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title

Chronic hepatitis C treatment in patients with HIV co-infection – results of the latest clinical trials

authors

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summary

Liver diseases, mainly viral hepatitis, recently have become the main cause of hospitalization and death in individuals with HIV infection. As HCV infection is predominant condition in this group of patients, treatment of hepatitis C is extremely important in halting hepatic injury. Large clinical trials (APRICOT, RIBAVIC, ACTG 5071) showed satisfactory efficacy and safety of therapy with pegylated interferon alpha and ribavirin. Other trials, searching ways to improve efficacy of chronic hepatitis C treatment in HIV co-infected individuals, are still running. Management possibilities include higher doses of ribavirin, prolonged course of treatment or higher doses of interferon in the early phase of therapy.

The article summarizes current state of knowledge in the field of chronic hepatitis C treatment in HIV-infected individuals.

key words

HIV and HCV co-infection, chronic hepatitis C, pegylated interferon alpha, ribavirin

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Recently viral hepatitis in HIV-infected individuals has become the main cause of hospitalization and death. Prevalence of HCV infection among HIV positive patients correlates with the percentage of HIV infection among intravenous drug users (IDUs). The highest ratio (above 70%) of HCV infection among HIV-infected individuals in Europe can be seen in countries with IDU as the main way of HIV spread (Belarus, Ukraine), and the lowest (10-15%) in countries with dominating sexual spread of HIV, mainly Austria and Germany. According to the Eurosida study, 61,2% of all HIV infected patients in Poland are also anti-HCV positive [1].

The progression rate to cirrhosis among co-infected patients is about three times higher than among patients monoinfected with HCV [2,3]. Liver failure can be attributed mainly to hepatotoxic properties of used drugs (antiretroviral, tuberculostatic, psychotropic and other agents), increased replication of hepatotropic viruses, immune deficiency, metabolic complications due to HAART and also alcohol or drug abuse.

Thus, treatment of hepatitis C in patients co-infected with HIV should always be taken into consideration as an extremely important factor in prevention of hepatic injury. However, access to treatment is still not satisfactory. The most important reasons are lack of sufficient motivation on the patient's side, advanced immune deficiency, AIDS defining diseases, anxiousness about the side effects and physician's insufficient conviction on expediency of such treatment. All these factors contribute to the fact that only 10% of co-infected patients in western countries receive optimal treatment, whereas in Poland, where interferon therapy coverage is insufficient, the percentage is still smaller.

In 2004 three clinical trials results were published (Apricot, ACTG 5071, Ribavic), showing that patients co-infected with HIV/HCV can be successfully treated with pegylated interferon and ribavirin. These trials analyzed diverse groups of patients (different CD4 count, different percentage of patients on HAART, different progression of liver disease and other factors). The percentage of sustained virologic response (SVR) was also diverse: indifferent of the genotype it was 27-40% and with genotype 2 and 3 it was higher, reaching 43-73%. Apricot trial with the largest number of participants (n=289) had shown the best results with SVR being generally achieved in 40% of patients and in 29% with HCV genotype 1. One quarter of patients were withdrawn from trial prematurely (36% in RIBAVIC trial). Thus, the percentage of co-infected patients achieving SVR was lower than among patients with only HCV infection [4-6]. However, the results were still promising and became the basis for therapeutic guidelines in this group of patients [7,8] and the point of reference for many other clinical trials. In March 2005 pegylated interferon alpha 2a and ribavirin were approved as treatment for chronic hepatitis C in patients with HIV co-infection.

In the last few years many clinical trials investigating possibilities for improving efficacy of chronic hepatitis C treatment in HIV co-infected individuals have been conducted. Many of them are still on-going. Management possibilities include higher doses of ribavirin, prolonged course of treatment and higher doses of interferon in the early phase of therapy. Experience gathered during treatment of co-infected patients allowed for optimal HAART guidelines to be drawn up which would minimize antiretroviral drugs interaction with ribavirin and maximize the efficacy of treatment.

Higher doses of ribavirin (RBV)

Adequate ribavirin dosing according to body mass (above 11,5 mg/kg) is essential for achieving SVR and is most important in the first weeks of therapy. RBV enhances mutagenesis and selects defective HCV strains, more susceptible to interferon [9]. That is especially important in co-infected patients. However, anemia is more frequent and more pronounced side effect of RBV treatment in these patients, especially among the ones receiving zidovudine as part of their HAART regimen. The poor results observed in Apricot and Ribavic trials can be attributed to constantly low RBV dose of 800 mg and to a high percentage of dose reduction due to anemia. It is important to set optimal RBV dose: for both efficacy and safety of therapy.

The largest efficacy study of higher than usual RBV dosing was conducted in Spain. PRESCO study analyzed patients with HIV/HCV co-infection with CD4 lymphocyte count above 300 cells/mm^3, non-cirrhotic and not receiving zidovudine or didanosine as part of their HAART regimen. Pegylated interferon alfa 2a (Pegasys) 180mcg once a week plus RBV 1000 mg for patients weighing below 75 kg and 1200 mg for patients above 75 kg were used. In the beginning the trial was supposed to last 48 weeks for patients infected with HCV genotype 1 and 4 and 24 weeks for genotype 2 and 3. In 2004 correction to the protocol was made, allowing treatment prolongation to 72 weeks with HCV genotypes 1 and 4 and to 48 weeks with genotypes 2 and 3. From all 398 patients taking part in the study, 192 infected with HCV genotypes 1 and 4 were treated for 48 weeks and 45 for 72 weeks while 96 infected with genotypes 2 and 3 continued therapy for 24 weeks and 56 for 48 weeks.

SVR has been achieved in 49,6% patients, 35,6% infected with HCV genotype 1, 32,6% with genotype 4 and 72,4% with genotype 3. The percentage of SVR was even higher with the prolonged treatment: 31% vs 53% in genotypes 1 and 4 and 67% vs 82% in genotype 3. However, the differences were not statistically important. On the other hand, using higher than usual doses of RBV (in RIBAVIC and APRICOT trials standard dose of RBV was 800mg) showed statistically significant higher efficacy. [10].

The optimal period of treatment

The optimal period of therapy in patients without HIV co-infection was set for HCV genotype 1 at 48 weeks and for genotypes 2 and 3 at 24 weeks. It is suggested (but still not recognized in therapeutic programmes) to shorten therapy period to 24 weeks with genotype 1 and to 12-16 weeks with genotypes 2 and 3 if rapid virologic response (RVR) has been achieved (negative HCV-RNA after 4 weeks of treatment). RVR is a very sensitive good prognostic feature and if it is achieved, there is a high and unchanged percentage of sustained virologic response even with preterm termination of therapy. Until recently, the decision to continue treatment was made upon virologic response after 12 weeks of therapy. However, RVR may become a useful parameter in this setting, allowing for individualizing therapy [11].

In the case of HIV co-infection setting the optimal period of therapy is especially important due to lower moti-

vation for treatment, interactions with antiretroviral drugs, greater mood disorders and other side effects.

Therapeutic guidelines for this group of patients suggested 48-week therapy indifferent of HCV genotype (i.e. prolonging therapy with genotypes 2 and 3 to 48 weeks) due to high percentage of relapse [12]. Even though HCV viremia in individuals co-infected with HIV is usually higher and response to treatment may be thus slower, RVR analysis can also be utilized in this group of patients. Retrospective analysis of co-infected patients with HCV genotypes 2 and 3 who achieved RVR showed good results after 24 weeks, stressing no need for prolonging therapy in all patients [13]. The results of still running EXTENT trial may confirm the possibility of individualizing therapy period depending on the baseline viremia and type of response. New therapeutic guidelines, based on currently available results, are described below.

Inducing doses of IFN

The REPEAT trial conducted among patients infected with HCV only confirmed higher efficacy of larger than standard doses of PEG IFN alpha 2a used in the first weeks of treatment [14].

In the Coral trial the possibility of better response in HIV co-infected individuals had been ascertained. However, the observed group was small and the results did not favour the inducing doses of PEG IFN [15]. The use of inducing doses of interferon in HIV/HCV co-infected patients needs further study.

Treatment guidelines for chronic hepatitis in HCV/HIV co-infected patients as for 2007

At The 3rd International HIV/HBV/HCV Co-infection Workshop the positive predictive value of RVR for achieving SVR led to the creation of new guidelines for treatment of chronic hepatitis C in HIV/HCV co-infected patients. Rapid virologic response as soon as in the 4th week of treatment was taken into account. In the case of negative HCV-RNA in patients infected with HCV genotype 2 or 3 treatment is continued for 24 weeks (only in patients with minimal baseline HCV replication and minimal histological liver changes), while in patients with genotypes 1 or 4 and others with the genotypes 2 or 3 the treatment is continued for 48 weeks with no further HCV viremia measurements in the course of therapy. In the case of positive HCV-RNA after 4 weeks of treatment, it should be assessed again after 12 weeks of therapy. If HCV-RNA falls at least 2 logs compared to baseline, therapy is continued and HCV-RNA assessed again after 24 weeks, however qualitatively this time. With negative HCV-RNA, therapy is continued until 48 weeks with genotypes 2 or 3 and until 72 weeks with genotypes 1 or 4. If the 24-week results are positive, therapy should be discontinued. It should also be discontinued if early virologic response (EVR) after 12 weeks of therapy is not achieved, meaning decrease of HCV-RNA by at least 2 logs compared with baseline viremia after 12 weeks of treatment [16].

Antiretroviral drugs (ARV) interactions with ribavirin

As data from treatment of HIV/HCV co-infected patients receiving ARV accumulate, new interactions between ARV and ribivarin come to light. Some nucleoside reverse transcriptase inhibitors (NRTI) like didanosine, zidovudine and stavudine have been known to be more toxic when combined with ribavirin. The added toxicity can have negative effect on the efficacy of treatment due to anemia and RBV dose reduction as mentioned earlier. The latest published data on ARV interactions with RBV include abacavir (ABC), another NRTI guanosine analogue. Both drugs require cellular phosphorylation, both occurring on the same metabolic pathways, which can hinder synthesis of active metabolites.

In RIBAVIC trial analysis one of independent factors for lack of early virologic response (EVR) was the use of abacavir [17]. Also in Spanish retrospective multicenter study of 426 HCV/HIV co-infected patients treated with Peg IFN alpha plus RBV, abacavir use was one of independent factors predicting lack of achievement of SVR in logistic regression analysis (p=0.03). However, statistical relevance was present only in the group of low serum RBV concentration. It could indicate competitive inhibition of phosphorylation meaning inhibition of RBV active metabolites synthesis by ABC and thus leading to ineffective therapy [18].

A multicenter Spanish study analyzing the influence of different factors on SVR achievement has shown that nucleotide reverse transcriptase inhibitor (NtRTI) as part of HAART, namely tenofovir, in combination with lamivudine or stavudine, increases the SVR rate. In a multivariate analysis it was one of independent factors (apart from HCV genotypes 2 and 3, low baseline viremia, high CD4 count and good adherence) for achieving SVR [19].

Nucleoside analogue sparing HAART regimens had been used to minimize toxicities during Peg IFN alfa/RBV therapy. In a randomized prospective German study of 120 both HIV negative and positive patients, the observed differences in the percentage of SVR achievement between patients receiving (including regimens without NRTIs) and not receiving HAART treatment were statistically relevant [20]. On the other hand, in a small group of 10 patients receiving as part of their HAART regimen 2 boosted protease inhibitors (PI) a small number of side effects was seen during Peg IFN plus RBV treatment. Only in one patient was it necessary to reduce RBV dose due to anemia. Due to very few interactions between PIs and RBV, this regimen can be a useful therapeutic option increasing the efficacy of hepatitis treatment with adequate RBV dosing [21].

Guidelines and clinical practice

Guidelines based on clinical trials are not always in accordance with clinical practice. It may be in part caused by differences between patients taking part in clinical trials and patients in "real life". The patients should be qualified

for hepatitis C treatment very accurately. Not only immune status and previous ARV treatment have to be taken into consideration but also patient's mental status, his motivation to stay in treatment, drug-free period, social status and other factors. Many patients co-infected with HIV and HCV stop their interferon and ribavirin therapy especially in the second 6 months of treatment. The new algorithm proposed by Soriano making provision for shorter therapy in case of rapid response is a move towards solving the problems.

An interesting Italian multicenter prospective observational OPERA trial – analysis of practical implementation of guidelines from 2005 European Consensus Conference (ECC) was conducted on all HIV/HCV co-infected patients treated for hepatitis C since 2005. Dosing regimens of Peg IFN alpha and ribavirin used in the study were different. Most patients (74,7%) treated with Peg IFN alpha 2b received too low doses according to their body weight while there were only 3% of patients treated with too low Peg IFN alpha doses. 30% of patients infected with HCV genotype 1 and with high HCV viremia received 800 mg/d or less RBV even though ECC advocates 1000-1200 mg/d in this group of patients. The authors conclude that the above mentioned discrepancies may result from ambiguous data from previous trials and guidelines on dosing regimens in HIV/HCV co-infected patients and from fear of side effects [22]. Further studies, also observational ones, are necessary to unify therapeutic regimens.

