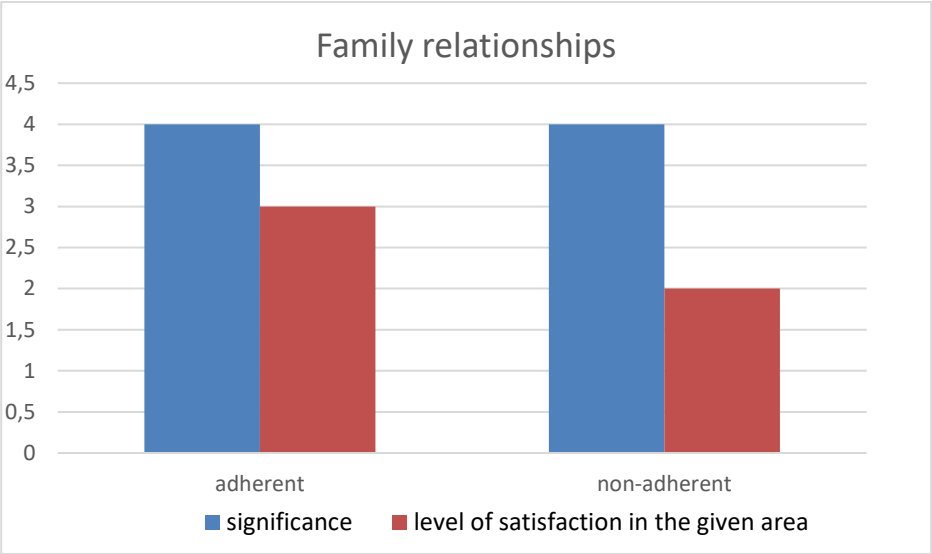

Figure 20. Family relationships. Evaluation of the QoL questionnaire.

Almost identical results in both groups of PLWH were also demonstrated in other monitored categories such as: raising children, pleasant living environment and housing, perception of one's own health and current state of health.

A persistent problem that PLWH still encounter is their rejection, for example, in connection with the provision of health care. Even after 40 years of addressing the issue of HIV/AIDS, there is a certain misunderstanding that can cause a feeling of stigmatization, in some cases a disadvantage for PLWH and thus a reduction in their quality of life. This can then be reflected in the adherence to the treatment itself [Chesney 2000, Frei 2014, Gurková 2011].

Furthermore, it was shown that social conditions have an influence on the subjective perception of the quality of life in both groups of PLWH, and thus also on adherence. Of the monitored specific indicators, this was particularly the case with the financial situation and material security.

Unexpectedly, it was found in our two sets that the level of education, partnership or marital status and employment do not have a significant effect on the subjective perception of the quality of life of PLWH.

We found that the overall subjective perception of the quality of life of non-adherent patients is lower than the subjective perception of the quality of life of adherent patients. That is, the quality of life can influence the level of adherence of PLWH. The very diagnosis

of HIV positivity is a significant stressor for PLWH. As expected, the psychological well-being of PLWH before the diagnosis of HIV infection was better than during the subsequent treatment, despite its maximum quality, availability and adherence.

Objective 3: Monitoring the emergence of HIV resistance over a longer period of time.

In the core group of 151 PLWH, we registered the development of resistance in 17 people during the 10-year follow-up (Tab. 16). In 6 people, resistance to some group of ART was already detected before the start of treatment, therefore, it could not be related to the treatment. In the remaining 11 persons, the connection of the development of resistance against some groups of ART due to non-adherence cannot be completely excluded. It is favorable that in this group only two people were repeatedly non-adherent.

Table 16. Proven HIV resistance to ART (n=17)

ART groups	PLWH with confirmed resistance
NRTI	9
NNRTI	10
PI	6
INSTI	2

The emergence of resistance can play a major role in treatment failure. Resistance often arises as a result of insufficient suppression of HIV replication, where more resistant HIV strains are selected due to subinhibitory levels of ART. Another reason for treatment failure may be the omission or incorrect use of medication. Here, although the selection of more resistant variants does not occur to a greater extent, the treatment results are unsatisfactory, as there is an increase in the viral load and a decrease in CD4+ T lymphocytes. The development of resistance will also be monitored in the future and will be the subject of further research.

