

# hiv & aids review

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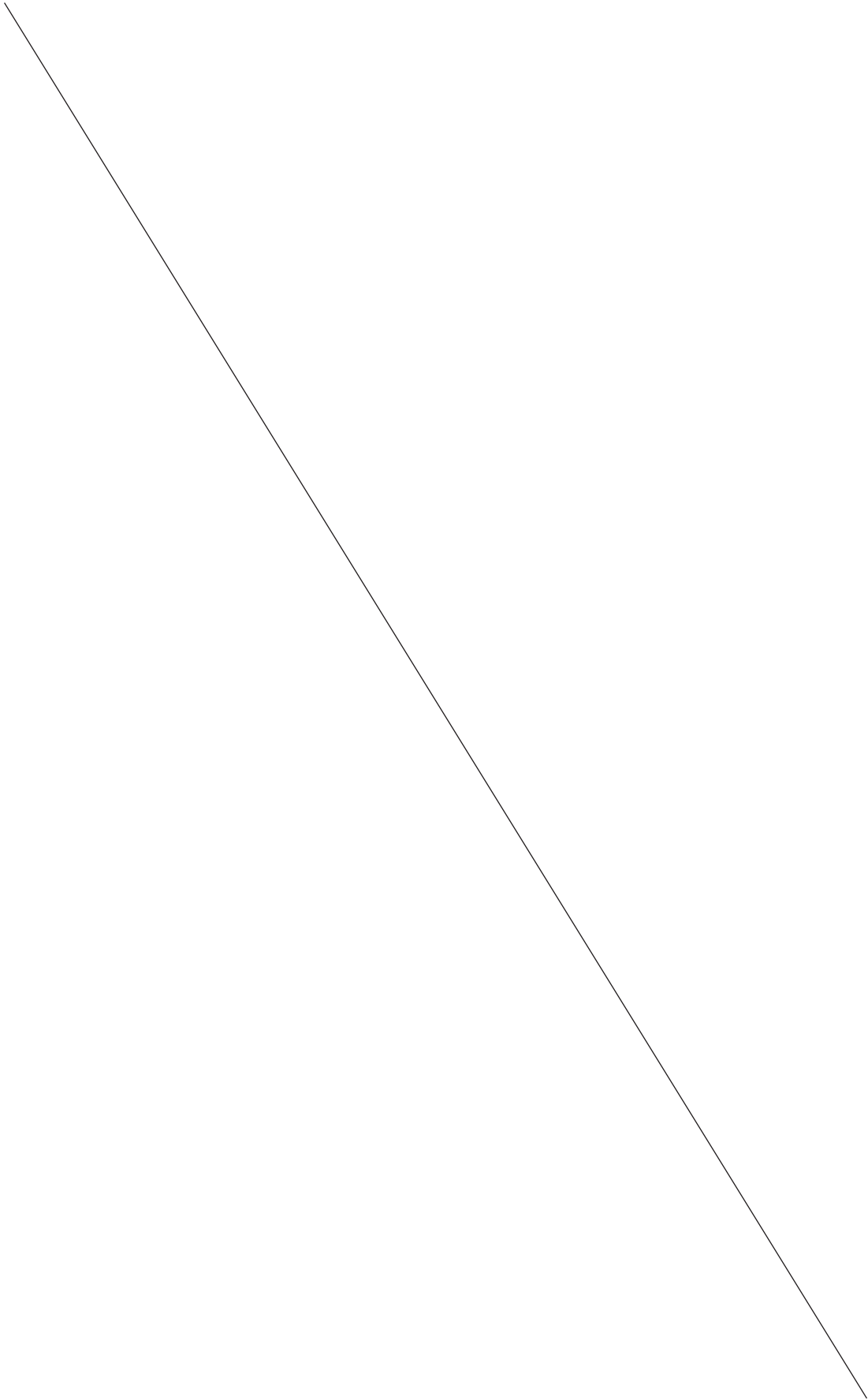
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## Dear Colleagues and Patrons, Dear Readers of HIV&AIDS Review,

It is a great pleasure to present to you this new issue of **HIV&AIDS Review** in my capacity as the new Editor-in-Chief. I trust that you are familiar with our journal, which has been published since 2002. This issue is the first to be published by the Polish Scientific Society of AIDS. We are one of the few journals combining immunology and other medical topics with coverage of the social problems experienced by HIV/AIDS patients.

**HIV&AIDS Review** is a journal focusing on HIV and AIDS treatment and research programs. The articles published in our journal are intended to serve the world-wide medical community as research and reference tools in dealing with the HIV/AIDS global pandemic. We would like to build a platform for sharing experiments, knowledge, and practical experience of dealing with HIV/AIDS. We are also maintaining a central focus on the wide spectrum of problems concerning testing, prevention, medical policies, and the resources available to people affected by HIV/AIDS.

The Journal publishes four issues per year. All papers considered for publication are peer-reviewed. Please allow me to take this opportunity to cordially thank the members of our International Editorial Board for their constant support and constructive collaboration in the time-consuming process of soliciting, evaluating, and reviewing papers. It is because of the devoted commitment of so many scholars that there is hope for people living with HIV/AIDS.

The papers in this issue reflect the current level of knowledge regarding the pathogenesis of HIV, lymphogranulomatous venereum. They examine the increasing problems among the HIV-infected, as well as the importance of physiotherapy for patients with lipodystrophy associated with HART.

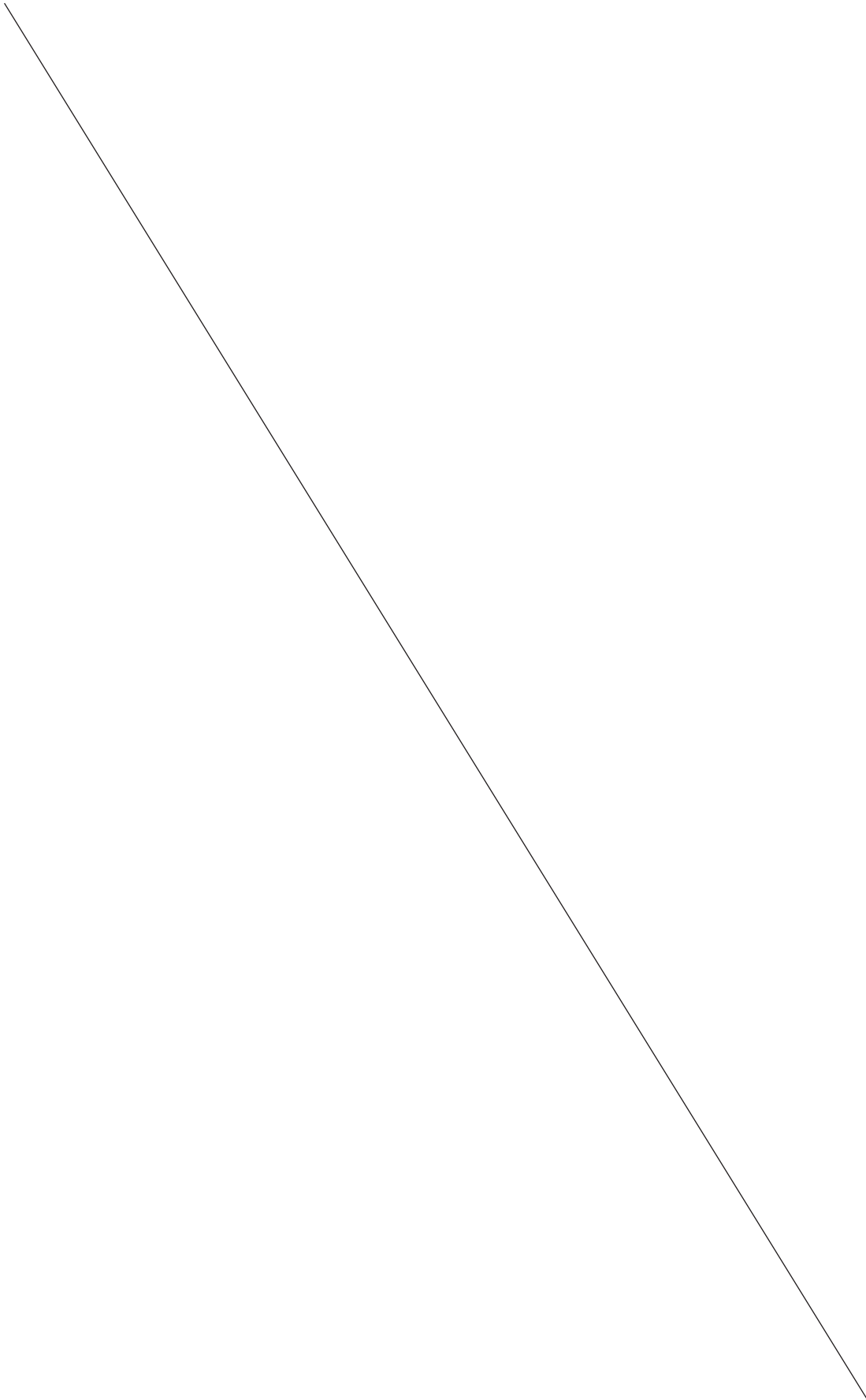
The original articles present new studies regarding the concentrations of IL-22, sFas and sFasL in HCV, HIV, and HCV/HIV infection, the genetic background of cardiovascular complications among HIV-positive patients, problem HIV-1 drug-resistance patterns among treatment-naïve and therapy-experienced patients in Poland, as well as the importance of Tuberculin Skin Test reactivity rates among adults with Human Immunodeficiency Virus in relation to age, transmission mode, and lymphocyte CD4 count.