Despite new data and advanced treatment regimens, hepatitis C treatment in HIV co-infected individuals still remains a complex and difficult field of expertise.

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title

Pharmacogenetics in HIV Clinical Practice

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summary

The best choosing of antiretroviral therapy for an individual patient is one that physicians have always tried to practice, making use of whatever knowledge or tools are available to guide treatment decisions. Individual genetic variations provide scope for a wide range of host-drug interactions that will differentiate therapeutic outcomes within an otherwise homogenous population. Pharmacogenetics looks set to become an increasingly important field to refine the medical tools to personalize antiretroviral treatment. The integration of pharmacogenetic tests into routine patient management has a great challenge for HIV – guidelines and for clinical care. Pharmacogenetics will increasingly impact on medical disciplines. The use of genetic tests in clinical practice improve drug prescribing holds great promise to improve the lives of individuals affected by HIV.

key words

genetic screening, HLA allels, genetic factors in HIV, hypersensitivity reaction

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INTRODUCTION

The term of "pharmacogenetics" was implemented by Friedrich Vogel in 1959 to denote the effects of polymorphism within a particular human gene on the disposition and action of drug [1]. It has long been recognized that individuals vary in their susceptibility to diseases and their response to drugs, but in the last years that progress has been made in elucidating the genetic basis of this phenomenon.

It is known numerous examples of polymorphisms in genes encoding drug-metabolising enzymes, drug transporters and drug targets (enzymes, receptors, etc.). Recent years have seen the mile stone in translating the pharmacogenetics into clinical practice through the use of molecular diagnostics (genotyping) to identify patients at risk of toxicity and drug reaction [2]. Pharmacogentetic tests to identify variations (polymorphism) in human genes can reliably predict the treatment efficacy and toxicity of clinically important medications. This is important area of intense research that is particularly relevant to HIV, given the need for chronic administration of multiple drugs to treat HIV infrction and the frequent need to discontinue or change regimens for the reason of safety and efficacy treatment. However, while multidrug of antiretroviral regimens provide considerable opportunity for host – drug interaction that may be affected by genetic variants, it is a challenge to elucidate the contribution of human genetic variability for each individual drug in the context of multidrug regimens.

Technological advances allowing the application of genome – wide approaches to identify the multiple genetic polymorphism that affect a drug response hold out promise for the identification of disease – susceptibility genes and genetic markers for drug efficacy, thereby opening the way for personalized drug therapy.

GENETIC POLYMORPHISM

An important of HIV research is the immune response and how HIV circumvents it to create a successful and chronic infection. Various studies have provided not only a basic understanding of " how HIV invade", but also clues for the development of vaccines to fight against AIDS. Although HIV initially evokes an immune system response, it later escapes and evades the immune system for a successful viral infection. Methods of escape from the immune response include rapid mutations altering the organization of cell surface receptors, alterations in the expression profile of human leucocyte antigen (HLA) and destruction of immune efector cells.

HIV infection of viral particles are counterattacked by CTL – mediated immune responses (CTL: cytotoxic T cells). Though the cellular immune response fails to control HIV infection completely in most infected individuals, its occurrence is evident in regulating viral load during chronic infection. The initial CTL response may be directed against a few epitopes, which subsequently broadens during prolonged antigen stimulation [3]. When CTL recognize self – HLA molecules loaded with foreign peptide, they activate *Fas* and secrete perforins and granzymes, which lyse target cell [4]. The CTL produces cytokines, such as interferon-α (IFN α) and tumor necrosis factor (TNF), that affect viral replication. HIV-1 – specific CTL also produce the CC chemokines macrophage inflammatory protein 1-α and 1-β and "rantes" which suppress HIV-1 replication [5]. Even with these various effector functions, CTL cannot completely check viral intrusion in the immune system.

During HIV infection, selective pressure imposed by CTL leads to the generation of various escape mutations and these variants may constitute the majority of the total viral pool. The role of CTL is selection pressure for occurrence and than for maintenance of these mutations. Later on, evidence of escape mutations in HLA – B8 restricted epitope in *Nef*, HLA-B44 restricted epitope in *Env* and HLA – B27 restricted *Gag* epitope [6]. After CTL response, HIV inhibits surface expression of the host major histocompatibility complex (MHC) class I.

THE ROLE OF HLA IN HIV INFECTION

Through various genetic factors have been associated with susceptibility to HIV. Table No.1 has shown the genetic factors in HIV infection susceptibility (tab. 1).

Table 1. Genetic factors in HIV susceptibility

Gene	allele	Impact on disease
CCR 5	Del 32	Prevent infection
CCR 5	P 1	Progression of disease
CCR 2	V 641	Delayed disease progression
CCL 5	In 1. 1C	Accelerate disease progression
CXCL 12	CXCL 12 3'A	Delayed disease progression
HLA-A, B, C	Homozygous	Disease progression
HLA-B	B*27	Delayed disease progression
	B*57	Delayed disease progression
	B*35	Rapid disease progression
HLA-G	G*0105N	Decreased risk of infection
HLA-E	HLA-EG	Decreased risk of infection

The role of HLA antigens has concentrated on three areas: zygosity of HLA loci, HLA-sharing alleles and specific HLA-allelic/halotyping association with the outcome of disease progression. It has been shown that homozygosity at the class I – loci is associated with relatively rapid progression to AIDS, compared with heterozygotes [7].

Another genetic component that predisposes to the progression to AIDS is HLA-sharing. If the MHC class I is common to the donor and recipient, the basis of successful transplantation, it would lead to increased susceptibility to viral infection. One natural model of viral transmission between HLA-sharing donor and recipient is mother-to-child transmission, which further supports increased transmission of HIV in these circumstances [8]. Significant increase in susceptibility to HIV has been shown to be associated with concordance at the HLA-B locus but not at HLA-A or HLA-C.

Various studies have confirmed the contribution of specific class I alleles and more particularly HLA-B alleles in the outcome of disease. This remarkable contribution of HLA-B may be because this group has the highest diversity among the class I antigens: approximately 661 alleles com-

pared with 372 in HLA-A and 190 alleles in HLA-C [9]. So, substantially, greater selection pressure would be imposed on HIV by HLA-B compared with other class I antigens. Delayed diseases progression has been seen with HLA-B*27 and HLA-B*57.

PHARMACOGENETICS AND ATIRETROVIRAL THERAPY

Genetic markers that can predict drug safety are particularly promising as candidates for eventual translation into clinical practice. This is very strong associations between specific genetic polymorphism and drug safety and toxicity. A number of pharmacogenetic markers have been reported among antiretroviral drugs including genetic predictors of protease inhibitor – associated hyperbilirubinaemia and dyslipidemia, efavirenz – associated central nervous system side effects, NRTI's – associated lipoatrophy and neuropathy, tenofovir – associated renal dysfunction and hypersensitivity reaction associated with nevirapine and abacavir (tab. 2).

Table 2. Association between allelic variants and HIV treatment response

drug	phenotype	gene
abacavir	Hypersensitivity	HLA-B*5701, hosp70 hom
atazanavir indinavir	Jaundice	UGT 1 1A1
NRTI's	Lipoathrophy	TNF-α promoter
nevirapine	Hypersensitivity Hepatotoxicity Pharmacokinetics	HLA-DRB 1*01 MDR 1 CYP 2B6
efavirenz	CNS side-effects, pharmacokinetics, viral response, resistance	CYP 2B6 MDR 1
nelfinavir	Viral response, pharmacikinetics	CYP 2C19
PI's	Dyslipidemia	Apo C, Resistin
NRTI's	Peripheral neuropathy	Mitochondrial haplogroup T HFE
HAART	CD4 recovery	Proliferation and apoptosis

Clinical practice in HIV therapy includes several examples of applied pharmacogenetics, although prospective genetic screening remains to be validated in randomized and adequately powered clinical trials. Licensing authorities currently recommend genetic information of relevance to drug safety which is increasingly appearing in prescribing drugs such as cytostatics, oral anticoagulants, antiarrythmics and other metabolized by the polymorphic enzymes in cytochrome P-450 (CYP), especially by 2C9 and CYP 2D6 izoenzymes.

Currently, the most promising application of pharmacogenetics to the field of HIV medicine, and one that readily lends itself to clinical investigation of its utility as a patient-management tool, is the identification of those individuals at greatest risk of genetically influenced drug toxicities. Potential genotypic-phenotypic correlations for drug – associated adverse events, or potential mechanisms for such events, have been elucidated for several antiretroviral agents, including nevirapine, atazanavir, efavirenz, tenofovir and abacavir.

Nevirapine – associated hypersensitivity. Australian cohort study suggest that early hepatitis and hepatitis-associated rash with nevirapine have a strong immunogenetic basis, with HIV-infected patients exhibiting the HLA class II alleles – HLA-DRB1*0101 and CD4+ cell count>25%, and having a 17-fold increased risk of developing these symptoms [10]. Recent data regarding Sardinian population indicate that the HLA class I alleles (HLA-Cw8 – B14) is additionally associated with hypersensitivity to nevirapine [11,12]. Moreover, nevirapine-related hypersensitivity appears to be susceptible to MDR1 gene polymorphism, with MDR1 3435 CT or TT genotypes conferring a significantly lower risk of hepatotoxicity than the CC genotype.

Atazanavir, Indinavir-related hyperbilirubinaemia and jaundice. Hyperbilirubinaemia affects 20-50% of atazanavir and 5-25% of indinavir treated patients. This effect depends on competitive inhibition by drug uridine diphosphate glucuronosyl transferase 1A1 (UGT 1A1) – mediated bilirubine conjunction and clearance[13]. This metabolic adverse event is especially common in the 5-10% of the population with Gilbert's syndrome, who have underlying genetic defect in bilirubine conjugation. In these patients, the incidence of atazanavir – or indinavir – related hyperbilirubinaemia varies according to UGT 1A1 promoter genotype, ranging from ~15% in those with the wild-type allele, to 90% in those homozygous for the UGT 1A1*28 allele. This latter group is more likely to develop experience of bilirubine levels increasing.

Efavirenz – related neurotoxicity. Efavirenz is completely metabolised by CYP 2B6. The CYP 2B6 516G>T polymorphism, which occurs more frequently in African-Americns than in Caucasian (20% vs 3% respectively), have the 516 T/T genotype [14] – is associated with increased plasma efavirenz exposure and a higher incidence of adverse central nervous system (CNS) effects during treatment.

Ritonavir – associated hyperglyceridaemia. Using ritonavir as a booster of protease inhibitors, is frequently associated with atherogenic lipid abnormalities; increased plasma triglyceride, high-density-lipoprotein cholesterol and apolipoprotein B levels. Polymorphism in the genes encoding apolipoproteines E and C3 (APOE, APOC3) and the promoter region of the TNF-α gene have been linked to development of dyslipidaemia with protease inhibitors experienced patients. The presence of variant APOC3 (455 T/C, 482 C/T, Sstl) and APOE (ε2, ε4) alleles has been associated with increased risk of severe hyperglycerydaemia in Caucasian patients, whereas variant APOC3 (455 T/C, 482 C/T, Intron 1G/C) alleles appear to confer protection against hyperglyceridaemia in Hispanic patients [15].

Tenofovir – related renal proximal tubulopathy. Renal proximal tubulopathy has manifested as acidosis, glycosuria and proteinuria. It is observed during long-term tenofovir therapy. The renal cytotoxicity has been asso-

ciated with a polymorphism of 1249 G/A in the ABC C2 gene encoding the MRP2 transporter [16]. ABC C2 halotype also appears to influence susceptibility to tenofovir – induced renal proximal tubulopathy.

Nucleoside reverse transcriptase inhibitors – related lipodystrophy and peripheral neuropathy. Lipodystrophy and peripheral neuropathy are manifestations of mitochondrial toxicity associated with NRTI's therapy, especially the thymidine analogues – stavudine, zidovudine, didanosine. In Caucasian, susceptibility to NRTI's-associated lipodystrophy has been linked to a promoter polymorphism in the tumor necrosis factor TNF-α gene (TNFα – 238A) [17,18]. Also the susceptibility to stavudine and didanosine –related peripheral neuropathy has been associated with polymorphism in the mitochondrial genome, especially with the mitochondrial haplogroup T [19].