6. Discussion and conclusions for practice

The aim of this dissertation has been to optimize the treatment approach in PLWH and point out on improoving of QoL using new trends of treatment strategies with an emphasis on ART-adherence. The trend in recent years has been to maximize the effectiveness of HIV treatment and to optimize it in order to be well tolerated by patients. Therefore, it is necessary to create simple therapeutic procedures which take this trend into account, and simultaneously are optimally aligned with current knowledge and possibilities in the field of antiretroviral therapy. If complete therapy in PLWH is simple and acceptable, it is better tolerated and observed, so the overall adherence increases. At the same time, the overall adherence can be relatively easily checked, for example by monitoring the levels of therapeutic substances in the patient´s body fluids.

The issue of HIV/AIDS is a very large area and it includes not only medicine and nursing, but also other scientific disciplines. In last years it has been proven to be essential not only to create new therapeutic, nursing and preventive practices, but especially their effectiveness and to ensure adherence of their application to the target population.

Compared to previous years, vast majority of care for PLWH has moved to the outpatient sphere. Only in the case of health complications that an HIV-positive person cannot manage to solve in a home environment is necessary to hospitalize these patients. This approach also reduces to some extent the risk of HIV transmission to healthcare professionals during care for PLWH with higher levels of HIV RNA [Cohen 2011, Snopková 2019].

The cure of HIV infection is not possible yet despite high-quality of ART, because of the rapid HIV replication which occurs after infection in target cells such as macrophages, CD4+ T cells and dendritic cells. Newly formed virions are then released into the blood and attack

other CD4+ cells, even those, that are behind some barriers in reservoirs e.g. “sanctuaries”. As a result, HIV can survive for long in these reservoirs, in spite the fact that the blood concentration of HIV RNA is almost undetectable [Chun 2015, De Cock 2009, Castro-Gonzalez 2018].

Currently, it is therefore considered very important to start ART as soon as possible after finding HIV positivity in both adults and children and adolescents. Early treatment and the required high degree of adherence contribute to minimizing the formation of reservoirs, from which the rebound phenomenon with newly increasing HIV viral loads occurs precisely in non-adherent PLWH [Brogan 2019, Frange 2021].

Solving the problem of adherence to ART is absolutely essential, as evidenced by, among other things, the number of articles dedicated to this issue. When entering the keywords HIV+ART+ADHERENCE, 2462 articles published during the last five years appear in the PubMed database alone. Most works follow the same methodological approach, based on subjective data from PLWH and their doctors.

There are, however, many other methods how to monitor and evaluate adherence, but in particular, it is necessary to make conclusions and new strategic recommendations for more effective management of the whole HIV/AIDS issue [Benitez 2020].

The reduced adherence is often assessed by how many times a week the patient admits to forgetting to take their medication. Based on single-dose daily regimens, one discontinuation of ART per week corresponds on average to 26/30, i.e. 86.7%, and one discontinuation of ART per month corresponds to 96.7% adherence. Thus, according to current recommendations, patients could theoretically skip one dose of ART per month without affecting the therapeutic effect.

In addition, in the long term, the attending physicians can monitor both the clinical status of their patients, supported by available results in individual time periods, and psychosocial influences that can negatively affect treatment adherence. Clinicians can use a simple three-level subjective division into good, unstable, and poor adherence [Boretzki 2017].

Persons in the care of our HIV center are asked during regular three-monthly check-ups about the diseases they had in the previous period, about the tolerance of the treatment, the regularity of its use and the number of remaining tablets. This can help in the indicative assessment of adherence. Fixed drug combinations ensure complete treatment only if they are used correctly. Administration of the drug in a regimen of once every 24 hours should not deviate by more than 15 minutes. This ensures a sufficiently effective level of the individual components throughout the day and a permanent suppression of the HIV life cycle.