In the case study report section of this issue, Knysz *et al* examine three different manifestations of immune reconstitution disease in an HIV-1-positive man receiving effective HART.

Starting this new and exciting venture, I would like to encourage you to send your manuscripts to be considered for publication in our journal. We welcome original articles, short communications, and case reports. In a spirit of openness, we would like to explore not only a wide range of clinical discussions, but also the social implications of living with HIV/AIDS. In this sense, our journal seeks to speak on behalf of people living with HIV/AIDS in order to reach out to the larger community. By providing a space for scholars, medical professionals, and people directly affected by HIV/AIDS, we hope to educate ourselves, to grow together, and to be of service to each other.

Thank you for your interest in our journal. Please feel encouraged to submit your papers and proposals. Your comments and suggestions would be greatly appreciated. Please do not hesitate to contact me if I can be of any assistance.

**Alicja Wiercińska-Drapała**  
Editor-in-Chief HIV&AIDS Review



## title

# Pathogenesis of HIV-1 infection – chosen aspects

## authors

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## summary

Immunopathogenesis of HIV-1 infection is very complex but its understanding is vital for creating new and effective antiretroviral treatments and specific prophylaxis. The authors wanted to give the latest insight into the pathogenesis of HIV infection.

## key words

**HIV-1, pathogenesis, immune activation, replication cycle**

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## HIV-1 REPLICATION CYCLE

The virus uses the cell machinery for its own replication leading to many viral and host interactions during its life cycle (1-8). Some of the cell factors (cellular co-factors) aid replication while others (restriction proteins) hinder it (2,3,4,5,8,9). In response, the virus utilizes mechanisms that inactivate restriction proteins allowing further replication.

All the mechanisms lead to immune activation, the key element in HIV-1 immunopathogenesis and disease progression (10,11,12). The elements of immune activation have been described in detail in the following parts.

### a) HIV-1 entry

Entry is facilitated by the CD4 receptor and co-receptors (2,13,14,15)).

CD4 receptors are surface glycoproteins of T cells, thymus and bone marrow precursor T cells, macrophages, monocytes, eosinophiles and microglia of the central nervous system. On binding with the viral glycoprotein gp120, CD4 receptor facilitates further conformational changes leading to the binding of the viral V3 loop with co-receptors (13). Then viral envelope and cell membrane fusion ensues.

Co-receptors which are vital for HIV entry are physiologically chemokine receptors (cytokine receptors that play key role in cell chemotaxis to the inflammation site): CCR5 for beta-chemokines and CXCR4 for alpha-chemokines (2,9,13,14). Depending on the cell tropism, two sets of distinct HIV strains can be described: M-tropic (R5) and T-tropic (X4). R5-tropic strains, which exhibit tropism for CCR5 co-receptors, are predominantly found in new infections and prevail in the early phase of disease (13). On the other hand, X4-tropic strains exhibit CXCR4 co-receptor tropism and are prevalent in the late stage of infection (14). However, R5X4 strains that can utilize both CCR5 and CXCR4 co-receptors have also been described.

Also other co-receptors have been found with a similar role in HIV infection like CCR5 and CXCR4: CCR3, CCR2, CCR8, CCR9 and APJ (in the central nervous system) (2,13,14,19).

Despite the high numbers of co-receptors, CCR5 and CXCR4 remain the most important ones in the pathogenesis of HIV infection. Homozygotes with deletion of the 32nd base pair (delta 32) in the gene encoding CCR5 co-receptor have been shown to be immune to HIV infection as such co-receptors have no outer membrane part. On the other hand, heterozygotes in the delta 32 base pair exhibit a decreased expression of CCR5 and predominate among long-term non-progressors who also tend to have better response to antiretroviral treatment (13).

There is a number of exogenous factors that already in this early phase activate the immune system by scaling up the expression of co-receptors in non-infected PMLs (6). This in turn increases their susceptibility to infection. Among these factors there are opportunistic infections and exogenous interleukin-2 (IL-2). Besides, CMV co-infection has been shown to facilitate HIV entry by coding and expressing co-receptors similar to CCR5 and CXCR4 while HHV-6 co-infection stimulates CD4 receptor expression on delta/gamma lymphocytes and NK (2,9). Other factors facilitating HIV entry include fusion-facilitating adhesion

molecules expression, expression of co-stimulating molecules, secretion of pro-inflammatory cytokines and close inter-cell interaction (2,9,12,15,16).

### b) Immune/infectious synapse

After fusion with dendritic cell (DC) HIV-1 infection or HIV-1 degradation and antigen presentation to T lymphocytes coupled with MHC class I and II or endocytic DC sequestration of viral particles and future infection of T cells ensue (17). DC-SIGN (DC surface adhesion molecule) fusion with gp120 leads to conformational change in the gp120 domains increasing the force of fusion with the CD4 receptor (2,9,15,18).

CD4 T cell infection can occur only if an immune synapse forms. The synapse is an interaction between non-infected CD4 T lymphocyte and an infected dendritic cell (9).

Lymphocyte chemokine surface receptors activation leads to polarization, a process during which adhesion and co-stimulating molecules together with receptors gather in one part of T cell's membrane. This cluster of adhesion and co-stimulating molecules together with transducing proteins close to receptors enables T cells to mount a more effective response even in the case of antigen scarcity (18).

An important factor for cell activation is the accumulation of micro-domains, structures rich in cholesterol and sphingolipids present in cell membranes (1). It is in these domains that proteins which transduce signal into the inner cell compartments harbor, intensifying each others action at the same time.

### c) Nucleocapsid's entry into the cytoplasm

Nucleocapsid releases viral genes and enzymes beginning the primary stage of reverse transcription. Recent data suggest an important role of a family of proteins named TRIM-5 alpha (4,5). Its role is to inhibit the release of nucleocapsids and thus hinder further replication. Interspecies differences in the structure of TRIM-5 alpha have been described adding yet another element to the explanation why humans cannot be infected with simian virus and the other way round (5). These proteins are now being thoroughly studied in the search for an effective antiretroviral treatment.

### d) Reverse transcription

The next step in replication involves reverse transcription, a process during which viral RNA is transcribed into proviral DNA. On the template of a RNA strand reverse transcriptase synthesizes a complementary DNA strand while the template RNA strand is degraded by ribonuclease H as the synthesis progresses. Then the second complementary DNA strand is synthesized. There are LTR on both ends of HIV's DNA which contain transcription initiation sequences and transcription factors binding domains (2,9,15,19).

APOBEC3G – cytidine deaminase is a restrictive protein incorporated into virions. It inhibits reverse transcription by deaminating deoxycytidine into deoxyuridine in the negative strand of DNA (3). Interaction of such a negative strand with a positive DNA strand leads to virion destruction. Vif – a regulatory viral protein which blocks



APOBEC3G integration into virions, can also cause APOBEC3G degradation allowing further HIV replication. Both APOBEC3G and *vif* are now being studied as promising ideas for antiretroviral treatment (6).

### e) Integration, transcription, translation and HIV-1 maturation

On reverse transcription completion viral pre-integration complex (PIC) is transported via the endoplasmic reticulum into the cell nucleus where it is integrated into the host's genome. However, for the integration to occur the cell has to be in active stage (11,16). In resting cells HIV's replication ends at this stage giving rise to a reservoir of HIV immune to antiretroviral treatment but capable of becoming activated and producing infectious virions (16).

The integration process includes the following steps :

- PIC transport to the nucleus
- viral integrase (IN) catalytic activation
- integration spot selection
- DNA repairs

There are many cellular proteins engaged in the integration process and they act either by direct interaction with IN or by binding with viral cDNA or by taking part in DNA repairs(20,21). They also prevent suicidal integration of DNA. Most of these proteins have been identified during *in vitro* studies and their significance *in vivo* remains unclear (8). Every one of them is being studied as a new target for antiretroviral therapy.