Abacavir – related hypersensitivity. Hypesensitivity reaction to abacavir (HSR) affect ~8% of treated patients, although symptoms are non-specific and can be difficult to distinguish from those of concomitant viral illness and/or similar reactions to concomitant antiretrovirals, other drugs etc. About 2-3% false-positive rate for diagnosis of clinical hypersensitivity to abacavir means that the true incidents of HSR is 5%. The syndrome of HSR is reversed on abacavir discontinuation. The genetic component of the syndrome was originally suggested by reports of a familial association and a lower incidence in men and individuals of African origin [20]. In Caucasians, abacavir hypersensitivity has been linked with HLA markers; with the HLA-B*5701 allele being most strongly implicated [21,22,23]. Recombinant mapping of allele 57.1 halotype, as well as presence of HLA-B*5701 in abacavir-tolerant individuals, suggests that HLA-B*5701 is necessary, but not sufficient by itself for causing abacavir HSR [24,25].

In contrast, the association between HLA-B*5701 genotype and abacavir hypersensitivity reaction appears to be appreciable weaker in individuals of African origin, although as wit the variable strength of association noted in Caucasians, it is currently unclear whether this is a true effect or a consequence of the difficulty of accurate clinical phenotyping. A retrospective analysis of 595 cases of suspected abacavir hypersensitivity indicated that no combination of markers offered superior predictive sensitivity of specificity to HLA-B*5701 alone (50% and 90% respectively) among Caucasians [23].

The early studies have been limited by their retrospective design, as well as by problems in defining precisely the phenotype of abacavir HSR. Results from an Australian cohort indicated that the HLA-B*5701, HLA-DR7 and HLA-DQ3 halotype, had a positive predictive value of 100% and a negative predictive value of 97% for abacavir hypersensitivity. In fact, the HLA-B*5701 occur in > 90% of the abacavir –sensitivity patients compared with fewer than 0,4% of abacavir-tolerant individuals [24].

Currently, two large studies has commissioned, which use abacavir skin patch testing to help provide greater diagnostic precision for HSR. The SHAPE study is a US-based retrospective case-control assessment of the association of HLA-B*5701 with HRS in both White and Black patients, while the European/Australian PREDICT-1 study is the first randomized, blinded, prospective study of the impact of screening on HSR incidence against a contemporary control population.

The abacavir hypersensitivity screening test in practice. Developing of HSR to abacavir can now be assessed with *a simple blood test.* This test meant to be used in individuals HIV-positive who have never previously been exposed to abacavir. The test should not be used in patients who has a diagnosis of abacavir toxicity reaction in the past. The test checks for the presence of specific genetic material: HLA-B*5701. The result can appear in one of two ways:

- negative – this does not meant that you will never get a HSR to abacavir. It does meant that there is a relatively low risk (less than 1%)
- positive – there is a high risk of having a hypersensitivity reaction to abacavir and you should not use this drug or any other drug containing abacavir.

A skin test, called an epicutaneous patch test (EPT), was developed in Canada and has been used to confirm the clinical diagnosis of abacavir hypersensitivity. The patch test is being used as a part of two major studies.

SHAPE study: an American study has looking at association between HLA-B*5701 and abacavir HSR in Black and White. The new preliminary study entitled "High sensitivity of HLA-B*5701 in Whites and Blacks in immunologically-confirmed cases of abacavir hypersensitivity (ABC HSR)" has been shown in 4th IAS Conference on HIV Pathogenesis, Treatment and Prevention, Sydney 2007 by M. Saag group [26]. This study estimates the sensitivity of HLA-B*5701 in Whites and Blacks using skin patch testing to supplement HSR diagnosis. These data suggest that prospective HLA-B*5701 screening may reduce ABC HSR rates in Whites and Blacks. Not all HLA-B*5701 positive patients were positive skin patch test. The authors conclude that HLA-B*5701 screening may augment role in HSR management, but must never substitute for clinical vigilance.

To complement these results, a large prospective study PREDICT-1 evaluating the clinical utility of HLA-B*5701 screening on ABC HSR has been completed. It was a first randomized, controlled study conducted throughout sities in Europe and Australia, to look at the usefulness of genetic screening for HLA-B*5701 in preventing abacavir hypersensitivity [27,28]. The PREDICT-1 study recruited 1956 abacavir-naïve subjects from 314 centers in Europe and Australia. Patients were randomized to start an abacavir regimen under the standard of care or to start abacavir with the standard of care plus HLA-B*5701 test got excluded, whilst those with a negative test continued treatment. For immunologically confirmed hypersensitivity, HLA-B*5701 had a negative predictive value of 100%, and for clinically suspected hypersensitivity, the genetic test had a negative predictive value of 96%. The HLA-B*5701 test is practically more useful for its negative predictive value; i.e. used to rule out patients (who are HLA-B*5701 positive) from receiving abacavir. However, that HLA-B*5701 negativity does not rule out the possibility of abacavir hypersensitivity.

Research on abacavir hypersensitivity reaction has been continued around the world with several teams, trying to find other ways of conducting genetic screening. Much of this work in Canada has involved collaboration with the Western Australian group to assess the quality and accuracy of diagnostics kits for HLA-B*5701. The quality, quicker test and cheaper methods for HLA-B*5701 will hopefully facilitate the incorporation to abacavir genetic screening into routine HIV care.

CHALLENGES FOR PHARMACOGENETICS IMPLEMENTATION INTO ROUTINE PATIENT MANAGEMENT

The application of pharmacogenetics to clinical practice is complicated by the lack of correlation between single polymorphism and plasma drug exposure, drug efficacy and/or tolerability. Pharmacogenetic association are typically derived from small or stratified population samples, and diverse ethnic groups is not clear. Despite the previously described established associations between specific polymorphisms and drug safety outcomes, most come with challenges for their potential implementation as patient-management tool.

HLA-DRB 1*0101 screening for nevirapine toxicity. The risk of nevirapine – related hepatitis and rash in Caucasian patients, suggesting that allele DRB 1*0101 may serve as an immunogenetic marker for nevirapine hypersensitivity. However, the recent identification of a different allelic marker (HLA-Cw8) in at least this patients, may complicate efforts to establish the utility of a universal prognostic marker.

UGT 1A1*28 screening for atazanavir/indinavir – related hyperbilirubinaemia. Prospective UGT 1A1*28 genotyping has been proposed to identifying patients at risk of jaundice and hyperbilirubinaemia during drug treatment. But, the influence of the P-glycoprotein 3435 C>T polymorphism on plasma atazanavir exposure and plasma bilirubine level introduces a potential second mechanism that requires separate assessment [29].

CYP 2B6 genotyping for efavirenz-related neurotoxicity. The high degree of overlap between CYP 2B6 genotypes and the multiplicity of factors affecting efavirenz exposure are likely to limit the value of CYP 2B6 genotyping to identification patients at risk of neurotoxicity.

MRP 2 genotyping for tenofovir-related renal proximal tubulopathy. In practice, the value of screening for the 1249 G>A polymorphism in the MRP2 transporter as a means risk tenofovir – related tubulopathy is uncertain, given the relatively low sensitivity of this marker.

HLA-B*5701 screening for predisposition to abacavir hypersensitivity. HLA-B*5701 is the extensively studied pharmacogenetic marker. This marker is also currently the most clinically promising pharmacogenetic intervention for improving patient safety. Observational findings suggest that prospective HLA-B*5701 genotyping and subsequent avoidance of abacavir use in HLA-B*5701-positive patients can reduce the incidence of HSR to less that 2% in Caucasian population. Moreover, HLA-B*5701 genotyping test appears to be a cost-effective strategy in this patients: result based on a meta-analysis of three cohorts showed that to prevent one case of hypersensitivity, eight HLA-B*5701-positive patients would be denied abacavir and that, to identify them, 48 patients would require testing [30].

In some HIV centers now routinely screen patients for HLA-B*5701, although the benefits of universal screening, particularly in non-Caucasian populations, are unclear. The predictive sensitivity of the HLA-B*5701 test is appreciably lower in Hispanic and Black than in Caucasian people.

The success in translating pharmacogenetic knowledge into clinical practice has been limited now to date, and a number of factors complicate. Realization of the full potential of pharmacogenetics in the clinic will require attention to appropriate phenotypic definitions, completion of large-scale well-controlled prospective studies and introduction of novel laboratory technologies.

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title

Lopinavir/ritonavir and rifampin: is coadministration possible?

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summary

The necessity of concomitant treatment of tuberculosis and HIV infection in the same patient creates a therapeutic challenge due to drug-drug interactions. The most problematic issue is coadministration of rifamycins with protease inhibitor (PI) – based antiretroviral regimens. One of the PIs, commonly used in Poland is lopinavir/ritonavir (LPV/r), and the only rifamycin directly available is rifampin. It is well known that rifampin dramatically decreases lopinavir plasma levels. In the view of different studies, coadministration of LPV/r with rifampin, despite attempts to compensate the interaction with dosage adjustment or additional ritonavir, is not advisable because of toxicity. In such situations, rifabutin should be a drug of choice. It can be taken as 1/4 of normal dosage. LPV/r dosage does not need to be changed. In Poland, rifabutin can be obtained through special importing procedure.

key words

lopinavir, ritonavir, rifamycins, rifampin, rifampicin, rifabutin, drug interactions

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The necessity of concomitant treatment of tuberculosis and HIV infection in the same patient is not a rare situation. Both infections require the use of combined therapy for the same reason: to prevent resistance. In either case, it means at least three agents given together. This leads to unavoidable problem of drug interactions.

In antiretroviral-naive patient with CD4+ count >50 cells/mm^3 and active tuberculosis, the DHHS Guidelines [1] recommend starting four-drug (rifampin or rifabutin, isoniazid, pyrazinamide, and ethambutol or streptomycin) antimycobacterial treatment, then to initiate antiretroviral regimen 4 to 8 weeks later. Such approach helps identify the causes of adverse reactions which are common in this group of patients; also, after this delay period, the number of antimycobacterials can be usually reduced to one of the rifamycins plus isoniazid. In patients already on antiretroviral therapy, a four-drug regimen is recommended for first 2 months, followed by 4-7 month therapy with rifampin/rifabutin plus isoniazid; therefore, the potential risk of drug interactions during first weeks of anti-tuberculosis therapy in these patients is even higher.

The most troublesome drugs are these metabolized via P450 cytochrome system (CYP450). Unfortunately, two basic anti-tuberculosis drugs: rifampin and isoniazid, are either metabolized that way, or at least they are CYP450 inducers. Among antiretrovirals, the situation is not better. Most potent agents – protease inhibitors (PI) and non-nucleoside reverse transcriptase inhibitors (NNRTI) – are all CYP450-dependent. In case of first-line NNRTI, efavirenz, it is possible to overcome the interaction with +33% dose adjustment; however, this simple approach does not work well with the PIs [1, 2].

One of the PIs, commonly used in Poland is lopinavir/ritonavir (LPV/r), and the only rifamycin directly available is rifampin (=rifampicin). The purpose of this paper is to review the current knowledge about possibility of coadministration of these agents.

Rifampin and other rifamycins

Rifampin is one of the rifamycins, the group of antibiotics active mostly against Gram(+) and acid-fast bacteria, including *Mycobacterium tuberculosis*. It is a first-line antimycobacterial agent, acting by inhibition of DNA-dependent RNA polymerase of mycobacteria. It is cheap, relatively safe and readily available worldwide, also as a fixed-dose combination with isoniazid, as both drugs can be given orally.

Rifampin is a potent CYP3A inducer, and this induction accelerates elimination of most other compounds which are metabolized by the same enzyme. Interestingly, rifampin metabolism itself does not depend on CYP3A [3, 4]. Adult dose of rifampin is 600 mg once daily (QD).

The compound which is chemically related to rifampin, and exhibits very similar antimycobacterial activity, is rifabutin. It is a relatively weak inducer of CYP3A, however, its metabolism is dependent on it; therefore, inhibition of this cytochrome leads to increase of rifabutin pharmacokinetic parameters [3, 4]. Adult rifabutin dose is 300 mg QD [5].

Third important member of rifamycin group, rifapentine, has very prolonged elimination, enabling twice-weekly dosage at 2-months initiation period, then once-weekly at maintenance therapy [6]. However, it proved to be inferior vs. rifampin in clinical trials due to high relapse rate [6], and currently it is not recommended in HIV+ patients because of resistance and adherence concerns [1]. Rifapentine is a strong CYP3A4 inducer [3, 4].

Lopinavir/ritonavir

Lopinavir (LPV), a HIV protease inhibitor, is metabolized mainly by CYP3A4. Ritonavir, another PI, is a strong inhibitor of this cytochrome. Therefore, coadministration of these compounds (with ritonavir given in much lower dose than lopinavir – the ratio is 1:4) enhances the pharmacokinetics of lopinavir, resulting in elevated and sustained LPV plasma levels. This beneficial interaction, occurring also between ritonavir and most other PIs, is known as "ritonavir-boosting", and is employed in a number of antiretroviral regimens. In case of lopinavir, it ended with the development of convenient, co-formulated tablet containing both lopinavir and ritonavir (LPV/r 200/50 mg). Adult daily dose of LPV/r is 800 mg of lopinavir and 200 mg of ritonavir, given as two tabs twice daily (BID) [7].