As a part of previous clinical studies, we had the opportunity to test special electronic monitoring closures of the Medication Event Monitoring System (MEMS), which record the time when the medication bottles are opened. It is not very suitable, however, for assessing adherence in routine clinical practice. We therefore decided on an objective method that we developed in collaboration with the Department of Clinical Pharmacology. In order to minimize the risk of HIV infection, we used the determination of individual substances in urine, which is known to contain a minimal amount of virions. We developed and published the original methodology earlier [Stehlík 2000, Sedláček 2001].

In our approach to the assessment of adherence, we were based on objective data, where we used HPLC to demonstrate selected nucleoside derivatives that are part of treatment combinations in the urine samples of patients treated with ART.

The introduction of this original methodology in 2000 allowed us to monitor the response to ART over a long period of time and thus to explain a number of discrepancies during the treatment of HIV positive persons. It was possible to regularly check the levels of selected drugs, but due to the multiple tablet regimens, we could not be sure that the patient was taking all the components of the treatment correctly. The patient could have discontinued some part of the treatment (e.g. due to suspected side effects, poorer tolerance, or for another reason) without it being possible to find out [Sedláček 2001].

The situation has changed when fixed combinations of at least two drugs, or even single-tablet regimens containing all three ART components simultaneously, entered the market. Moreover, these are in most cases administered in one daily dose. In these cases, the determination of one component from the combination gives clear information about the correct use of the entire drug.

For the purpose of objective evaluation of ATR levels in urine, we divided the PLWH of the core group according to the measured values into subjects with zero adherence (they had zero levels of the drug in the urine, even repeatedly), individuals with low adherence (they had detectable levels of the drug in the urine up to 10 mg/ l) and persons with good adherence (had drug levels in urine higher than 10 mg/l). 404 samples from 151 subjects were examined.

We compared the results obtained from groups of people with zero and low adherence using statistical methods suitable for small numbers from the point of view of differences in viral load, CD4+ and CD8+ T lymphocyte counts. The assumption was that if statistically significant differences between people with zero and low adherence are proven, there will also likely be differences in PLWH with good adherence. In both studied groups, we tried to demonstrate statistically significant differences between viral load levels and absolute CD4+

and CD8+ T-lymphocyte counts. Viral loads on the day of urine collection for testing drug levels were statistically significantly higher in subjects with zero adherence than in subjects with a low degree of adherence. Differences in absolute numbers of CD4+ and CD8+ T lymphocytes were not statistically significant at the 5% level in both sets.

To make it easier to compare our results from the questionnaire survey with other publications, we partially used the procedure according to Byrd et al. [Byrd 2019]. During the 6-12 month follow-up, individual patients reported the regularity, possible discontinuation or forgetting of the use of the treatment during check-ups. Adherence was estimated and recorded according to their data. PLWH were divided into three groups. Group A – with adherence above 90% (87 people), group B – with adherence 80-90% (49 people) and group C – with adherence below 80% (15 people).

By comparing the results of the subjective assessment of adherence by the PLWH themselves (Fig. 21) and objectively determined data (Fig. 22), we found that 136 (90%) or 133 (87.8%) persons had a good adherence during the monitored period, but from the point of view of current requirements (i.e. 95%), insufficient treatment adherence. The detected difference between the subjective and objective assessment of non-adherence is not statistically significant. The difference is evident in the subjectively assessed reduced adherence compared to the objectively proven fact (32% vs. 13.2%).