Recently a new protein LEDGF/p75 located in the nucleus has drawn scientists' attention because of its anti-apoptotic and pro-transcriptic characteristics (8). During HIV-1 infection it protects IN from enzymatic degradation and takes part in binding PIC with the right transcriptionic region. Having been integrated, if not expressed, the provirus can be passed on during mitosis. Latency is a term describing the presence of proviral DNA in the cytoplasm or integrated with the host's genome (latent HIV-1 reservoir) (19,20).

The most widely studied cofactor of HIV-1 transcription is the nuclear transcription factor NF<sub>k</sub>B. It is found in the cytoplasm bound with inhibitory proteins. After activation as a result of TNF-alpha, Il-1, antigen presentation or tat protein exposure it is transported from the cytoplasm to the nucleus(10,12,20). Numerous microorganisms can promote HIV-1 replication by stimulating TNF-alpha secretion (10,22). In the nucleus NF<sub>k</sub>B binds with HIV's LTR and with enhancers present in, among others, Il-2 gene and starts transcription (21). In the early phase genes for regulatory transcriptic proteins like tat (transcriptionally activating all viral genes) and rev (activating structural and enzymatic proteins' genes, suppressing nuclear mRNA degradation by cellular enzymes and transporting mRNA into cytoplasm) are transcribed (20).

After that Gag ang Pol genes follow which encode precursor proteins cleaved into p24, p17, p9 and p7 later in the cytoplasm by the HIV's protease and also env genes encoding glycoprotein gp160 cleaved into gp120 and gp41. This multi-step transcription process reduces the time during which viral proteins are exposed to immune mechanisms and allows more efficient HIV-1 replication(8,11,20,21,23). Finally an infectious viral particle arises incorporating into its envelope during budding host's proteins like MHC, adhesion molecules and complement inhibitors and leading to cell death. Incorporation of cellular membrane molecules facilitates later fusion rendering the virus more infec-

tious. The scale of immune response depends on the type of cellular membrane molecules incorporated into the viral particle. The most activating and pro-apoptotic are the viruses with MHV class II and CD86 molecules (24).

## IMMUNE ACTIVATION

Non-specific immune activation is essential for HIV-1 replication and its pathogenesis (10,11,17,25,26). The activation is directly proportional to the scale of replication. During replication the scale of expression of adhesion molecules in lymphoid tissue as well as the number of circulating lymphocytes rise. The activation is the result of many factors:

- directly viral (HIV-1 particles or gp120, HIV's regulatory proteins) (10,11)
- pro-inflammatory and regulatory cytokines (TNF-alpha, IL1, IL2, IL6) (17)
- other antigens and genes (CMV, HBV, HSV, parasites) (22)

The main immune activator is the virus itself, especially through gp120 (16,26). This glycoprotein can activate lymphocytes, macrophages and pro-inflammatory cytokines secretion. It also directly stimulates peripheral PMLs to secrete TNF-alpha whilst TNF-alpha chronically stimulates HIV transcription. Elevated TNF-alpha concentration is the most vital primary immune disturbance leading to immune activation and as consequence progression of infection (17).

As a result of immune activation there is a rise in concentration of pro-inflammatory cytokines and as the disease progresses Th2 dependent cytokines (IL4, IL10) become predominant, lymphocytes exhibit surface markers of activation (HLA II, CD38), there is an increased cell proliferation, LT together with LB and macrophages express surface CD95 receptors and their ligands which leads to the apoptotic destruction of both infected as well as uninfected cells („bystander cells”), the thymic maturation is inhibited as well as hemopoiesis and some HIV characteristic genotypic and phenotypic changes appear (11,26,27,28,29).

As the result of non-reactiveness to certain antigens the immune activation can also cause clonal deletion. The immune activation clinically leads to disease progression (29,30).

## HIV-1 INFECTION — STAGES

HIV-1 infection can be characterized by 3 stages:

1. Primary infection
2. Latent stage, chronic
3. Clinical stage, AIDS

### 1. Primary infection

After infection the first encountered cells are dendritic cells (DC), macrophages and naive CD4 T cells (after activation) in *lamina propria* of mucous membranes (16,31).

Initially the number of infected cells is relatively small. After this initial phase HIV spreads to the local lymph nodes where it replicates (about 5-7 days after infection) and spreads systemically (2,15).

Dendritic cells play an important role in HIV-1 infection. Naïve DC are present mainly in mucous membranes of the intestines, genital and respiratory tract and also in lymphoid tissue (16). Fusion with the virus leads either to HIV-1 replication or its degradation and antigen presentation (see immune synapse). In this time they migrate to lymphoid tissue and mature. Interaction between DC and CD4 cells which should physiologically activate specific immune responses actually leads to the infection of CD4 cells (32,33). In one hour one DC can spread the infection to tens of cells. By eliciting both non-specific (IFN alpha, cytokines 1,6-10,12,15,18, chemokines and TNF) and specific immune responses DCs are the cause of immune stimulation (16,17,26).

The main characteristic of PHI is the decline of CD4 T cells count, phenotypically CD45Ro+, key cells in the HIV-1 replication, naturally residing in mucous membranes (16). This process is best seen in the intestinal lymphoid tissue (GALT-gut associated lymphoid tissue) as 60% of all CD4 T cells are localized in the GALT. It takes place 4-6 months after infection irrespective of the route. The described CD4 memory T cells loss, especially central ones, is irreversible and its scale determines future HIV infection course (16,31). The loss of CD4 memory T cells during primary infection is caused mainly by direct cytopathic effect and not by apoptosis of uninfected CD4 T cells ("bystander cells") (16).

After viral entry the number of CD8 T cells rises (with surface activation markers). CD8 T cells control HIV-1 replication by cytolysis with perforin (MHC I), stimulation of apoptosis (fusion of Fas ligand – FasL – of CD8 T cell with Fas-CD95 receptor on the surface of target cells), cytokine synthesis (IFN gamma, chemokines) and CAF synthesis – cell antiviral factor – which inhibits HIV transcription (34,35). There is a correlation between the appearance time of HIV-1 specific CD8 T cells and viral load decrease (34,35). Despite all of the above, a question remains why HIV-1 eradication is impossible in spite of all the multidirectional immune responses.

There are two most probable explanations, one being fast rate of HIV mutation and the other one CD8 T cells dysfunction (diminished cytolytic activity and proliferation and decreased signal molecules expression) (11,27,28). The dysfunction can arise due to large and chronic antigen abundance, decreased helper T cell activity and chronic inflammatory state (10). Recently an increased expression of PD-1 receptor (programmed death) on the surface of CD4 and CD8 T lymphocytes in this stage of infection has been described (36). Fusion with PD-L1 or 2 disrupts CD8 T cells function (decreased proliferation and cytokines synthesis).

The larger the decrease of CD8 T cell count and bigger viral load the more pronounced the PD-1 expression (36).

## 2. Chronic infection

In this stage of infection chronic immune activation as well as gradual CD4 T cells decline are the major characteristics (10,25,26,31,37,38). It has been noted that the observed CD4 T cells decline is much bigger than would be expected from the number of infected cells. In the chronic stage, unlike in PIH, most CD4 T cells die by apoptosis of

bystander cells (29,30,38). Most commonly activated memory cells with CD38, CD4Ro and Fas expression are involved (30). The cumulative decline of CD4 T cells is due to the apoptosis of both infected and uninfected cells (direct HIV-1 effect and immune activation) (28,29,39). HIV-1 replication is a very dynamic process with a high turnover rate of CD4 T cells (destruction and regeneration) (37). However the compensatory mechanisms are not efficient enough to stop the progression of infection (11,27). Life span of infected cells averages 2,2 days. It is estimated that about a billion CD4 T cells die each day (37).

During the chronic asymptomatic stage Th1 lymphocytes producing IL-2 and IFN gamma predominate and activate T cytotoxic lymphocytes, NK cells and macrophages (. As the infection advances the immune homeostasis is lost and the number of Th2 T lymphocytes producing IL-4, IL-10, IL-5 and IL-6 rises (12,27,40).

## 3. Symptomatic stage/AIDS

It is inadvertently bound with immune suppression and AIDS. It manifests itself with severe functional immune dysfunction, intensive HIV-1 replication, disrupted antigen presentation and increased susceptibility to infections (11).

## IN CONCLUSION

Chronic immune activation which aids HIV replication is the basic pathogenic factor in the course of infection.

During the course of HIV-1 infection there is a gradual T lymphocyte decline, first only CD4 cells then also CD8 ones, and many cellular and viral factors interact on different levels aiding or hindering HIV's replication.

The pronounced decline of CD4 T cells happens due to the destruction of both infected and uninfected cells mainly because of activated cells' apoptosis, diminished hematopoiesis and thymic maturation.