Ritonavir, given in low dose as a pharmacokinetic booster, exhibits no antiviral activity of its own.

Lopinavir/ritonavir and rifampin

First studies [8] of the pharmacokinetic interaction between rifampin and LPV/r showed that the area under the pharmacokinetic curve (AUC) and the minimum concentration in plasma (C_{min}) for lopinavir in healthy subjects were decreased by 75 and 99%, respectively, as a result of coadministration of rifampin at 600 mg QD with standard dose of LPV/r. This results from strong induction of CYP3A by rifampin, which overcomes the inhibition of this enzyme by low-dose ritonavir. It is clear that rifampin, combined with standard-dose LPV/r, makes the latter drug almost completely inactive.

The next major study by la Porte et al. [9] tried to establish whether modifying the LPV and ritonavir dosage could overcome the interaction with rifampin. In standard-dose regimen, they basically confirmed the results of Bertz et al. [8]: LPV C_{min} levels in steady-state have dropped by 93%. However, in their study, the observed lopinavir exposure was substantially higher compared to the historical data [8]. The dosage adjustment investigated by the authors (LPV/r BID, respectively: 667/167 mg, 800/200 mg, 400/300 mg, 400/400 mg; rifampin always 600 mg QD) showed that while the concentrations of lopinavir could be significantly increased, especially in 400/400 mg BID group, it could not be demonstrated that these regimens were equivalent – particularly with respect to C_{min} – to the standard dose of LPV/r without rifampin. This indicates that the simple dose adjustment (either with LPV/r ratio maintained, or ritonavir dose increased to 1:1 with LPV) may not be capable of complete compensation for the accelerated metabolism of lopinavir by rifampin. Moreover, in order to approach LPV plasma levels achievable without rifampin, the authors had to administer 800 mg of ritonavir daily; due to nonlinear pharmacokinetics of ritonavir, this resulted in unproportionally high ritonavir exposure [9]. According

to Abbott, the ritonavir manufacturer, such dosage may be used only under strict safety monitoring due to tolerance issues. It should be noted that both ritonavir and rifampin can cause liver function tests elevations, and, after increase in the dose of ritonavir, the combined effect of both drugs can be deleterious. Because of toxicity, nine of 29 (31%) studied healthy volunteers discontinued therapy; the same number of subjects experienced a ≥grade 2 increase in ALT and/or AST. Most discontinuations due to hepatotoxicity occurred in the arm with the highest ritonavir daily dose (800 mg) [9].

From the other hand, no evidence of statistically significant changes in rifampin pharmacokinetics was observed, regardless from the dosage regimen tested [9]. This confirms already known fact that rifampin metabolism is not affected by LPV or ritonavir coadministration.

Recently, a South African study tried to establish if there is a similar rifampin-induced decrease in LPV plasma concentration in children, as it was seen in adults, and if this could be ameliorated by additional ritonavir. The results showed that it was partially possible when ritonavir dose was increased to the same as lopinavir (1:1 ratio) [10]. This again raises safety concerns.

It should be noted, however, that the data regarding the possibility of compensation of the interaction between LPV/r and rifampin are limited. In particular, it is not clear what is the role of each agent (lopinavir, ritonavir and rifampin) in observed liver enzyme elevations. Further studies on this subject are warranted.

In the light of above, current recommendation of Polish Scientific AIDS Society [11], which proposes concomitant use of rifampin with increased dose of LPV/r (from 800 mg/200 mg to 1600/400 mg daily) or with standard dose of LPV/r augmented with additional 600 mg of ritonavir daily, should be taken with caution.

Other protease inhibitors and rifampin

An increased hepatotoxicity associated with coadministration of rifampin 600 mg once daily and saquinavir 1000 mg/ritonavir 100 mg twice daily has been observed. Liver enzymes elevations (up to 5-fold) were detected in 40% of the HIV-seronegative subjects evaluated. It is recommended that saquinavir/ritonavir and rifampin not be used together [12]. This finding was confirmed by another study involving HIV+ patients with tuberculosis [13].

A study involving 71 HIV-negative volunteers revealed that coadministration of rifampin and atazanavir 300 mg/ritonavir 100 mg decreased atazanavir exposure. This reduction could not be compensated by an increase in the atazanavir and ritonavir doses to 400 mg and 200 mg, respectively. Concurrent use of atazanavir/ritonavir-based regimens and rifampin should be discouraged [14]. Similar results were obtained in ACTG 5213 trial, completed by 10 HIV-negative individuals. In this study, the authors unsuccessfully tried to restore desired ATV plasma levels in the presence of 600 mg rifampin QD by administering unboosted atazanavir in the dose of 300 or 400 mg BID [15].

All other PIs are listed as producing unacceptable interactions with rifampin [16].

Lopinavir/ritonavir and rifabutin

According to the CDC [17], current DHHS Guidelines [1] and lopinavir/ritonavir drug manufacturer recommendation [7], the rifamycin to be used together with LPV/r is rifabutin. Despite some problems with predictability of pharmacokinetic parameters [3, 18, 19], a guide for dosage adjustment has been established [7]. When given concomitantly with LPV/r, rifabutin dose should be slashed by 4, i.e. only 25% of usual dose should be used. The plasma levels of rifabutin, due to pharmacokinetic interaction with ritonavir, a potent CYP3A inhibitor, are expected to be within therapeutic range, and the C_{min} and AUC of lopinavir should not be impaired [1, 7].

Despite clear guidance from LPV/r manufacturer, some authors suggest twice as much rifabutin dosage, i.e. only 50% reduction [2]. This recommendation was based on a very small (n=4) Bonora study [19], made on HIV+ subjects with tuberculosis; this study gave rather inconclusive results because of wide inter-subject variability. The results of another, more recent study [20], done on five patients, also suggests that – in some cases – the 25% dosage may be too low. Though these data are not large enough to modify current dosage recommendation, it seems advisable to perform therapeutic drug monitoring of rifabutin levels where available.

Rifabutin usual adult dose is 300 mg QD; the product is available as 150 mg capsules. In order to approach the dosage of 75 mg daily when co-administered with LPV/r, thanks to long rifabutin half-life (around 45 hours [5]), one capsule can be taken every second day (QOD).

Rifabutin is not registered in Poland; this is also the case in many other countries. Even in the U.S., rifabutin is approved only as MAC prophylaxis for HIV patients [5, 21], though its use against latent or active tuberculosis in HIV patients treated with PIs or NNRTIs is advised by many [1, 2, 8, 17, 21]. Unfortunately, its cost is much higher than that of widely available, generic rifampin: in the U.S. it is around USD 8 per one capsule [21], in Poland – according to the information collected in several Polish hospitals – it is around PLN 17 per capsule (~ USD 6.15). This leads to monthly therapy costs around PLN 260 (~ USD 90) when rifabutin is given together with LPV/r; in comparison with the monthly cost of typical PI-based regimen (LPV/r + zidovudine/lamivudine; PLN 3030 or ~USD 1080) [22], it means 8.6% additional expenditure.

There is a procedure enabling importation and use of rifabutin in Poland after getting special permission from the authorities; this procedure is commonly used by pneumonologic wards and hospitals. Therefore, the use of rifabutin in HIV+ patients with tuberculosis, concomitantly treated with LPV/r, should become a rule, instead of taking the risk of coadministration of rifampin with increased dose of ritonavir or LPV/r.

Conclusions

The patient, who needs concomitant antimycobacterial and anti-HIV drugs remains a therapeutic challenge. The use of efavirenz, which appears to be logical first choice, may not be always possible due to its CNS side effects, or because of already present resistance (K103N mutation).

The next alternative, triple-NRTI regimen, is currently regarded as inferior [1]. PI-based antiretroviral regimens often are the best option, with LPV/r being one of the most common. However, in the view of currently available data, coadministration of LPV/r with rifampin, despite attempts to compensate the interaction with dosage adjustment, is not advisable. In such situations, rifabutin should be a drug of choice. Similar opinion can be found in recently published Medscape CME activity [23].

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title

The influence of protease inhibitors on a frequency of lipid metabolism disturbances occurrence in HIV-1 infected patients

authors

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summary

Highly active antiretroviral therapy (HAART) has had a significant impact on the natural history of human immunodeficiency virus (HIV) infection, leading to a remarkable decrease in its morbidity and mortality. Progressive changes in the distributions of body fat and severe alterations of lipid, glucose and lactate levels challenge the success of a modern antiretroviral therapy. The aim of our study was to asses the risk factors and frequency of lipid metabolism alterations and fat tissue redistribution in HIV infected patients with ongoing HAART including protease inhibitors (PI). In our study hypercholesterolemia as well as lipodystrophy syndrome were observed in approximately 30% of studied population. Both of those disorders were characterised with high coincidence ratio and shared similar risk factors, which include PI administration, HAART continuation for over 24 months, as well as age of 40 or more. Patients yielding those risk factors should be monitoring thoroughly during antiretroviral treatment. In the process of monitoring the dynamics of serum total cholesterol increase can be a helpful marker.

key words

HIV infection, protease inhibitors, hypercholesterolemia, lipodystrophy syndrome

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Introduction

The introduction of new class of an antiretroviral therapy (ARV) – protease inhibitors (PI), in 1996 gave the rationale of modern, highly active antiretroviral therapy (HAART) of HIV infection. Implementation of PI's was a breakthrough in HIV infection therapy, which significantly influenced long-term and durable HIV viral suppression and markedly reduced AIDS related mortality [1, 2]. However, the new ARV regimen associated with the decrease in opportunistic infections prevalence as well as significant life extension, challenged medicine with long-term medication toxicities and adverse effects.

Long-term application of an antiretroviral therapy may be associated with toxic activity in various organs and systems, including liver, pancreas, central nervous system and bone marrow. Among them lipid and glucose metabolism disturbances and fat tissue redistribution – lipodystrophy syndrome (LD) yield important significance [3, 4]. Abovementioned complications are associated with the increase of cardiovascular risk and notably affect quality of life in HIV infected individuals [5].

Many authors linked lipid metabolism disturbances with protease inhibitors administration, nonetheless the exact mechanism is complex and not fully understood [3, 6-8]. Among many factors that contribute to lipid metabolism alterations the use of other antiretriviral therapy classes (nucleoside reverse transcriptase inhibitors – NRTI's and non-nucleoside reverse transcriptase inhibitors – NNRTI's) are listed [9]. Moreover the influence of factors other than ARV for example age or body mass index (BMI) is underlined [10]. Current concepts concerning this issue are reviewed in Wohl DA et al. [11]. Taking into consideration multivariable model of lipid metabolism alterations during ARV the evaluation of potential the risk factors seems to be of a great importance.

The aim of this study was to evaluate the prevalence and risk factors of lipid, glucose metabolism alteration and fat tissue redistribution in HIV infected patients undergoing HAART including PIs.

Patients and methods

Studied population consisted of 57 HIV-1 infected patients undergoing HAART including at least one PI or NNRTI. Patients were evaluated before ARV introduction and in the third, sixth and every six months following. The mean period of observation was 19 months (min. 6, max. 45). During each visit physical examination was performed and total cholesterol and its fractions, triglycerides and fasting glucose were measured. Hypercholesterolemia were defined as total cholesterol exceeding 240 mg/dL (6,2 mmol/L, grade 2) observed at least two times [12]. Clinical characteristics of studied population are presented in Table 1.

In order to evaluate ARV effectiveness HIV-RNA was measured by use of RT-PCR (Amplicor, Roche Diagnostics, Switzerland) with range of 50 – 75,000 cp/mL and CD4 and CD8 T lymphocyte count by use of three-color flow cytometry (Beckton-Dickinson, Franklin Lakes, NJ USA).

Existence and severity of fat tissue redistribution was evaluated on the base of physical examination and patient self-assessment questionnaire. Lipodystrophy syndrome was defined as coexistence of peripheral lipoatrophy and central lipohypetrophy. Patients receiving other than ARV medication that could contribute to lipid metabolism and fat distribution alterations were excluded. Informed consent was obtained from each patient.