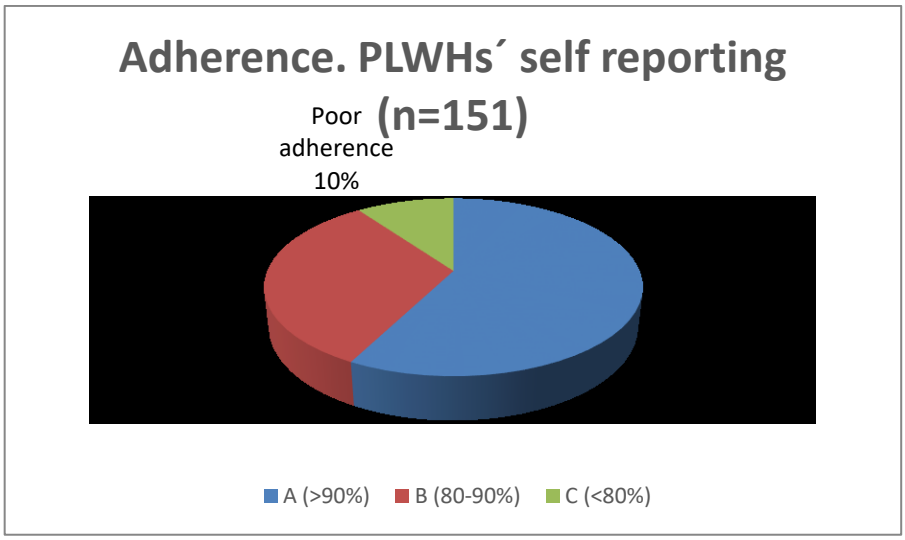

Figure 21. Adherence level according the patients´ data

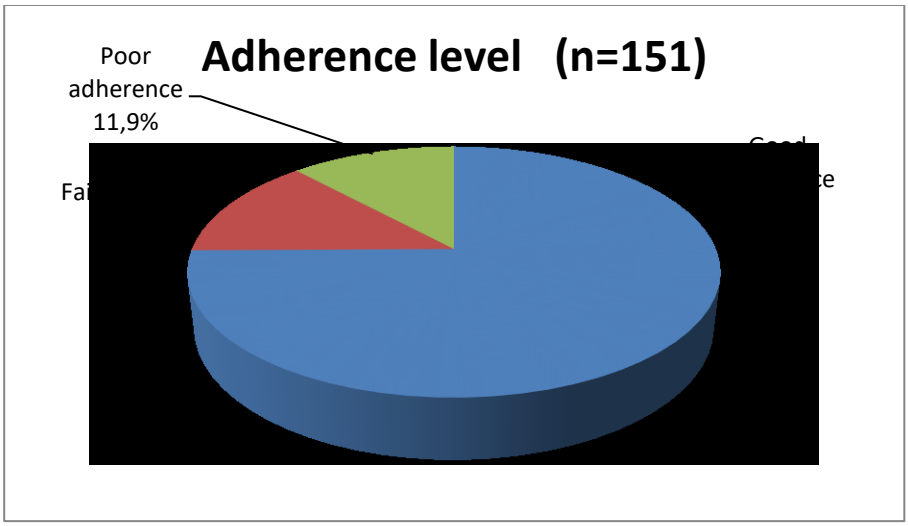

Figure 22. Adherence level according the urine nukleoside level

Table 17. Comparison of adherence results in some countries.

Country	File (n=)	ART Adherence (%)	Bad adherence in:	How to improve the adherence?	Citation
India	116	91,25	Poverty, alcohol, drugs depression, stigma	Solve barriers	Achappa 1203
Brazil	253	71,6	age <35 years, smoking, sedentary lifestyle, lack of medication and of knowledge regarding the patient's HIV status, on the part of the patient's partner or family	To improve adherence	Albuquerque Soares 2019

Ethiopia	329	>95% in 83,3%	Age <39, > 49, living in country, uneducated, comorbidities, AIDS status, CD4+<500	Solve barriers	Angelo 2021
Ghana	397	44,6	job losses, poverty, ART side effects, great distance to the physician	Increase clients' confidence in the effectiveness of treatment, remind patients	Addo 2022
Nigeria	550	92,6	No money for transportation to the hospital, traveling, forgetting, avoiding side effects and being seen	monitoring plans, home visit and care	Anyaike 2019
USA	169545	45-74	Female gender, non-white race, low education, poverty, unemployment	Solve barriers	Benson 2020
Germany	215	79,4	30 years old, AIDS and psychiatric disorders, efficacy of ART, HIV stigma, ART toxicity beliefs alcohol, drugs, dissatisfaction with ART regimen	Identification of factors associated with poor adherence	Boretzki 2017