During the primary infection there is a rapid decline of memory CD4 T cells in the GALT, where their numbers are greatest. This reservoir of cells is never fully repopulated even with suppressive antiretroviral therapy. As there are few or virtually no CD4 cells left in the GALT, bacteria can translocate through intestinal mucous membranes and cause disseminated disease. The loss of CD4 cells in the GALT gives rise to a new question: what effect does translocation have on immune activation and HIV infection progression? Translocation has been described in AIDS patients but whether it also takes place in healthy HIV positive individuals causing a more rapid progression to AIDS by immune stimulation remains to be seen.

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title

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# HIV-Associated Anorectal Lymphogranuloma Venereum: An Emerging Epidemic

authors

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summary

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Anorectal Lymphogranuloma Venereum (AR-LGV) has recently been recognized as an emerging problem among HIV-infected men who have sex with men (MSM). AR-LGV may resemble other conditions such as Crohn's disease, resulting in misdiagnosis and the potential for the development of disabling and irreversible long-term complications. Prompt, appropriate antibiotic therapy is essential. We review the epidemiology, pathology, clinical presentation, diagnosis, treatment and public health concerns of AR-LGV.

key words

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Anus, Chlamydia, Crohn's, HIV, Lymphogranuloma Venereum, Rectum

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## INTRODUCTION

Lymphogranuloma venereum (LGV) is a sexually transmitted disease (STD) caused by the invasive serovars L<sub>1,3</sub> of *Chlamydia trachomatis*. Until recently, LGV was considered a rare disease in developed countries. Following the outbreak of anorectal LGV (AR-LGV or LGV proctitis) in 2003 in the Netherlands<sup>1-3</sup>, and the subsequent report of additional cases in other countries, LGV has emerged as a significant problem among men who have sex with men (MSM). The majority of these patients were co-infected with HIV. These recent outbreaks have brought renewed attention to LGV. As a result, there has been a recent increase in the number of publications on AR-LGV. However, there has been no comprehensive review dealing with this subject. Therefore, we set out to review the literature regarding the current epidemiology, pathology, clinical presentation, diagnosis, treatment and public health concerns of AR-LGV.

## EPIDEMIOLOGY

The more well known sexually transmitted *Chlamydia trachomatis* infections (serovars D-K) occur worldwide, while LGV infections (*Chlamydia trachomatis* serovars L<sub>1,3</sub>) are endemic in Africa, India, South and Central America, Asia and the Caribbean.<sup>4</sup> Only in the pre-antibiotic era was LGV also endemic in Europe, the USA and Australia. In recent times, LGV in these latter industrialized countries was rare. Kornblith (1936) provided one of the earliest accounts of rectal involvement in LGV.<sup>5</sup> Very little was published on AR-LGV since then, until the 1980's when there were sporadic reports of LGV proctitis occurring in MSM in non-endemic countries.<sup>6-9</sup> In 2003 there was a disquieting outbreak of AR-LGV reported in the Netherlands among gay men.<sup>1-3</sup> Shortly thereafter, following warnings launched by national and international health authorities such as the European Union and to the European Surveillance of Sexually Transmitted Infections Network (ESSTI), there were accounts of AR-LGV affecting hundreds of MSM across several European countries (Belgium, France, Germany, Sweden, Italy and Switzerland), the United States, Canada and Australia.<sup>10-20</sup>

Many of the European cases were found to be caused by a newly discovered *Chlamydia* variant L2b (the Amsterdam variant). Interestingly, this L2b variant was traced back and isolated from anal swabs of MSM who visited a STD clinic in San Francisco in 1981.<sup>13,21-22</sup> New cases of this slowly evolving epidemic continue to be reported. However, the true extent and actual number of persons with AR-LGV is likely to be considerably larger than has been reported thus far. This is because AR-LGV is difficult to diagnose and/or mimics other conditions, and physicians may not even be aware of its existence. Risk factors for acquiring AR-LGV include unprotected anal intercourse in MSM, HIV seropositivity, multiple sexual partners, concurrent STDs, and particular sexual activities such as fisting or sharing of sex toys.<sup>1-3,16,23-25</sup> So far, neither young age nor ethnicity has been identified as risk factors.<sup>26</sup>

## MICROBIOLOGY

*Chlamydia trachomatis* is a small Gram negative, obligate intracellular bacterium that replicates within membrane-bound inclusions (**Figure 1**). They cannot grow outside living cells because they are unable to synthesize their own ATP. Within these inclusions elementary bodies become reticulate bodies. After having multiplied, up to 1000 elementary bodies may burst out of the host cell in order to infect more host cells. *Chlamydia* can enter their human host via breaks in the skin or mucosa. The non-LGV serovars (designated A to K) cause genital tract infections, trachoma, and pneumonia. LGV, on the other hand, is the result of infection with the more virulent *Chlamydia* serovars L1, L2 and L3. So far, most of the reported cases of AR-LGV in MSM have been caused by the L2 serovar<sup>26-28</sup>, which is clinically more severe than L1 infections.<sup>29</sup> All LGV serovars tend to cause severe inflammation and invasive infection, pass through the epithelial surface to regional lymph nodes, and may cause disseminated infection often with systemic symptoms.<sup>30</sup> Tissue damage is immune mediated. While host immunity ultimately limits *chlamydia* multiplication, it does not entirely eliminate these microorganisms. Therefore, it is not surprising that in some patients infection may persist for even up to 20 years.<sup>31</sup> Immunity to infection is also not long-lived. As a result, reinfection is possible. Bacterial superinfection of AR-LGV may occur and further complicate the disease course.

## CLINICAL FINDINGS

The clinical manifestations of LGV depend on the site of *chlamydia* entry (i.e. the sexual contact as a site of micro-trauma) and the clinical course<sup>32-34</sup>, which can be conveniently divided into three stages: (i) a primary stage that involves the site of inoculation; (ii) a secondary stage that occurs 2-6 weeks later, in which the inguinal lymph nodes and/or anorectum are affected; and (iii) a tertiary stage in which there may be late sequelae of the genitals and/or rectum. In rare cases, LGV may remain asymptomatic but detectable.<sup>21</sup>

Following exposure, the incubation period is 3-30 days.<sup>34</sup> Primary infection is characterized by a self-limited, painless mucosal inflammatory reaction (papule) or ulcer at the site of inoculation, i.e. genital, anal, or adjacent skin. Hence, such lesions often go unnoticed. This stage is similar in the HIV-positive and HIV-negative. In one study comparing 45 HIV-infected patients to 8 non-HIV-infected patients the clinical presentations of LGV and non-LGV ulcers were similar.<sup>32</sup>

Two to six weeks later, inoculation of the penis, vulva, or vagina leads to an inguinal syndrome, whereas infection of the rectal mucosa (through anal sex or migration from the cervical lymphatics or posterior vaginal wall) causes an anorectal syndrome. A pharyngeal syndrome with enlarged nodes in the neck is rare, and is due to infection of pharyngeal tissue following inoculation during oral sex. The inguinal syndrome is caused by inguinal bubos (painful, enlarged, and fluctuant nodes that may rupture to form discharging sinuses). The presence of lymphadenopathy above and below the inguinal ligament gives rise to a characteris-

tic groove sign. In MSM, classical inguinal presentation with concomitant anorectal involvement is uncommon.<sup>33</sup> A concomitant inguinal syndrome and proctitis in women is not an uncommon presentation of LGV.<sup>34</sup>

LGV proctitis or the anorectal syndrome following anal infection is characterized by proctitis, proctocolitis, and/or symptoms related to an inflammatory anorectal mass. This is typically accompanied by perirectal, deep pelvic, and/or lumbar lymphadenitis.<sup>35</sup> Involvement of these deep nodes may lead to pelvic pain, lower abdominal tenderness, low back pain, and/or deep seated abscesses. Patients with AR-LGV may also present with a purulent or mucous rectal discharge, anal/rectal pain, anal pruritus, tenesmus, and rectal bleeding. They may even have signs of lower gastrointestinal inflammation including diarrhea, constipation and abdominal cramps. On digital rectal examination there may be localized tenderness and/or narrowing with spasm of the anorectum. Perirectal abscess formation, manifesting as a tumor in the distal rectum, can occur.<sup>36</sup> Endoscopy may reveal an inflamed and friable rectal mucosa with hyperemia, a mucopurulent exudate, thickening due to edema, and ulceration. This is usually confined to the distal 10-20cm of the anorectal canal.<sup>37</sup> Ulcers may appear aphthoid (small and discrete), linear, stellate or be more irregularly shaped. When present systemic symptoms include fever, weight loss, decreased appetite, and malaise.<sup>38</sup> Systemic spread may also occur resulting in arthritis, pneumonitis, and hepatitis. Erythema nodosum also occurs in a small subset of cases.