Table 1. Clinical characteristics and laboratory parameters of studied population prior to ARV introduction

Clinical characteristics	
Sex (%) • Men • Women	 37 (65%) 20 (35%)
Median age, years (min., max.),	34 (21-45)
Route of HIV infection • IVDU • Sexual	 39 (68%) 18 (32%)
HIV and HCV co-infection	41 (72%)
Mean period of observation, months (min., max.)	19 (6-45)
Laboratory parameters prior to ARV	
CD4 count /mL, (median, 95% CI)	122 (96-185)
HIV-RNA, cp/mL, (median, 95% CI)	65,000 (46 – 182 x103)
Total cholesterol mg/mL, (median, 95% CI)	155 (144-178)
HDL cholesterol mg/mL, (median, 95% CI)	49 (44-63)
Triglycerides mg/mL, (median, 95% CI)	165 (146-182)

Data were presented as means (±SD) or median (95% CI). The significance of differences was calculated by non-parametric Mann-Whitney U test. For correlation analysis, the Spearman non-parametric correlation was used. For multivariate analysis linear multiple regressions were performed. A $P < 0.05$ was considered statistically significant. Statistical analyses were performed with Statistica 5.0 for Windows software (Statsoft Inc., Tulsa, USA).

Results

During the antiretroviral treatment gradual increase in mean total cholesterol and triglycerides concentrations was observed. The mean increase in total cholesterol levels was 21% in third month, nearly 30% in 12-th, over 50% in 24-th and over 70% in 30-th month ($P < 0.001$ in respect to initial concentration). Similar trend was observed in respect to triglycerides concentration, $P < 0.01$ between initial concentrations and those observed in 18-th month, and $P < 0.001$ in 24-th and 30-th month of ARV (Figure 1). Further analysis included differences between PI and NNRTI-based regiments. The statistical significances in total cholesterol concentrations were obtained in 3-rd (215 v. 163mg/dL, respectively, $P = 0.005$) and 6-th month of ARV (234 v. 151mg/dL, respectively, $P < 0.001$).

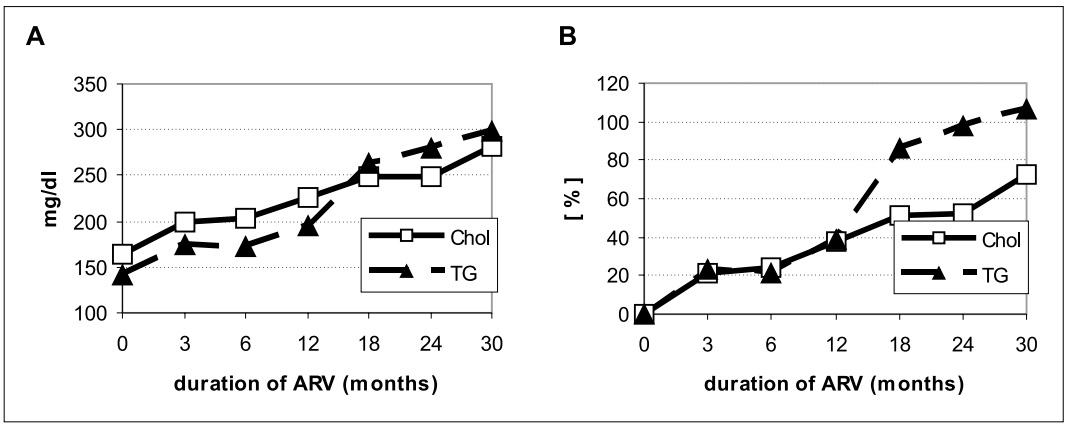

Figure 1. The mean total cholesterol and triglycerides concentrations (A) and their mean increase (B) during ARV in studied population

Table 2. Comparison of selected clinical and initial biochemical parameters in individuals without fat tissue redistribution (LD-) and with lipodystrophy syndrome diagnosed (LD+)

	LD – (n = 41)	LD + (n = 16)	P
Sex (%) • Men (n = 37) • Women (n = 0)	 28 (68%) 13 (32%)	 14 (87.5%) 2 (12.5%)	NS
Age, years (median, min., max.),	33 (21-45)	40 (31-51)	0.003
Route of HIV infection • IVDU (n = 39) • Sexual (n = 18)	 32 (78%) 9 (22%)	 9 (56%) 7 (44%)	NS
HIV and HCV co-infection	32 (78%)	7 (44%)	NS
BMI (median, 95% CI)	22.8 (20,8-23,9)	21.7 (18,3-24,9)	NS
CD4 count/mL (median, 95% CI)	96 (86-185)	128 (64-279)	NS
Cholesterol mg/mL (median, 95% CI)	159 (142-189)	166 (121-215)	NS
HDL mg/mL (median, 95% CI)	55 (51-65)	39 (24-51)	0.01
Triglycerides mg/mL (median, 95% CI)	92 (85-138)	208 (151-311)	<0.001

Table 3. The risk factors of hypercholesterolemia and lipodystrophy syndrome developement in studied population

Risk factor	Hypercholesterolemia		Lipodystrophy syndrome	
	Relative risk (RR)	P	Relative risk (RR)	P
Age over 40	7,5	<0.001	3.35	0.03
Sexual route of HIV-infection transmission	3.0	0.02	2.8	0.02
Treatment with PIs	1.5	0.05	6.58	0.01
Duration of HAART > 24 months	5.0	0.003	13.6	0.02

The mean total cholesterol and triglycerides concentrations exceeded high values (ie. 200 mg/dL and 240 mg/dL [12]) in 18-th month of ARV. The positive correlation between total cholesterol concentration and age of patient was shown ($r = 0.56$, $P = 0.002$ in 6-th month and $r = 0.48$, $P = 0.03$ in 12-th).

During the period of observation hypercholesterolemia was diagnosed in 15 (26.3%) patients. The incidence of this complication was associated with fat tissue redistribution ($\chi 2 = 11,7$, $P < 0,001$), on the contrary no association with sex, CD4 count or HIV viral load were shown. The risk factors of hypercholesterolemia development in our study were: age over 40 (RR = 7.5), sexual route of infection (RR = 3.0), HAART lasting over 24 months (RR = 5.0) and PI-based ARV (RR = 1.5), (Table 3). On the contrary NNRTI-based regiments were associated with lower risk this complication (RR = 0.3).

In the observation period no significant increase in mean glucose concentration was observed, however the values exceeding 110 mg/dL were significantly more frequently observed after 12-th month of treatment (in 4% of patients in 6-th, 14% in 12-th, 16% in 18-th and in 12% in 24-th month of ARV).

Lipodystrophy syndrome was diagnosed in 16 (28%) patients. Demographic characteristics and selected biochemical parameters of are presented in Table 2. Every patient with LD diagnosed was treated with at least one PI (RR = 6.58). Among other risk factors age over 40 (RR = 3.35) or HAART duration over 24 months (RR = 13.6) were identified, (Table 3).

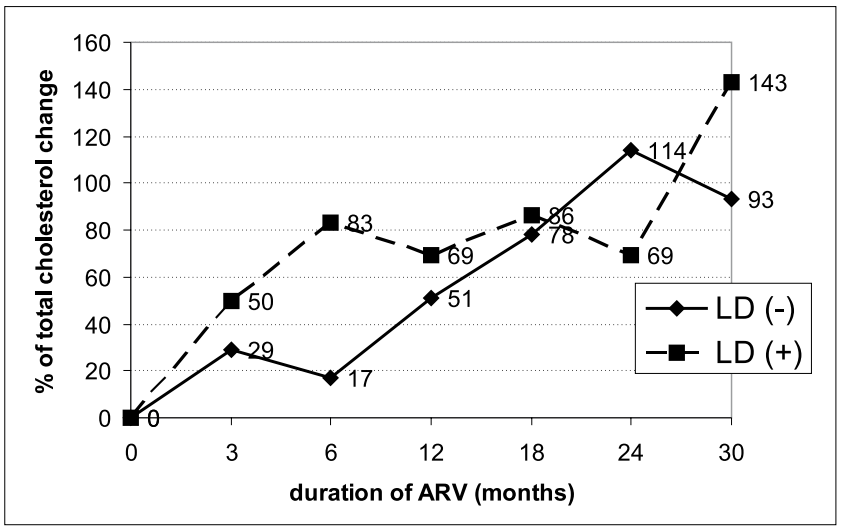

Figure 2. The change of mean total cholesterol concentration in relation to initial values in patients witout (LD-) and with lipodystrophy syndrome (LD+)

Nevertheless the initial total cholesterol concentrations did not differ in patient with lipodystrophy syndrome and without fat tissue alterations, in the first group author observed more rapid dynamics of total cholesterol increase in first 6 months of ARV (Figure 2). For that reason we introduced the parameter of >50% increase in total cholesterol during first 6 months of HAART. This parameter was associated with increased risk of lipodystrophy syndrome occurrence (RR = 3.5) and also yielded high positive predictive value (PPV = 82%) whereas negative predictive value of LD was 62,5%.

Discussion

Lipids metabolism alterations in HIV-infected patients were described prior to era of modern ARV treatment. Increased triglycerides concentrations as well decrease in HDL cholesterol were observed in advanced stages of an HIV infection [13]. However the introduction of HAART put this issue in special interest of clinicians and scientists. The majority of publications linked lipids disorders with PI administration [3-6]. The results of our study as well showed that PI administration significantly increases the risk of hypercholesterolemia as well as fat tissue redistribution. The prevalence of those disorders according to different authors varies between 28–80% of that hypercholesterolemia affects 10-50% of patients receiving PI-based HAART [3, 4, 14]. In our study hypercholesterolemia and lipodystrophy syndrome affected around 30% of studied population. There were no differences between various used PI's and the prevalence of abovementioned disorders (data not shown).

Another factor affecting lipid metabolism and fat distribution in HIV-infected patients is duration of ARV. Martinez et al. calculated that every six months of HAART increases the risk of lipodsytrophy syndrome occurrence 1.57 times. In our study the duration of HAART exceeding 2 years was the strongest prognostic factor and increased the risk of hypercholesterolemia five times and lipodsytrophy thirteen times. Furthermore mean concentrations of total cholesterol and triglycerides exceeding high values (ie. 200 mgl/dL and 240 mg/dL respectively) in 18-th month of ARV.

It was shown that age and initial BMI before ARV introduction have a significant predictive value of lipids metabolism disturbances occurrence [10]. We found a positive correlation between total cholesterol and age. Moreover age over 40 significantly increased the risk of hypercholesterolemia (RR = 7.5) and lipodsytrophy syndrome development. Interestingly, in our study the risk of metabolic disturbances was associated with sexual route of HIV infection acquiring. Similar relation was showed by Martinez et al. [4].

In our study hypercholesterolemia and fat tissue redistribution more often coexisted together. Despite of the lack of differences in initial total cholesterol serum concentrations in patients with and without lipodystrophy syndrome, the first group showed higher dynamics of cholesterol raise during ARV. Introduced parameter of over 50% total cholesterol increase during first 6 months of HAART showed high positive predictive value for lipodystrophy syndrome development (PPV = 82%). The possibility of prediction of the lipodystrophy syndrome occurrence seems to be of a great importance, especially when the irreversibility as well as pharmacotherapy difficulties are taken into consideration [15, 16].

In summary, hypercholesterolemia and lipodystrophy syndrome affected almost one third of studied HIV-infected population during ARV. Both disorders showed high coincidence and common risk factors, among them age over 40, PI-based ARV and HAART duration over 24 months seemed to be of a major magnitude. Individuals possessing those risk factors should undergo close monitoring, in which the dynamics of total cholesterol increase could be helpful.

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title

Assessment of metformin therapy effect on selected parameters of glucose and lipid metabolism and the course of lipodystrophy in HIV-1 infected patients receiving protease inhibitors – pilot study

authors

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summary

Background

Abnormalities in glucose and lipid metabolism as well as the occurrence of central adiposity are the most serious adverse effects of HAART (*highly active antiretroviral therapy*). They occur as the result of insulin resistance and hyperinsulinaemia. Metformin can reduce these abnormalities.

The aim of the pilot study was to find out if there was any influence of metformin therapy on insulin level and selected lipid metabolism parameters as well as the possibility of stopping lipodystrophy progression in HIV-1 infected patients receiving protease inhibitors.

Material and methods

Twenty four HIV-1 infected subjects receiving protease inhibitors were enrolled into the study. Among them were 13 patients who got metformin and 11 patients of a control group. The time of observation was 6 months. At the beginning of the study and after 6 months the following parameters were tested: plasma HIV-RNA level, CD4(+) T cell count, level of insulin, free fatty acids, total cholesterol, HDL-cholesterol fraction, triglycerides, as well as BMI, the ultrasound assessment of the adipose tissue. Statistical analysis of the results was then performed.

Results

After 6 months of follow-up there were statistically no significant changes in insulin, total cholesterol, triglycerides, free fatty acids and HDL-cholesterol levels, compared with the results obtained at the beginning of the study both in the group receiving metformin and in the control. There were also no other statistically significant differences between the two groups. Additionally a positive influence of metformin on redistribution of abdominal adipose tissue and reduction of subcutaneous adipose tissue was observed in a group of patients with lipodystrophy.