World studies show that adherence to antiretroviral therapy, especially in some countries, is still not ideal nowadays [Achappa 2013, Albuquerque 2019, Altice 2019, Addo 2022]. A study from the Houston Health Services Research and Development Center of Excellence also shows that adherence to HIV/AIDS treatment and quality of life is dependent on self-monitoring of patients. If self-monitoring is regularly carried out, e.g. patients are regularly invited to screening and are both educated and checked for drug-using, treatment adherence is approximately 95% [Nelsen 2013]. This figure is very close to the investigation carried out at our department. Monitoring and evaluating adherence to treatment and nursing care has proven to be indispensable, both for health and economic reasons. This is the only way how to ensure that the resources as well as human resources are used efficiently throughout the whole health system, not only when working with HIV-positive patients**.** In addition, the use of all currently available antiretrovirals ensures that the overall treatment regimen will be most effective for patients and thus clearly beneficial and with positive impact on their quality of life.

Does a zero level always mean nonadherence?

In 18 persons out of 151 (15.4%) tested samples, we found that they violated the treatment schedule at least once (on the day of examination of ART levels in urine). We were interested in the causes of non-adherence. Eight patients did not have their medication because they postponed their regular check-up for various reasons. Four of them are persons with repeated adherence problems. Another three people stayed abroad longer than planned and did not have enough medicine with them. For the last eight, we were unable to prove the reason for non-adherence. The results of the determination of nucleosides in the urine are only available at the next check-up, usually in three months, and patients usually no longer remember the details. Some admitted that they might inadvertently forget to take their medication. However, by studying the medical reports, we found that three of these eight patients could no longer have their medication on the day of the check-up.

Since 2000, hundreds of urine samples from people treated with ART have been tested for the presence of nucleoside analogues at our institution. All results were compared with anamnestic data obtained from patients, clinical picture and laboratory findings (mainly CD4+ and HIV RNA). Extremely low or zero urine values correspond to low serum values, therefore ineffective treatment.

The fate of AZT, 3TC, FTC and other nucleoside/nucleotide analogues can be influenced by a number of situations. Absorption can be reduced in GIT diseases (acute diarrheal diseases, non-inflammatory bowel diseases - IBD, liver diseases). Distribution is enabled by binding to plasma proteins, and nucleoside levels may be altered in dysproteinemias. Metabolism can be affected by many interactions with other drugs inducing/inhibiting some necessary enzymes. Nuleosides or their metabolites are excreted in the bile and then in the feces or urine. Disturbances of the individual mechanisms can manifest themselves at a low level of the relevant analyte in the urine.

In these cases, it is more appropriate to determine nucleosides directly in the blood. We are currently preparing this method for further use in connection with the acquisition of a new HPLC instrument.

There are many factors influencing adherence both in a negative and positive sense, and a number of them are relatively easy to be influenced [Watson 2015].

Already at the end of the last century, Paterson et al. demonstrated that adherence to protease inhibitors statistically significantly reduces viral load and increases the number of CD4+ T lymphocytes. With high adherence (>95%), virological failure occurred in 22% of people, with reduced adherence (80%-94.9%) already in 61% of people and when adherence fell below 80%, virological failure was the rule in up to 80%. Also, the length of hospital stay for those with high adherence averaged only 2.6 days per 1000 days of follow-up, compared with 12.9 days per 1000 days of follow-up for those whose adherence was reduced below 95% (P = 0.001). No opportunistic infections or deaths were observed during follow-up in those with high adherence (>95%) [Paterson 2000].

How to increase long-term adherence to ART?