The late (tertiary) stage of infection is characterized by a vigorous immune mediated inflammatory response that leads to lymphatic obstruction and fibrosis. Chronic untreated LGV can lead to the formation of fissures, strictures and anogenital fistulas.<sup>39-40</sup> Widespread destruction (esthiomene) and elephantiasis of the external genitalia may occur typically following the inguinal syndrome of LGV infection.<sup>41</sup>

## PATHOLOGY

LGV proctocolitis in the early stages of infection is characterized histologically by marked mucosal and submucosal inflammation (**Figure 2**), focal cryptitis, and crypt distortion.<sup>30,37,42-43</sup> The inflammatory infiltrate is comprised of neutrophils, plasma cells, lymphocytes, occasional eosinophils, and histiocytes. Reactive follicular lymphoid hyperplasia scattered throughout the submucosa is common.<sup>44</sup> Non-necrotizing granulomas may be seen (**Figure 3**), but are not a prominent feature. Granulomas may contain giant cells. Pseudomembranes and focal small vessel inflammation (endophlebitis)<sup>42</sup>, as well as prominent hypertrophy and proliferation of nerves has been noted by some investigators.<sup>44</sup> Chlamydia inclusions present in epithelial cells and later in tissue histiocytes are rare<sup>30</sup>, and unlikely to be found in routine surgical biopsy material. Biopsies taken from endoscopically uninvolved areas so far have yielded unremarkable findings.<sup>37</sup> In the tertiary stage of AR-LGV disease, there may be considerable fibrosis of the bowel to form rectal strictures, perirectal abscesses, and anal fissures. In women, inflammation may provoke the formation of sinus tracts and potentially cause rectovaginal fistulas. Death may occur from bowel obstruction or perforation.

## DIFFERENTIAL DIAGNOSIS

The differential diagnosis of LGV in the HIV-positive patient is the same as the HIV-negative patient and depends on the site of inoculation and clinical stage of infection. Thus, the ulcerative primary stage carries the differential diagnosis of genital ulcer disease, which includes syphilis, chancroid, donovanosis (granuloma inguinale), herpes simplex virus infection (HSV), candidiasis, scabies, common skin infection (e.g. staphylococcal), aphthous ulcer, Behçet's – the letter ç instead of c syndrome, fixed drug eruption, Reiter's syndrome, or trauma.<sup>44-47</sup>

The differential diagnosis of inguinal lymphadenopathy includes lower extremity infection, cat scratch disease, HSV, syphilis, chancroid, and occasionally neoplasm. The inguinal syndrome of LGV is typically unilateral and only bilateral in one third of cases, while the inguinal nodes associated with penile HSV are frequently bilateral.

The differential diagnosis of proctitis in homosexual men is dominated by the sexually transmitted infections: gonorrhea, herpes simplex, chlamydia, and syphilis though enteric pathogens such as Shigella, Campylobacter, Salmonella, and Entamoeba histolytica may also cause proctitis and be transmitted sexually. The acute inflammatory exudate in gonorrheal proctitis is usually more extensive than LGV, and may be accompanied by liquefactive necrosis of the bowel mucosa.<sup>48</sup> In syphilitic proctitis a lymphoplasmacytic infiltrate predominates and granulomas may also be seen.<sup>49</sup> Additional non-sexually transmitted infections such as Eschecheria coli, Clostridium difficile, and cytomegalovirus may also cause a proctitis or proctocolitis-like syndrome and require evaluation. Schistosomiasis too may involve the rectum and is often accompanied by a granulomatous reaction to parasitic ova, with possible fissuring and stricture formation.

Of the noninfectious causes of proctitis, inflammatory bowel disease (IBD) including Crohn's disease and ulcerative colitis (UC), as well as anorectal carcinoma and lymphoma are important conditions to exclude when evaluating proctitis. An anorectal mass (pseudotumor) secondary to AR-LGV can become quite large, and may be mistaken for a neoplasm.<sup>50</sup> LGV can mimic IBD clinically, endoscopically and even on rectal biopsy<sup>51</sup>, resulting in a delay in diagnosis.<sup>52</sup> Unlike LGV, IBD is a relapsing condition with flares that are poorly responsive to antibiotic therapy. The lesions of UC are largely located within the mucosa. Table 1 compares AR-LGV to Crohn's disease. While Crohn's disease may involve the entire GI tract from the mouth to the perianal area, LGV is largely limited to the rectum. However, symptoms and signs related to perianal disease occur in up to one third of patients with Crohn's disease, and may dominate the clinical picture. Like AR-LGV, these may include perianal pain, drainage, anal fissures, perirectal abscess and anorectal fistulas. Also, granulomas in tissue biopsies can be identified in up to 30% of patients with Crohn's disease. Interestingly, antibodies against Chlamydia have been detected in many patients with Crohn's disease, suggesting a causal relationship between LGV and Crohn's disease.<sup>53</sup> Unlike Crohn's disease, AR-LGV is not associated with the development of rectal carcinoma. However, we are aware of at least one report of a case of rectal LGV associated with rectal adenocarcinoma.<sup>54</sup> Finally, ischemia and in the appropriate setting radiation-induced proctitis can be included in the differential of AR-LGV.

**Table 1.** Comparison between LGV proctitis and Crohn's disease

	LGV Proctitis	Crohn's Disease
<b>Etiology</b>	Chlamydia infection	Unknown
<b>Clinical presentation</b>	Rectal discharge, bleeding, anal pain or pruritis, diarrhea, constipation, tenesmus, pelvic pain, fistulas, perianal mass, fever, weight loss and malaise	Diarrhea, abdominal pain, rectal bleeding, sinus tracts, fistulas, bowel perforation, weight loss and fever
<b>Gastrointestinal site involved</b>	Limited to anorectum	Anywhere from mouth to perianal area
<b>Endoscopic findings</b>	Inflammation, edema, exudate, and ulceration	Inflammation, ulceration, cobblestone mucosa, and pseudopolyps
<b>Gross Pathology</b>	Proctitis, anal fissures, fistulas, perianal abscesses (deep pelvic bubos)	Skip areas of small and large bowel involvement, strictures, fissures, fistulas
<b>Histopathology</b>	Transmural inflammation, ulceration, granulomas, rarely inclusions, nerve hypertrophy, and fibrosis	Focal transmural inflammation, ulceration, fibrosis, and granulomas
<b>Extraintestinal disease</b>	Rarely inguinal bubos (in women), arthritis, hepatitis, pneumonitis, and erythema nodosum	Uveitis, erythema nodosum, pyoderma gangrenosum, clubbing, polyarthritits, sclerosing cholangitis, amyloidosis
<b>Complications</b>	Abscess, fistulas, strictures, genital elephantiasis, esthiomene, frozen pelvis, and infertility	Malabsorption, intestinal obstruction, perforation, fistulas, abscess formation, toxic megacolon and adenocarcinoma
<b>Laboratory diagnosis</b>	Serology, molecular tests, tissue culture and biopsy	Serology (e.g. p-ANCA, anti-OmpC; chlamydia may be positive), and biopsy
<b>Therapy</b>	Antibiotics and surgery	Anti-inflammatory, corticosteroids, immunosuppression, antibiotics, and surgery

## DIAGNOSIS

Establishing a definitive diagnosis of AR-LGV can be difficult for several reasons. Not only is the clinical presentation non-specific, but laboratory procedures are not well standardized.<sup>24</sup> Moreover, microorganisms may be difficult to culture, there is often serologic cross-reactivity between LGV serovars and non-LGV serotypes<sup>55</sup>, and many of the tests do not distinguish recent from past infection. There are also considerably fewer detectable organisms with disease progression.

In the past, LGV was diagnosed by a Frei skin test. Today we rely on clinical suspicion, epidemiologic clues, and ruling out other etiologies (of genital or rectal ulcers, proctitis, or inguinal lymphadenopathy), along with the identification of *C. trachomatis* in appropriate clinical samples and supportive serologic data.<sup>24,33,56-57</sup> As of June 2006, a reliable polymerase chain reaction (PCR) test became available through the efforts of the Centers for Disease Control and Prevention (CDC) and may be available through one's local department of public health (DPH). If PCR is positive, subsequent restriction fragment-length polymorphism (RFLP) analysis can be done to determine the specific serovar.<sup>58</sup> In addition, genital and lymph node specimens (i.e. lesion/rectal swab or bubo aspirates) may be tested for *C. trachomatis* by culture, direct immunofluorescence, or nucleic acid detection. Although nucleic acid amplification tests (NAAT) have become the mainstay of diagnosis for urethral and cervical infection with *C. trachomatis*, in the USA they are not yet FDA-cleared for testing of rectal specimens.<sup>59</sup>

Routine analysis of urine or samples obtained from the urethra may fail to detect LGV.<sup>2,32</sup> Chlamydia cannot be

cultured on artificial media. They need to be isolated in tissue culture, using HeLa-229 or McCoy cell lines, or they can be identified by direct fluorescent microscopy. Enzyme immunoassays so far have not been shown to distinguish between infections with different chlamydial species.