Conclusions

1. Metformin is not a useful drug in the therapy of metabolic abnormalities (glucose and lipids) due to protease inhibitors; 2. Metformin influences adipose tissue redistribution and thus can be recommended in the treatment of HIV-1 infected patients with lipodystrophy, to improve adherence and quality of life.

key words

HIV infection, protease inhibitors, metformin, lipodystrophy

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Introduction

Metabolic abnormalities in glucose (hyperinsulinaemia, insulin resistance, impaired glucose tolerance) and lipid (hypercholesterolaemia, hypertriglycerydaemia) metabolism are the most serious adverse effects of HAART (*highly active antiretroviral therapy*). The lipodystrophy syndrome also includes the occurrence of central adiposity characterized by the accumulation of visceral fat with secondary ailments such as a feeling of fullness and flatulence worsening quality of life [1,2,3].

The observed lipid abnormalities among patients receiving antiretroviral therapy are mainly due to insulin resistance and hyperinsulinaemia. However, the pathomechanism of these abnormalities is not understood in detail. They are known to be caused by the use of protease inhibitors, which are often the basis of antiretroviral therapy [2,4]. Hypercholesterolaemia and hypertriglycerydaemia can result in cardiovascular complications, including myocardial infarction, and pancreatitis. Extremely advanced lipodystrophy can lead to serious disability [5]. As yet, there is no effective method to treat metabolic abnormalities caused by protease inhibitors. The only way to stop lipodystrophy is to cease antiretroviral therapy, which quickly results in irreversible progression of HIV infection and worsening of immune function [2,6]. Attempts to use fibrate derivatives based therapies in cases of high cholesterolaemia and/or triglycerydaemia were made. However, due to their limited effectiveness, ie. little influence of this group of drugs on stopping the progress of lipodystrophy, numerous interactions between fibrates and antiretroviral drugs, as well as their high cost, their use is limited in clinical practice [1,2,7].

In the last few years reports have been made on biguanide derivatives' (metformin) positive effect on glucose and lipid metabolism deranged by antiretroviral therapy as well as the possibility of putting stop to or even regression of central adiposity [1,8,9,10,11,12,13,14]. Metformin acts by restoring sensitivity of insulin receptors, and thus normalizing insulin concentration as well as other parameters of lipid metabolism. All this leads to a stop or regression of lipodystrophy in HIV-1 infected patients receiving protease inhibitors as part of their antiretroviral therapy.

Aim of the study

The aim of the pilot study was to find out if there was any influence of metformin therapy on insulin and selected lipid metabolism parameters as well as the possibility of stopping or even regressing lipodystrophy in HIV-1 infected patients receiving protease inhibitors. Study was made with the consent of Ethic Committee of Medical University in Wroclaw.

Material and methods

Twenty four HIV-1 infected patients receiving HAART containing protease inhibitors were enrolled into the study. Patients with diabetes were excluded.

The follow-up period lasted 6 months. We decided on this follow-up period after other researchers, also assuming that this is the optimal time to observe the effects of metformin on the parameters under study [8,9,15,16].

The patients were divided into two groups:

1. group receiving metformin (Metformax, Polfa Kutno S.A) 500 mg twice a day for 6 months (13 patients)
2. control group (11 patients).

All the patients enrolled into the study continued the previous antiretroviral therapy with 2 nucleoside analogues (zidovudine + lamivudine) and 1 protease inhibitor (saquinavir) boosted with ritonavir.

The following examinations were performed at the beginning of the study and at the end after 6 months:

- plasma HIV-RNA level by polymerase chain reaction method (RT-PCR; Amplicor HIV-1 Monitor®; Roche Molecular Systems),
- T CD4(+) lymphocyte count in peripheral blood with the use of the flow cytometry method (Becton Dickinson FacsCount),
- insulin fasting level in peripheral blood with the use of the immuno-enzymatic method (MEIA); Abbott Diagnostics,
- fasting: fatty acids, total cholesterol, HDL-cholesterol fraction, triglycerides levels in peripheral blood,
- physical examination,
- BMI (body mass index)
- ultrasound

The ultrasound assessment of the adipose tissue was performed at the beginning of the study and after 6 months of follow-up. The assessment was performed at three points:

- point 1 – subcutaneous adipose tissue, measurement 5 cm from the navel
- point 2 – subcutaneous adipose tissue, measurement 2 cm from the left nipple
- point 3 – abdominal adipose tissue in the omentum region, measurement between the posterior surface of the left lobe of the liver and the anterior wall of the aorta.

The subcutaneous adipose tissue measurement was performed in 13 patients (8 in metformin group and 5 in control group) with the use of a 7.5 MHz linear probe, and the abdominal adipose tissue measurement was performed with the use of a 3.5 MHz Convex probe. An Aloka SSD 1100 apparatus was used. All patients under study were right-handed, which excludes any possible differences in subcutaneous adipose tissue thickness of the left nipple region among right- and left-handed patients.

Statistical analysis

For the comparison of average results of feature distribution which was approximately normal ($p > 0.05$) in both groups, the *t*-Student test was used.

For feature distribution other than normal ($p<0.05$) in either of the groups, the non-parametric *U* Mann-Whitney test was used.

For the results of the insulin, triglycerides, total and HDL cholesterol as well as free fatty acids levels between the group receiving metformin and the control the Wilcoxon matched pairs test was used.

Results

Both groups under the study (patients receiving metformin and the control group) did not differ according to the age (av. 35 vs. 33.6 years), gender, duration of HIV-1 infection and previous antiretroviral therapy, including protease inhibitors (av. 18 months), T CD4(+) lymphocyte count (av. 454 vs. 437 cell/µl.) and HIV RNA level (av. 6189.1 vs. 21193.7 copies/mL – statistically insignificant difference).

In 3 out of 13 patients receiving metformin the drug was stopped due to diarrhoea. Other patients tolerated the therapy well and no adverse events were observed.

After 6 months of therapy no statistically significant differences in insulin, triglycerides, total and HDL cholesterol as well as free fatty acids levels between the group receiving metformin and the control were observed. The detailed results of the study are shown in Table 1. Glucose levels, before treatment and after 6 months were within the normal ranges.

Table 1. Patients characteristics (average results)

	group receiving metformin	control group
Number	10	11
Age	35	33,6
Gender	7M 6F	8M 3F
T CD4 lymphocytes (cells/uL)	454.3	437.5
HIV RNA (copies/mL)	6189.1	21193.7
BMI *at the beginning of the study* *after 6 months*	 23.26 23.16	 23.86 22.81
Insulin (mIU/mL) *at the beginning of the study* *after 6 months*	 11.9 26.97	 11.1 13.0
Apolipoprotein A (g/L) *at the beginning of the study* *after 6 months*	 2.386 1.563	 2.028 1.806
Apolipoprotein B (g/L) *at the beginning of the study* *after 6 months*	 1.244 0.997	 1.048 1.193
Free fatty acids (µmol/L) *at the beginning of the study* *after 6 months*	 0.59 0.459	 0.444 0.633
total cholesterol (mg/dL) *at the beginning of the study* *after 6 months*	 216.4 208.8	 202.5 185.0
HDL-cholesterol (mg/dL) *at the beginning of the study* *after 6 months*	 44.21 43.2	 43.65 43.03
triglycerides (mg/dL) *at the beginning of the study* *after 6 months*	 369.6 305.48	 244.01 190.5

Table 2. Ultrasound adipose tissue examination

	Group receiving metformin	Control group
Number	8	5
Age	22-49	32-50
Gender	7 M, 1F	5M
Initial examination (mm)		
Point 1	av. 15.9 /11.2 – 19.2/	av. 14.24 /7.4 – 22.9/
Point 2	av. 15.97 /8.4 – 25.4/	av. 13.6 /9.0 – 18.5/
Point 3	av. 21.28 /11.7 – 33.2/	av. 16.42 /11.0 – 23.1/
Follow-up examination (mm)		
Point 1	av. 13.22 /9.4 – 17.1/	av. 6.42 /0.84 – 14.2/
Point 2	av. 11.3 /4.9 – 20.2/	av. 3.25 /0.68 – 12.9/
Point 3	av. 18.46 /10.2 – 33.2/	av. 24.6 /19.0 – 28.07/

In both groups no differences in thickness of subcutaneous and abdominal adipose tissue were seen in the examination performed at the beginning of the study.

A reduction in subcutaneous tissue in both groups was observed in the follow-up examination. However, this effect was more visible in the control group.

A reduction in abdominal adipose tissue was seen in the follow-up examination of the group receiving metformin (6 patients). A reduction in thickness of abdominal adipose tissue by over 50% compared to the measurements performed at the beginning of the study was observed in 5 patients administering metformin. In this group, an average reduction in the thickness of adipose tissue in the omentum was approximatelly 3 mm. This effect was not shown in the control group where the adipose tissue growth in the omentum was observed in the majority of the patients (an average of 8 mm after 6 months). The results are presented in Table 2.

Discussion

Use of protease inhibitors is regarded as the main cause of central adiposity syndrome with the excessive growth of visceral adipose tissue [2,3,4,5,6,10,11], usually accompanied by an increase in cholesterol and triglycerides levels. The main reasons for these abnormalities are insulin resistance and hyperinsulinaemia [8,11,13]. The etiopathogenetic relation between these symptoms and antiretroviral therapy remains unexplained. The existence of two mechanisms is stipulated: the direct one which assumes an impairment of cellular glucose use as a result of action of antiretroviral drugs and the indirect mechanism related to the redistribution of adipose tissue: central adiposity and/or peripheral lipoatrophy. [13] Among drugs that restore sensitivity of peripheral receptors to insulin, and thus reduce insulin levels, hypertriglycerydaemia and stop lipodystrophy, metformin is the most promising one [8,15,16].

Based on the results of previous reports, taking care of an increasing number of patients with lipodystrophy syn-

drome due to antiretroviral therapy, we started to use metformin to diminish the symptoms of this abnormality. We also assessed the effects of the drug on selected biochemical parameters and thickness of adipose tissue in selected body regions. The choice of biochemical parameters was dependent on the pathomechanism of lipodystrophy and the mechanism of metformin action [7,9,10].

Our pilot study has not shown any noticeable effects of metformin treatment on the reduction of insulin levels. In the study of Hadigan et al. and Kohli et al, in a group of patients similar in number and duration of follow-up treated with metformin, a significant reduction in insulin levels was observed in comparison to the control [8,11,15]. Considering our results and those presented by Hadigan et al. [8,15], it will be possible to draw unequivocal conclusions only after studying larger groups of patients.

As for parameters of lipid metabolism, throughout the 6-months follow-up period, no statistically significant changes were observed both in the group receiving metformin and in the control. Similar results were obtained by Calza et al. [7], Hadigan et al. [15] and Kohli et al. [11]. On the other hand, other authors reported a reduction in total cholesterol, HDL-cholesterol and apolipoproteins A and B levels as the effect of metformin treatment. However, according to most researchers there were only minor fluctuations [2,5,9,11,17]. It is well known that in HIV-1 negative patients with type II diabetes, metformin normalizes lipid metabolism parameters such as total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides. There is lack of such an effect in HIV-1 infected patients with mixed hyperlipidaemia [15]. In our opinion the 6-months treatment period, long enough for an improvement in the control group but perhaps too short for achieving the same effect in HIV-1 infected individuals, is less significant.

Ultrasound measurement of adipose tissue, both subcutaneous and abdominal, is a valuable complementary method for the assessment of thickness of adipose tissue in patients with lipodystrophy as a result of antiretroviral therapy [18,19,20].

Ultrasound examination performed in the course of the study and after the 6-month follow-up period in the group receiving metformin showed a significant reduction in the thickness of abdominal adipose tissue.

Our results corroborate the study of Hadigan et al. and Kohli et al., according to whom metformin treatment was related to the reduction in the volume of abdominal adipose tissue and reduction of subcutaneous adipose tissue [8,11].

According to literature, the choice of measurement region is of great importance. In the study of Martinez et al. [16,19] ultrasound measurements of subcutaneous adipose tissue at the navel, humerus and zygomatic bone regions as well as abdominal adipose tissue were performed. The most useful were arm and zygomatic bone regions, regardless of sex. In our study, due to the predominance of male patients, other measurement points were selected for the group receiving metformin, which according to authors can be equally useful in the assessment of redistribution of adipose tissue. This was corroborated by the results obtained in our study which also indicates a reduction in subcutaneous adipose tissue at navel and nipple regions in the group receiving metformin.

As metformin treatment of HIV-1 infected patients runs a risk of lactic acidosis [3,14,21], the patients were also screened for the symptoms typical for acidosis which was not observed in any of the patients.