Current optimized management of patients with confirmed HIV infection was published recently [Hofman 2022]. In the past, a screening examination for anti HIV antibodies took place with confirmation of the result, which is communicated to the patient. With the help of epidemiologists, nowadays, the investigations focusing on contact tracing will take place. PLWH are cared for in one of the eight HIV centers that they choose (often according to their place of residence for easier accessibility, but this is far from the rule, also for reasons of anonymity). After the necessary laboratory, imaging and clinical examinations, early treatment is started at the HIV center [Cohen 2015, Snopková 2019]. The patient is monitored and controlled clinically and

laboratory after three months, later after achieving stability of laboratory parameters after six months. At each check-up, the attending physician addresses, among other things, the patient's adherence to treatment, the plan for the next check-up (retention), questions about a healthy lifestyle, sports, the need for psychosocial or economic support. Our results demonstrate a lack of adherence in the entire population of PLWH, especially among those in whom we demonstrated nonadherence using objective methods or who admitted it during regular HIV cetrra visits. For us, this is an important signal to improve the care of PLWH in the sense of higher control, better information and, if possible, simplification of treatment so that, if possible, there are no treatment failures and thus the risk of developing HIV resistance and subsequent treatment failure.

From the point of view of the physician, only a few basic and simple principles have to be followed. In relation to patients, it is always necessary be tacit and professional. Inappropriate behavior or manners may reveal the patient's diagnosis and thus leads to loss of trust in a health care professional. This can result to the failure of both treatment effectiveness and adherence already achieved. This also could contribute to the deterioration of their health condition and, in some cases, even directly to endanger their lives. The patient should not experience negative feelings in terms of excessive or inadequate protection from medical staff practices. It may reduce confidence and raise doubts about the professionalism of health professionals. Quality care usually results in good overall adherence as well as a positive impact on the patient's quality of life due to his overall medical condition.

We therefore used the findings of the study to improve adherence among those PLWH where it appeared to be insufficient. These patients were re-educated on the correct use of medication at the next visit. It was again explained to them that violation of the treatment schedule is one of the main reasons for the emergence of HIV resistance and subsequent treatment failure. In the next period, we repeated the tests with some nonadherent PLWH often with surprisingly good results.

The right motivation for treatment can also be induced in the homeless and other marginal groups of people [Bangsberg 2000]. In patients who are repeatedly non-adherent, the additional point of prescribing expensive treatment needs to be considered. According to Act No. 258/2000 Coll. on the protection of public health as amended, the patient is obliged to undergo medical supervision and examination and treatment, which he may refuse [Sbírka zákonů ČR 2000].

In the long term, we also looked at the possibility of HIV resistance to ART. In the years 2010 - 2020, we monitored the possible development of resistance in both non-adherent and highly adherent people. The development of resistance and its possible relationship to non-adherence of PLWH is shown in Tab. 16. In an attempt to limit the further development of resistance, it is necessary for some subjects to continuously adjust the treatment according to the current recommended procedures [Bangsberg 2008].

Conclusions

AIDS is still incurable disease and its course and duration depends on many factors. It has, however, already been proven that besides the actual administration of specific drugs, the major impact on the course of infection has the grade of adherence of ART treatment. The possibilities of objective measurement of adherence are limited. The methodology introduced by us for determining ART concentrations in urine has a very good informative value, is reliable, has high sensitivity and specificity. Likewise, the adherence to treatment affects the prognosis of disease progression and a number of other related problems and complications in the overall life of PLWH. The minimum adherence that ensures optimal results of antiretroviral therapy was already set at 95% many years ago [Paterson 2000, Nachega 2011]. Other authors refer good efficacy of ART using NNRTI even if the adherence is much lower than 95% [Bangsberg 2006]. We are also

approaching this value in our group of patients. The results obtained were used in consultations with patients in an effort to improve adherence, especially in less cooperative persons. Currently one of the priorities in the strategy of the treatment is monitoring and evaluating the adherence of PLWH and monitoring their quality of life. Data analysis of our cohort helped to improve access to all PLWH and provide them with better treatment conditions with long-term favorable results and thus with a greater chance of living to an age comparable to the general population.

Limitations

The study was conducted in one center, therefore there is a certain limitation in the number of included patients who meet the relevant criteria. However, the relatively low number of non-adherent persons testifies to the good motivation of the majority of PLWH, in the monitored center. A certain limitation can also be seen in the subjective evaluation of the questionnaire by the patients themselves. The answers can only reflect the current state of moods and feelings, and their answers at another time could be diametrically different.

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8. Attachments: tables, graphs, CD ROM