Two main serologic tests exist. The complement fixation (CF) test is genus specific, and therefore does not distinguish between different *Chlamydia* infections. Because it is more invasive, LGV usually leads to higher titers. A titer of > 1:256 strongly supports the diagnosis, while a titer of < 1:32 rules it out except in the very early stages of the disease. Serial titers demonstrating a titer rise are confirmatory. While the microimmunofluorescence (MIF) test can distinguish between infections with different chlamydial species, it is not readily available. A MIF IgG titer of > 1:128 strongly suggests LGV.

When available, histopathological examination of biopsy specimens can support the diagnosis. Typing of LGV in tissue specimens with a monoclonal antibody (if available) supports the diagnosis. Although molecular testing can be performed on tissue biopsies, it is not unusual for PCR identification of chlamydia DNA in tissue samples to be negative.<sup>36-37</sup>

Finally, evaluation of patients with AR-LGV should include anoscopy and/or sigmoidoscopy. Patients with anorectal smears that have > 10 white blood cells/high power field (WBC/hpf), although not diagnostic, are reported to be 3.5 times more likely to be infected with LGV.<sup>23</sup> Rarely, smears taken from a rectal discharge and stained with fluorescent monoclonal antibodies may show inclusion bodies.<sup>60-61</sup> CT scan findings in AR-LGV have not been routinely reported, but in the few cases reported to date have not proved additive.<sup>36</sup> Magnetic resonance imaging findings in selected cases showed diffuse mucosal wall thickening, submucosal edema and perirectal adenopathy.<sup>2</sup>

## TREATMENT

Spontaneous remission is possible. Nevertheless, appropriate treatment is essential to reduce inflammation, diminish the duration of infection, prevent chronic complications, and reduce subsequent disease transmission. In fact, a complete cure can be obtained with early and suitable antibiotic treatment of early disease while results are variable on the manifestations of late disease such as strictures. It is recommended that HIV-positive persons with LGV be treated similar to their HIV-negative counterparts. Doxycycline (100 mg PO BID for 21 days) is the preferred treatment for LGV, including those who are HIV-positive.<sup>1,24</sup> Erythromycin is an acceptable alternative. Ciprofloxacin and metronidazole, which are usually given for Crohn's colitis flares, are ineffective in LGV. Once the diagnosis of LGV is established, treatment can then continue for 21 days, or for as long as symptoms persist. Many patients with LGV proctitis may have concomitant STDs<sup>62</sup>, and if detected should also be treated. Although several of the HIV-positive patients that contracted AR-LGV were receiving HAART<sup>2</sup>, no reports on the effect of antiretrovirals on LGV have yet been reported.

While late complications such as rectal stricture may improve with antibiotic therapy, this does little to reverse fibrosis. Patients with chronic infection including abscesses, fistulas, strictures with bowel obstruction and esthio-mene often require surgical intervention. We are aware of two cases of AR-LGV presenting with large stenosing masses in the distal rectum that resolved following long-term oral doxycycline therapy.<sup>50,63</sup>

## PUBLIC HEALTH

The recent increase in cases of LGV and high HIV prevalence in these patients has resulted in great public health concern. Proctitis is a marker of other STDs, and its presence increases the risk of their transmission, including Hepatitis C viral hepatitis C.<sup>10</sup> LGV may even be contributing to the epidemic of HIV infection by facilitating transmission.<sup>64</sup> However, it appears that many clinicians and MSM are not fully aware of this re-emerging disease. Fortunately, substantial effort was made after the recent outbreaks in Europe to promote awareness of AR-LGV. For example, in 2004 the Health Protection Agency (HPA) in England sent out an alert to genitourinary medicine clinicians and established a case definition, reference service, and reporting system for LGV. Similar efforts were made by the CDC in the USA.<sup>1</sup> In addition, briefings were produced for use in clinics and leaflets handed out in gay venues. Positive cases of LGV should be reported to appropriate central agencies and/or local health authorities.<sup>1</sup> As with many STD's, sex partners of patients who have LGV should be examined and tested for urethral or cervical chlamydial infection. They should be given antibiotic therapy (azithromycin 1g PO once or doxycycline 100 mg PO BD × 7 days) if they had sexual contact with the patient during the 60 days preceding onset of symptoms in the patient.<sup>1,24,34</sup>

## MANAGEMENT RECOMMENDATION

Just as the clinical findings and differential diagnosis of LGV infection depend upon the site of inoculation and clinical stage, so does specific management. Recommendations for patient testing may depend on the local DPH and should be guided by local authorities, e.g. some local authorities have validated NAAT rectal sample testing. A patient presenting with symptoms of proctitis, as the bulk of patients in the recent outbreaks have, requires detailed sexual, dietary, antibiotic exposure, family, and travel history taking for consideration of a prodromal ulcer, potential enteric pathogens acquired locally or abroad, *Clostridium difficile* infection, and family history of IBD as clues to the etiology. In addition, close physical examination looking for the presence of unilateral or bilateral inguinal lymphadenopathy, single or multiple genital ulcers, and the findings of pelvic, digital rectal and anoscopic exam (potentially with biopsy) are all critical to the thoughtful evaluation of proctitis.

For proctitis without inguinal lymphadenopathy, rectal specimens should be cultured for enteric pathogens, gonococci, HSV, and Chlamydia. A separate rectal swab for LGV PCR should be provided to the local DPH or referred directly to a national body like the CDC. Serum samples should be sent for RPR testing and also chlamydial serology, preferably MIF if available. Based on the patient's history, one should consider sending stool for *Clostridium difficile* toxin and *Entamoeba histolytica* antigen testing.

Empiric treatment should begin while awaiting test results: ceftriaxone 125 mg intramuscularly remains the mainstay of gonococcal treatment and doxycycline 100mg orally twice a day is preferred for Chlamydial therapy. One can also consider 7-14 days of empiric HSV therapy (acyclovir 400 mg po every 8 hours, famciclovir 500 mg po every 12 hours, or valacyclovir 1000mg po every 12 hours). Once the test results are known, one may curtail anti-chlamydial therapy at 7 days for serovars D-K or extend therapy to 21 days for appropriate LGV therapy. Further antibiotics may be necessary depending upon resolution of symptoms. Also, whether by DPH staff or the treating clinician, the patient's sexual contacts within 60 days of symptom onset should be screened for infection. Asymptomatic contacts should receive prophylaxis with 1gr of azithromycin or a week of doxycycline 100 mg bid while symptomatic individuals should undergo evaluation.

## DISCUSSION

It is clear that AR-LGV has re-emerged as an important STD to medical practitioners in the developed world. As with syphilis, the emergence of AR-LGV is mainly affecting HIV-positive MSM. It is possible that LGV has been present and endemic for some time in this HIV-infected population, with many cases going misdiagnosed. Should screening of men, especially those who are HIV-positive MSM, therefore be advocated? Because of its variable presentation and similarity to other conditions such as Crohn's disease, a high index of suspicion is essential in order to avoid misdiagnosis. LGV should always be included in the