The possible positive effect of metformin on lipodystrophy syndrome would be an extremely valuable observation. Attempts to use other drugs (e.g. growth hormone, androgens) did not yield such good, if not thoroughly unsatisfying, results. The high cost and numerous adverse events are just few of additional problems with growth hormone [16]. Most of the studies (including ours) are unfortunately based on small groups of patients, which is an additional factor that makes it difficult to make a complete assessment of metformin's influence on lipodystrophy syndrome in HIV-1 infected individuals receiving antiretroviral therapy. Yet it justifies the need for further studies.

Conclusions

1. Metformin is not a useful drug in the therapy of metabolic abnormalities (glucose and lipids) due to protease inhibitors.
2. Metformin influences adipose tissue redistribution and thus can be recommended in the treatment of HIV-1 infected patients with lipodystrophy, to improve adherence and the quality of life.

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title

HIV-Associated Plasmablastic Lymphoma Following HAART-Related Immune Reconstitution

authors

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summary

Plasmablastic lymphoma is a unique AIDS-related lymphoma. Data from the pre-highly active antiretroviral therapy (HAART) era shows that they portend a poor prognosis. However, recent reports suggest that these lymphomas may respond to HAART. We describe a case of EBV-associated plasmablastic lymphoma in a 39-year-old homosexual man with concomitant pulmonary cryptococcus that was aggravated following the initiation of HAART. The exacerbation of plasmablastic lymphoma following HAART in this case may represent a novel manifestation of the immune reconstitution inflammatory syndrome. This report highlights the fact that the optimal time to commence HAART in this setting remains to be established.

key words

HAART, lymphoma, reconstitution

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Introduction

Plasmablastic lymphoma is a unique AIDS-related non-Hodgkin lymphoma (NHL) that presents as a rapidly growing destructive tumor, typically arising in the setting of a low CD4 count.[1] These lymphomas have been reported to occur in several locations including the jaws and oral cavity, lung, mediastinum, bowel, nose, skin, testes, soft tissue, lymph nodes, brain and bone marrow.[2-5] Lymphoma cells are characterized by a plasmacytoid morphology and plasma cell phenotype. Unlike myeloma, the lymphoma cells are EBV positive and often HHV8 negative.[5]

To date, there have been too few cases of plasmablastic lymphoma reported to reliably determine their optimal management. Available data from the pre-HAART era shows that these novel lymphomas carried a poor prognosis, with a dismal median survival of approximately 5 months.[6] Based on only a few case reports, it has been proposed that immunologic and virologic control with HAART may be beneficial in the treatment of plasmablastic lymphoma, as well as may help maintain continued complete remission.[7-8] Nasta and colleagues described an HIV-infected man who presented one month after discontinuation of HAART with plasmablastic lymphoma of the mediastinum.[7] After restarting their patient on his HAART regimen, the bulky mediastinal mass completely resolved. Treatment with CHOP chemotherapy was only started by Nasta *et al* when the patient presented with recurrent disease, which resulted in a further remission. Lester *et al* reported another HIV-positive patient with plasmablastic lymphoma who had a complete response to HAART lasting 14 months, followed by relapse several months after an antiretroviral holiday.[8] Their patient went into complete remission eight months from the diagnosis of relapse after having received a full course of combination chemotherapy with modified. CHOP.

We report a case of HIV-associated plasmablastic lymphoma exacerbation following HAART, which may represent a unique manifestation of the immune reconstitution inflammatory syndrome.

Case report

A 39-year-old homosexual man presented with severe fatigue, fevers, night sweats, and a 15-pound weight loss. He was found to be HIV positive with an initial CD4 count of 3 cells/μm (CD4/CD8 ratio of 0) and HIV viral load of 321,000 copies/mL. He weighed 139 pounds and appeared cachetic. There was no palpable lymphadenopathy or organomegaly. Laboratory tests showed a decreased hematocrit of 22.7%. Bone marrow biopsy showed a high-grade plasmablastic lymphoma occupying up to 30% of the marrow cellularity. He was started on antiretroviral therapy with boosted atazanavir, tenofovir and emtricitabine. Shortly thereafter he was diagnosed with pulmonary cryptococcus for which he received fluconazole. Prior to starting a full chemotherapy regimen (2 months since his initial presentation), the patient experienced dysphagia, and he developed right supraclavicular and cervical masses. His CD4 count at this time was 47 cells/μm and HIV viral load 169,000 copies/mL. Excisional biopsy of the neck mass revealed a plasmablastic lymphoma (Figure 1) similar to that detected in the prior bone marrow biopsy specimen. By immunohistochemistry, tumor cells were demonstrated to be negative for B-cell (CD20, CD79a), T-cell (CD3, CD4, CD5, CD8), and NK cell markers (CD56) as well as negative for cytokeratins (AE1/AE3, CAM 5.2), bcl-2, PAX-5, kappa, lambda, and TdT. Immunoperoxidase stains showed them to be positive for the plasma cell markers CD138 and MUM-1, and a subset were also immunoreactive for CD43 and CD10 (dim). MIB-1 staining showed a proliferative fraction of greater than 90%. Tumor cells were positive for Epstein Barr virus (by EBER in-situ-hybridization), but negative for HHV8 (by LNA immunohistochemistry). A lumbar puncture was negative for lymphoma. The patient was started on systemic chemotherapy consisting of EPOCH (etopside, doxorubicin, vincristine, prednisone and cyclophosphamide) and he also received intrathecal cytarabine for CNS prophylaxis. After the first cycle of chemotherapy, his fevers and night sweats disappeared, and the neck and supraclavicular masses resolved. His CD4 count at that point was 12 cells/μm and HIV viral load undetectable.

Discussion

We report a case of HIV-associated, EBV-related plasmablastic lymphoma in which the initiation of HAART was temporarily associated with an exacerbation of the patient's lymphoma. Gilaberte *et al* described a similar case of a 44-year-old man with AIDS that developed cutaneous plasmablastic lymphoma following treatment with HAART.[9] Similarly, other authors observed four cases of non-Hodgkin lymphoma (NHL) following combination antiretroviral therapy.[10] As a result of potent antiretroviral therapy these four patients reached viral suppression in a mean time of 15 weeks followed by a rise in CD4+ T cells within 16.5 weeks. The diagnosis of NHL was established at a mean time of 36 weeks after HAART was introduced, and 20 weeks after the CD4+ T cell increase was achieved.[10] The authors of this previously reported series believe that the immune reconstitution following HAART was a predisposing factor for the development of NHL in their patients. Also, they report prompt progression of lymphoma in their patients. The outcome in all four of their cases was fatal. Such prior reports support the proposal that lymphoma exacerbation in our case could be ascribed to immune enhancement by HAART, rather than lymphoma progression due to a lack of response to HAART.

It has been shown by other investigators that, despite a decline in HIV viral load and restoration of total CD4+ T cell numbers in patients treated with HAART, EBV load remains unaltered, even after 5 years of therapy.[11] Moreover, those researchers found that for individuals who receive HAART late during HIV progression, as did our patient, lymphoma may develop shortly after initiation of HAART.[11] We are now aware of the fact that the initiation of HAART may, in certain circumstances, worsen clinical findings as a result of an immune reconstitution inflammatory syndrome (IRIS).[12-13] The exacerbation of plasmablastic lymphoma in our patient, and possibly the atypical lymphoid infiltrates present in his gastrointestinal tract, may have been part of an IRIS. Risk factors for IRIS include a low CD4 count, the presence of infection(s) such as cryptococcus and herpes viruses, and a robust virologic and immunologic response to HAART.[13-14] Indeed, our patient

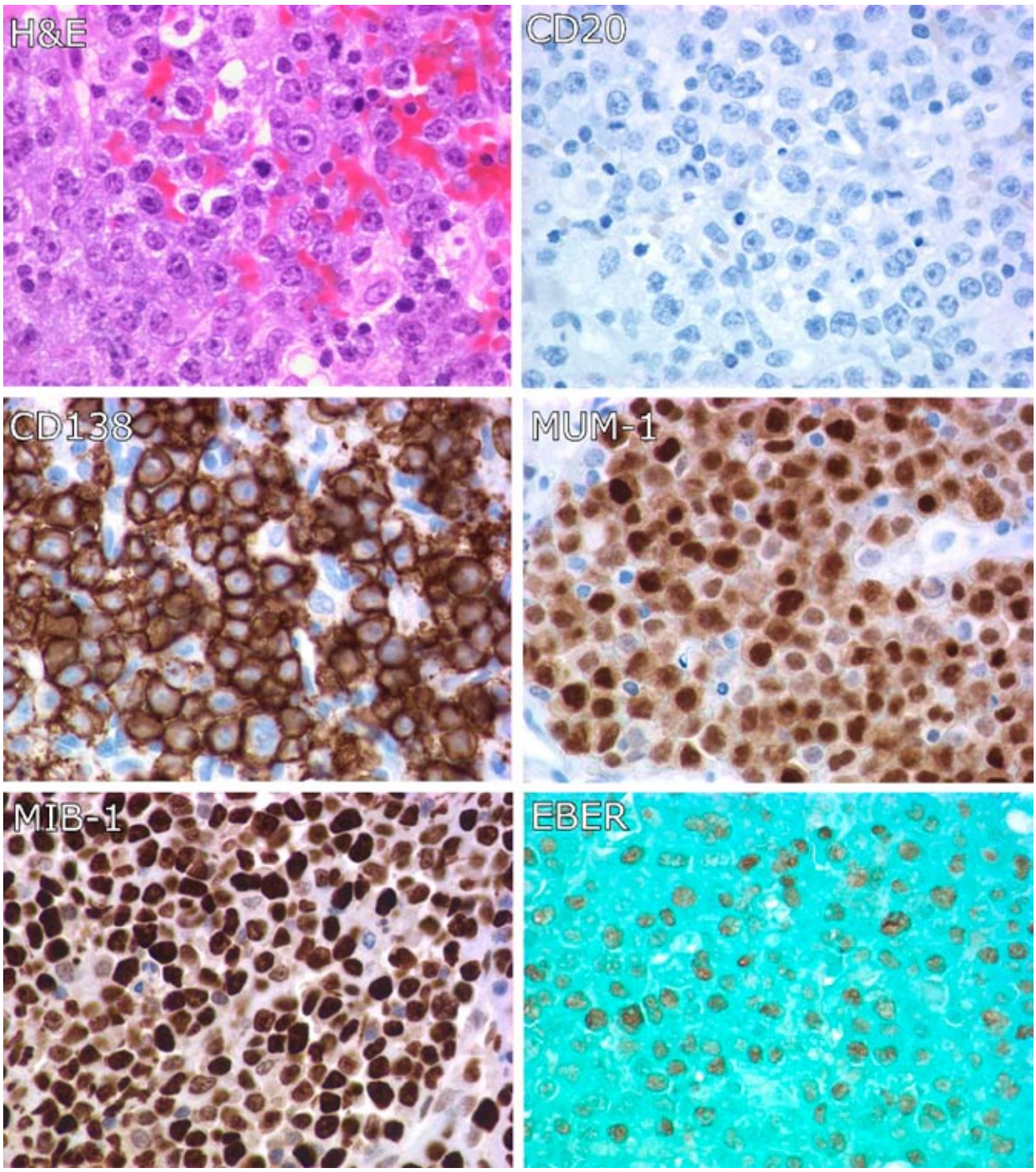

Figure 1. Plasmablastic lymphoma pathology

Top left: High-grade lymphoma comprised of plasmablasts with prominent nucleoli (H&E stain). *Top right:* Lymphoma cells are negative for the B-cell marker CD20. *Middle left:* Lymphoma cells demonstrate cytoplasmic immunoreactivity for the plasma cell marker CD138. *Middle right:* Lymphoma cells are positive for MUM-1. *Bottom left:* Mib-1 staining shows a very high proliferation rate in tumor cells. *Bottom right:* EBER in-situ hybridization is positive in lymphoma cells.

had an abrupt decline in his HIV viral load and rise in his very low CD4 count following HAART, as well as pulmonary Cryptococcus and evidence of a latent EBV infection. Therefore, we speculate that the exacerbation of lymphoma observed in this case may have been avoided by delaying HAART, at least until the cryptococcal antigen load in our patient was reduced by effective antifungal therapy. The optimal time to commence HAART in co-infected patients, especially when they have advanced HIV disease with low CD4 counts, needs to be established.

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title

Slowly progressing cutaneous T-cell lymphoma in HIV infected individual

authors

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summary

We present a case of HIV-infected individual on HAART diagnosed with mycosis fungoides (MF) – a variant of cutaneus T-cell lymphoma (CTCL), in which the prominent increase of peripheral blood CD4+ count was noted although the haematological form of this lymphoproliferative disease – Sézary syndrome (SS) was not confirmed.

key words

T-cell lymphoma, HIV infection

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A 50-year-old homosexual HIV-positive white man presented in November 2002, with multiple erythematous exfoliating pruritic macules localized in the face, trunk, groin, axillae and purulent lesions in perineal region. His past medical history included syphilis successfully treated 20 years earlier. He was in stage B3 of the clinical category of the HIV infection (according to CDC classification) based on fever lasting longer than 1 months. His CD4+ lymphocyte count was 168 cells/mL, CD4+/CD8+ ratio – 0.1 and HIV-viral load – 122 copies /mL. The HAART was introduced (ddI, d4T, NFV). Blood test showed a normal white blood cell count (WBC) with moderate eosinophilia, mild normocytic aneamia, accelerated erythrocyte sedimentation rate (ESR) (115/122 mm) and increased C-reactive protein (143 mg/L). Liver and renal function tests, coagulation screen, platelet count, electrolytes, and urinalysis were within normal limits A diagnosis of generalized seborrhoeic dermatitis was confirmed and topical corticosteroids, a*ntifungals and keratolytics were started resulting only in a mild remission. An antihistaminic drugs were administered to reduce pruritus.*

Over the next two years his skin condition deteriorated and the development of the generalized erythroderma with pruritus and scaling was observed (Fig. 1). Cutaneous eruption became more infiltrative with the thickening of the skin folds in the face (leonine – like facies appearance) (Fig. 2), ectropion of the eyelids, blepharitis. Scattered violaceous plaques and exophytic ulcerated tumours with excoriations were observed on the trunk and extremities. The palms and soles were affected with hyperkeratosis with fissuring of the skin and the onychodystrophy (Fig. 3). The peripherial lymphadenopathy was reported. Repeated skin biopsies were performed, but the diagnosis was equivocal. Microscopy of the first cutaneous biopsy specimen from the dorsum showed hyperkeratosis and focal parakeratosis, epidermal proliferation, massive infiltrate of lymphocytes and histiocytes along with vascular proliferation in the dermis. Skin biopsy repeated in 2006 eventually revealed findings consistent with CTCL corresponding to incipient MF. Immunocytochemical staining showed evidence of CD3(+) CD20(-) lymphocytes. Histopathological examination of lymph nodes biopsies revealed non–specific inflammatory infiltrates. Laboratory parameters taken in April 2006 showed accelerated ESR (62/78 mm), elevated level of C-reactive protein (140 mg/L), increased activity of lactate dehydrogenase (501 U/L). The remaining blood tests: WBC, RBC, platelet count, liver and renal function tests, urinalysis were within normal limits through the four–year observation. Although periodically transient intermittent mild eosinophilia was noted. Moreover some of the serologic tests towards syphilis, USR (unheated serum reagin test), FTA-ABS (fluorescent treponemal antibody absorption test), TPHA (T. pallidum hemagglutination test) were transitory positive, whereas VDRL slide test (venereal disease research laboratories) remained negative. Serological treponemal and non-treponemal tests repeated routinely every 6 months were negative before and after this single incident. Therefore, this phenomenon was explained as a trace of past lues or rather immunological phenomenon concomitant with the presence of cutaneus lymphoma. Interestingly, in the observed period the CD4+ lymphocyte count measured at least twice a year was consistently increasing to 1260 cells/mm^3 and CD4+/CD8+ ratio rose to 0,9. The last measured CD4+ count, in April 2007, was 740 cells/mm^3 and CD4+/CD8+ ratio 1,29 (Fig. 4). This might suggest the development of haematological variant of MF – Sézary syndrome. Therefore the cytometric evaluation of peripheral blood leucocytes was performed. The result was: CD19+ – 10,2%, CD3+ – 86%, CD3+/CD4+ – 39,2%, CD3+/CD8+ – 46,1%, CD16+ 56+ – 2,4%.

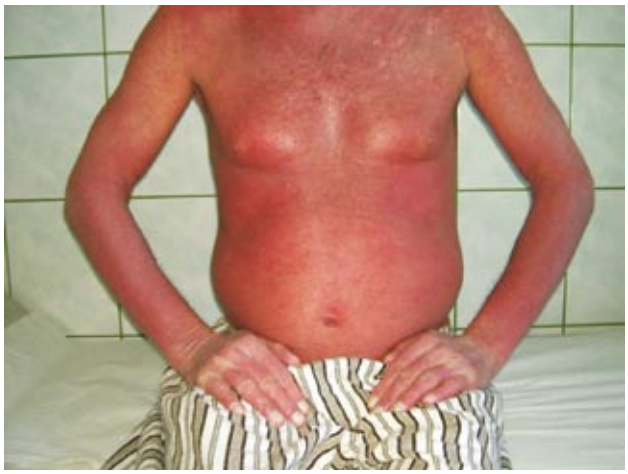


Figure 1.

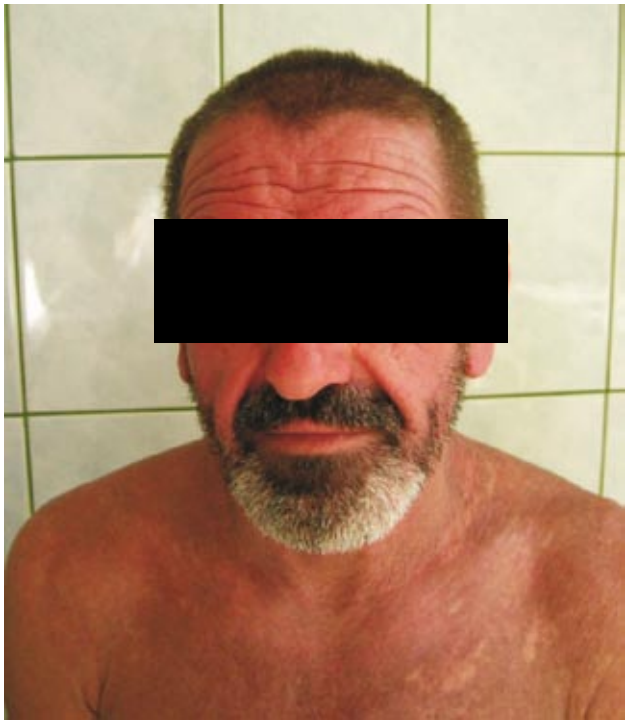
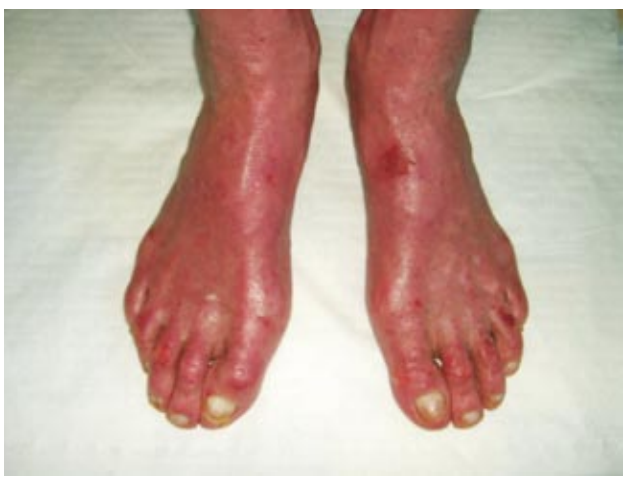


Figure 2.

Figure 3.



This finding was not consistent with the criteria for SS diagnosis. The HIV viral load was 122 copies/ml in the 3rd month of HAART (ddI, d4T, NFV). After the 30 months of viral suppression with undetectable HIV RNA (limit of detection – 50 copies/ml) the rebound was seen and drug regimen was changed to 3TC, TDF, NFV. The viral suppression was achieved and the evaluation of HIV RNA repeated every 6 months was below the LOD. The chest roentgenogram did not disclose any abnormalities. Abdominal ultrasonography revealed mild splenomegaly. No signs of the visceral involvement were present. Since the establishment of the diagnosis, the patient was given three courses of ultraviolet A treatment enhanced with 8-metoksypsoralen (PUVA) and gradual, but transitional improvement was observed. So far, he has not developed any opportunistic infection or has been diagnosed with other AIDS defining illness.

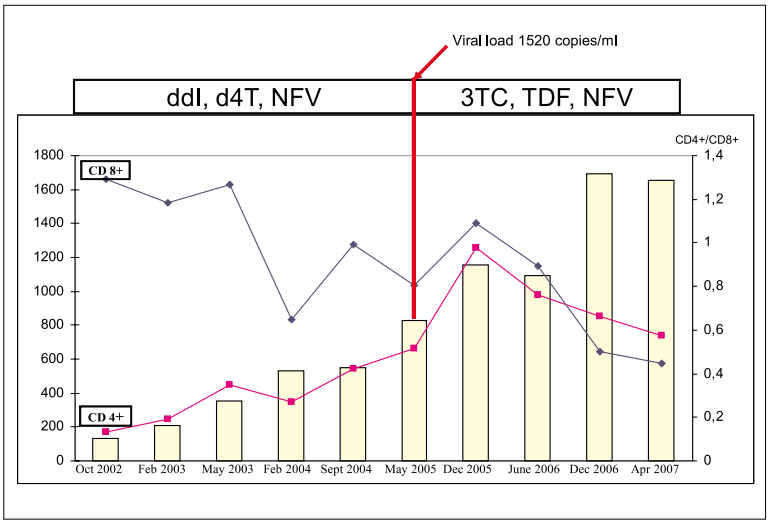


Figure 4.

In HIV infected individuals B-cell NHLs are predominant. However, it was shown that HIV infected individuals are at increased risk of T-cell lineage lymphoproliferative disorders, including MF, SS, peripheral and cutaneus lymphomas, adult T-cell leukaemia/lymphoma[1]. A few cases of CTCL in HIV infection have been reported, among which the predominant were CD8+ T-cell lymphomas[2-5]. In the case of CD4+ T-cell MF followed by SS the CD4+ T-cell infiltrates were shown in skin biopsies and the increase in blood CD4+ count and decrease in blood CD8+ count was observed parallel with the disease progression. Despite the remarkable increase in blood CD4+ count resembling clone expansion, the criteria for SS diagnose were fulfilled in advanced stage of disease. The authors suggest that in condition of CTCL the CD4+ count might be useless to stage the HIV-infection[2]. Mycosis fungoides, which is the established diagnosis of this patient, is a systemic disease even when it appears to be clinically limited only to the skin. MF is a malignant lymphoma manifesting with expansion of the T-cells clone. These cells infiltrate the skin in physiological conditions. Malignant CD4+ cells lose their CD2+, CD5+ or CD7+ T-cells antigens and express cutaneous lymphocyte antigen (CLA) which promotes their adhesion to endothelial selectins in cutaneous postcapillary venules. Although malignant T-cells could leave the skin via lymphatic vessels to the lymphatic nodes and blood, haematological variant of MF called Sézary Syndrome, occurs only in 5% of MF cases. Blood criteria for SS are the following: more than 1000 per mm^3 atypical T lymphocytes with cerebriform nuclei circulating in the peripheral blood or other evidence of a significant increase in relative or absolute T-cell count with the presence of malignant T-cell clone in the blood such as clonal T-cell gene rearrangement (identical to that found in the skin). The T-cell gene rearrangement revealed by molecular or cytogenetic techniques is an expansion of T-cells immunophenotype with aberrant expression of pan T-cells markers (an increase of $CD4^+$ cells, the CD4/CD8 ratio >10, and/or an expansion of T cells with a loss of 1 or more of the normal T-cell antigens is observed in laboratory tests)[6, 7].

There are many unanswered questions until now with regard to this case. Skin biopsy established diagnosis of T-cell lymphoma consistent with MF. Cytometric evaluation of peripheral blood leukocytes did not confirmed the SS, which if positive could explain prominent increase of CD4+ count. Immunological phenomena appearing as a transitory positive syphilis tests could suggest lymphocytes abnormalities in the course of the disease. On the other hand the significant rise in CD4+ count and CD4+/CD8+ ratio might indicate vivid response to the HAART introduction. Thus, immune restoration diseases (IRD) might be considered as well. There are reports suggesting the link between EBV infection and occurrence of HIV-associated B-cell lymphomas despite treatment with HAART. However in HIV related T-cell lymphomas similar association was not proven. The vigorous rise of CD4+, high baseline CD8+ count, effective HIV suppression and progress of skin disease might support the diagnosis of paradoxical immune response termed IRD. Although, in this patient the first dermatological symptoms appeared about one month prior to HAART introduction. His skin condition gradually deteriorated in the course of the disease, despite the transient rebound in HIV viral load in the beginning of 2005 (Fig. 4). Hence, this makes the diagnosis of IRD unlikely.

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I look forward to welcoming you to Warsaw in 2008.

Sincerely yours,

Andrzej Horban, Conference Chairman