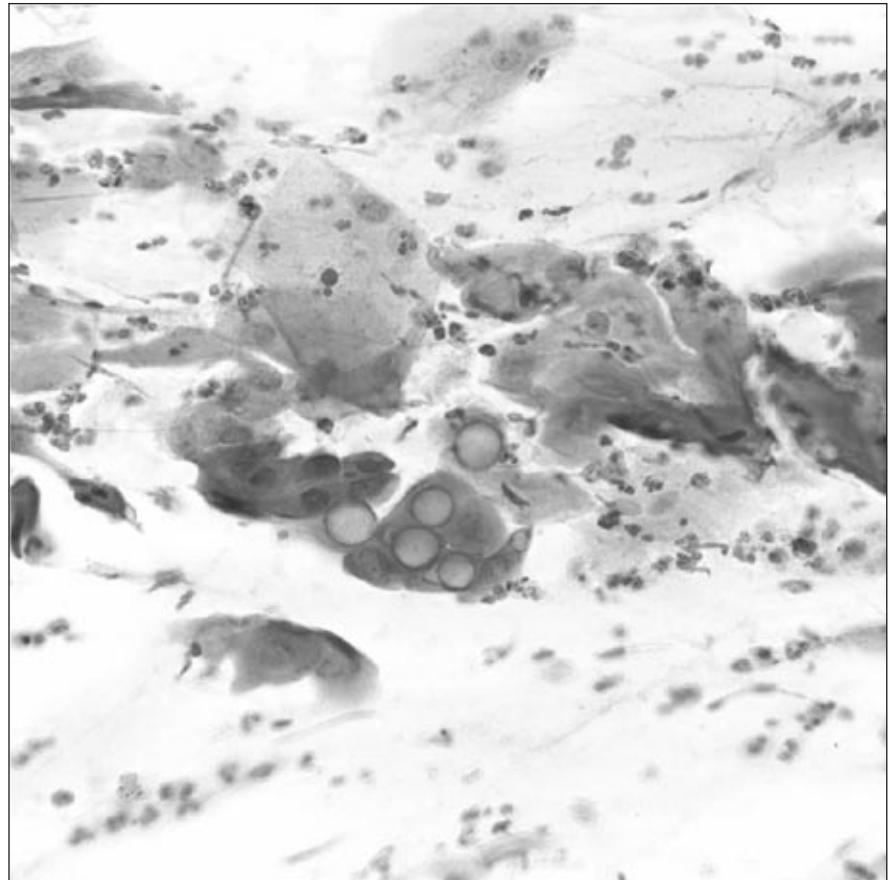
differential diagnosis of anogenital erosions, inguinal lymphadenopathy, and especially proctitis, particularly in homosexual patients and HIV-infected patients. Clinicians need to remember that rectal strictures are uncommon in young patients without a history of malignancy, IBD, prior surgery and/or radiation therapy. Prompt and appropriate antibiotic therapy for AR-LGV should lead to complete mucosal healing, which will typically prevent long-term complications. A delayed diagnosis can lead to disabling long term sequelae. STDs with ulcerative lesions such as LGV also increase the risk of contracting multiple STDs including HIV itself and Hepatitis C viral or HCV. Increased awareness, enhanced surveillance, and further collaborative research are required.

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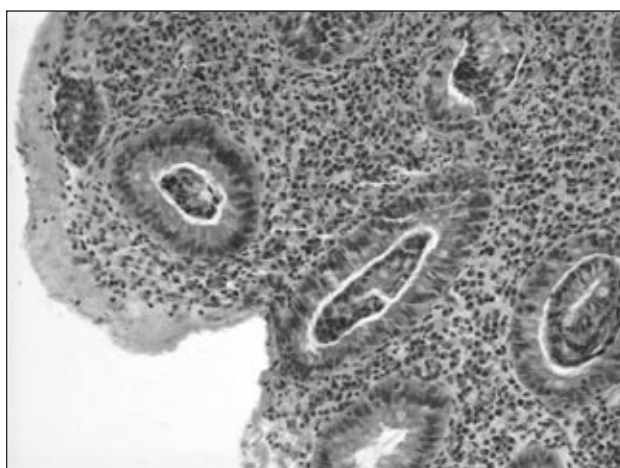
**Figure 1.**

Epithelial cells with Chlamydia inclusions (magnification x600; Papanicolaou stain)



**Figure 2.**

Acute and chronic proctitis with surface ulceration in an HIV-infected man with AR-LGV, mimicking ulcerative colitis (magnification x400; H&E stain)



**Figure 3.**

LGV proctitis with focal granuloma formation in an HIV-infected man, mimicking Crohn's disease (magnification x100; H&E stain)



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title

# Physiotherapy of patients with lipodystrophy associated with HAART

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summary

Among HIV (+) patients treated with antiretroviral therapy lipolytic disorders called lipodystrophy have been described. Majority of patients with HAART-associated lipodystrophy are women. The symptoms of lipodystrophy manifests as: dorsocervical fat accumulation ("buffalo hump"), central adiposity, peripheral fat waisting (face, lower and upper limbs) and associated laboratory abnormalities such as hipertriglyceridemia with low HDL, elevated LDL and total cholesterol levels and insulin resistance or type II of diabetes mellitus. Treatment of patients with lipodystrophy is very difficult and must include specialized medical and dietary cares, accurate physiotherapy and psychological help. Appropriate exercises and diet should be considered as a part of the treatment. In this review we present problems, connected with physiotherapy of this group of patients in the light of the recent publications.

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key words

physiotherapy, lipodystrophy, HIV/AIDS, physical training, aerobic training, resistance training

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## BACKGROUND

Clinical cases of lipolytic disturbance called lipodystrophy have been described among patients treated with highly active antiretroviral therapy (HAART). There has been still no obligatory definition of lipodystrophy. Because of that, the data regarding to the frequency of lipodystrophy among these patients oscillates from 6 to 80%. The great majority of patients with such disorders are women [1]. The symptoms of lipodystrophy manifests as: dorsocervical fat accumulation ("buffalo hump"), central adiposity, peripheral fat waisting (face, lower and upper limbs) and associated laboratory abnormalities such as hypertriglyceridemia with low HDL, elevated LDL and total cholesterol levels and insulin resistance or type II diabetes mellitus [2-9].

The redistribution of fat often manifests as accumulation of visceral fat ("protease paunch") or/and over the dorsocervical spine (resulting in a "buffalo hump"), enlargement of the breast, especially in women, rarer in men and subcutaneous lipodystrophy which is most noticeable in the limbs and face (sunken cheeks). All these changes cause significant deformation of silhouette.

Patients with extreme large "buffalo hump" complain on headaches, insomnia, difficulties with head, neck and arms motions and also psychological discomfort [10]. For these reasons the treatment and physiotherapy in form of physical training for this group of patients is very difficult and exhausting. It must be complexly done, including specialized medical care, dietary care, accurate physiotherapy and psychological help.

The medical care should include first of all:

- Clinical evaluation of lipodystrophy
- BMI (body mass index)
- Periodical control of cholesterol and triglycerides
- Cardiological control

Dietician's tasks should include appropriate diet and education of the matter of choosing appropriate products (low fat) and counting of energy expenditure.

Psychological care should affect on psychological and emotional sphere.

Physiotherapy must contain:

- Motivation of a patient to exercise
- Individually planned exercises
- Measuring the girth of abdomen, hips, legs and neck
- Indications about daily activity to raise energy expenditure

## METHODS OF TRAINING IN HIV(+) PATIENTS WITH LIPODYSTROPHY

### Aerobic Training

Aerobic training is considered by the scientists to be the most effective method of physiotherapy among patients with lipodystrophy. This training affects whole organism, especially the circular, muscular and respiratory systems. The aerobic exercises rise the frequency of breathing and heart rate and improve the blood circulation in the work-

ing muscles, therefore they are better supplied with oxygen. These exercises also rise capacity of submaximal efforts (90% of maximal load) and rise capacity of maximal oxygen uptake ( $VO_{2max}$ ). Endurance training increases use of fat acids by muscles. Thanks to that the aerobic metabolism of substrates in muscles increases. The muscle oxygen uptake raises too.

A typical time of aerobic training is from 20 to 60 minutes. It must be considered that HIV(+) patients with lipodystrophy belong to the group of the higher risk of physical exertion. The intensity of training should reply on patients' fitness ability. Middle intensity exercises are recommended in this group of patients (30-70% of maximal load). Jogging, marshing, bike riding and swimming are typical aerobic efforts [11, 12].

### Resistance Training

Resistance training contains static and dynamic efforts. Resistance exercises raise strenght and mass of muscles, bone endurance and metabolism. They also improve endurance of tendons and ligaments, as well as reduce fat, raise motivation for further exercises and improve the body composition. At the same time resistance training effect on raising muscle mass, lowering plasmatic cholesterol level, improving glucose tolerance and insulin sensitivity. Numerous studies indicates that resistance training have great influence on cardiovascular system and the level of cholesterol, but it also helps in reduction of total body mass, what in patients with lipodystrophy has a huge meaning [12].

The exercise prescription both for aerobic and resistance training should target the specific muscle groups of the body, as it is affected by fat accumulation – "buffalo hump" and "protease paunch".

Exercises for reduction of "buffalo hump" should involve particular muscles [13]:

- *musculus trapezius pars superior et media*
- *musculus levator scapulae*
- *musculus rhomboideus major*
- *musculus rhomboideus minor*
- *musculus splenius cervicis*
- *musculus splenius capitis*
- *musculus semispinalis capitis*
- *musculus rectus capitis lateralis*
- *musculus obliquus capitis superior*
- *musculus longus capitis*
- *musculus transversus nuchae* (muscle is present in ~ 25% of population)

Exercises for reduction of "protease paunch" should involve particular muscles, especially [13]:

- *musculus rectus abdominis*
- *musculus obliquus externus abdominis*
- *musculus obliquus internus abdominis*
- *musculus transversus abdominis*

## EFFECTS OF TRAINING IN HIV(+) PATIENTS WITH LIPODYSTROPHY

A positive influence of exercises and low-fat (lipid) diet involves the improvement of regulation of levels of total cholesterol, LDL fraction, HDL fraction, triglycerides and glucose [14]. These effects are presented in Table 1.

**Table 1.** Most important metabolic effects of physical training [14]

Parameter	Effect of physical training
Total cholesterol level	↓
LDL fraction level	↓
HDL fraction level	↑
Triglycerides level	↓
Glucose concentration	↓

## CONCLUSIONS

Many scientists from all over the world came to the same conclusion, that beside pharmacological treatment physical training is very important in HIV(+) patients with lipodystrophy. Two separately working groups of scientists directed by Roubenoff (USA) and Thöni (France) came to one conclusion, that physical training reduces total body fat [15, 16, 17]. Both aerobic and resistance trainings are recommended by the scientists. Roubenoff worked on resistance training and recommended a training system in which patients had been training 3 times per week for 8 week. Thöni – based on aerobic training for 16 weeks. Both studies have shown, that exercise training can help reducing total body and visceral fat, as well as increase muscle strenght in HIV(+) group of patients with lipodystrophy [15, 16, 17, 18].

Physiotherapy of patients with lipodystrophy is very important factor improving general health by better self feeling, reducing fear and symptoms of depression. Many investigations have proven that exercises reduce visceral adipose tissue, lipemy and have positive influence on metabolism of carbohydrates [15, 19,20, 21]. The others showed also the benefit of various forms of physical exertion in the aspect of metabolic disorders – lipodystrophy. The best results were achieved using the aerobic pattern of exercising, although form of interval or resistance training was used as well [16, 17, 21].

In conclusion exercises and appropriate diet should be considered part of the treatment for lipodystrophy. There should be taken some trials to create an optimal model of exercises, which would improve reducing of fat accumulation in HIV(+) patients with lipodystrophy. Therefore further investigations may approach the significant achievements.

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## title

# The concentrations of IL-22, sFas and sFasL in HCV, HIV and HCV/HIV infections

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## summary

IL-22 is a cytokine regulates acute phase protein synthesis, inhibits the processes of necrosis and apoptosis and activates damaged hepatocytes repair. The aim of the study was to assess the concentrations of IL-22, sFas and sFasL in sera of HCV, HIV, HCV and HIV infected patients.

The study included 21 HCV, 27 HIV and HCV and 10 HIV infected patients. The highest IL-22 concentration was detected among HIV infected patients with no HCV infection (33.5 pg/ml), and the lowest among HCV infected patients before antivirus treatment (10.9 pg/ml). The concentration of sFasL was significantly higher among HIV infected patients in comparison to those infected with HCV (0.37 pg/ml vs. 0.03 pg/ml). A significant relationship between the concentration of IL-22 and the concentration of sFasL ( $r = 0.429$ ;  $p < 0.0006$ ) was observed. Antivirus treatment of HCV infected patients resulted in an increase in the concentrations of IL-22 (10.9 pg/ml vs. 15.2 pg/ml,  $p < 0.03$ ). We did not observed correlation among concentration IL-22, sFas, sFasL, and viraemias of HCV or HIV.

A high concentration of IL-22 and sFasL is detected among HIV infected patients. The IL-22 is an index of improvement of liver function in period of therapy interferon maybe.

## key words

HIV, HCV, IL-22, sFas, sFasL

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IL-22 is a cytokine classified as a member of the IL-10 family. Besides IL-22, IL-19, IL-20, IL-24, IL-26, IL-28 and IL-29 are also classified as members of the same family<sup>1</sup>. It is believed that IL-22, discovered in 2000, is synthesized by active Th1 lymphocytes, NK cells<sup>2-3</sup>. Wolk et al.<sup>4</sup> proved that IL-22 does not have any effects on immune cells in vitro or in vivo. The authors indicate the significance of IL-22 in the innate, nonspecific immunity of tissues. A reduction in IL-22 endogenous synthesis has an influence on the decrease in IL-4, IL-13 and IFN- $\gamma$  syntheses<sup>5</sup>. IL-22 has an influence on acute phase protein synthesis regulation<sup>5</sup>. Together with IL-6 it most probably plays a significant role in the "acute phase" type defense against pathogenic factors damaging the skin, intestines, liver or pancreas<sup>6</sup>. Andoh et al.<sup>7</sup> describe the stimulating and modifying influence of IL-22 on the activity of pro-inflammatory cytokines and matrix-degrading molecules in human colonic subepithelial myofibroblasts obtained from patients with inflammatory bowel disease. In experimental studies, Radaeva et al.<sup>8</sup> showed inhibitory properties of IL-22 as regards necrosis and apoptosis processes in hepatocytes. IL-22 activates damaged hepatocytes repair. The studies of Pan et al.<sup>9</sup> determining the role of IL-22 in the liver showed its stimulating properties as regards the syntheses of the anti-apoptotic STAT3 transcription factor and apoptosis-inhibiting proteins, i.e. Bcl-xL, Bcl-2 and Mcl-1. Apoptosis inhibition by IL-22 takes place not only through Bcl-xL and Bcl-2 syntheses stimulation but also through blocking of Fas-type membrane programmed death receptors. Programmed hepatocyte death plays an important role in the development of chronic HCV infections, especially among HCV and HIV infected patients. It seems that studies focused on the activity of IL-22 can be significant in the broadening of knowledge related to the development of chronic hepatitis C.

The aim of the study was to assess the concentrations of IL-22 in sera of patients infected with HIV, patients co-infected with HCV and HIV as well as HCV infected patients treated antiviral. Correlations between the concentration of IL-22 and the concentrations of sFas, sFasL, as well as between HCV and HIV viraemias were determined. The concentration of the cytokine under study in relation to the applied therapy was analyzed.

## MATERIALS AND METHODS

The study included 58 patients: 21 (10 women and 11 men, aged 23 to 65 yrs) with chronic viral hepatitis C, 27 (1 woman and 26 men, aged 19 to 45 yrs) co-infected with HIV and HCV and 10 (1 woman and 9 men, aged 25 to 54 yrs) infected with HIV.

The patients infected with HCV were treated with interferon with pegylated IFN alfa 2b (PegIntron, Schering, USA), applied in a dose of 1.5  $\mu\text{g}/\text{kg}/\text{week}$  and ribavirin in a dose of 1000 to 1200 mg/day (Rebetol, Schering, USA).

The examinations among HIV infected patients, and those co-infected with HIV and HCV were performed only once. Among HCV infected patients and those treated with pegylated interferon the examinations were performed before the treatment and after 1 and 3 months of therapy.

### • HIV infection

HIV infection diagnosis was based on double anti-HIV antibodies detection by use of immunoassay (ABBOTT, USA) and confirmed by a positive Western-blot result (Cambridge Biotech Corporation, USA) [The Western-blot test was performed in Laboratory and Experimental Institute of Department of Venerology Medical University of Warsaw, Head: Z. Solibórska, MD, PhD]. The percentage and absolute counts of peripheral CD4 (+) and CD8 (+) T-cells were determined by means of 3-color flow cytometric analysis (Beckton-Dickinson, Franklin Lakes, NJ USA). Serum HIV-1 RNA was evaluated using Cobas Amplicor HIS 1.5 system (Roche Diagnostics, Basel, Switzerland), with sensitivity range between 50 and 100,000 RNA copies per mL<sup>1</sup>.

### • Studied lymphocytes count

CD3, CD4 and CD8 counts were determined in HIV infected patients' blood by means of a Becton Dickinson flow cytometer. [The examination was performed in Department of Immunology and Molecular Diagnosis of Regional Infectious Hospital in Warsaw, Head: J. Stańczak, MD, PhD.]

### • HCV infection

HCV infection diagnosis was based on anti-HCV antibodies (MEIA method, ABBOTT, USA) and HCV-RNA in the serum-based RT-nested-PCR detection (Syngen Biotech, USA). Detection range of the described method was 50 HCV-RNA copies per 1 mL. [The examination was performed in Department of Clinical Molecular Biology, Medical University of Białystok, Head: Professor Lech Chyczewski, MD, PhD]

### • IL-22 concentration

IL-22 concentration was measured in duplicate by use of enzyme immunoassay technique (ELISA, R&D Systems GmbH, Germany)

### • sFas and sFasL concentrations

sFas and sFasL concentrations were measured in duplicate by use of enzyme immunoassay technique (ELISA, Bender MedSystems, Austria).

### • Control group

The preferred values of IL-22, sFas and sFasL in serum were determined in 10 healthy persons, the blood donors (6 men and 4 women aged 21 to 38 yrs).

All the patients and persons from the control group gave their consent to take part in the study. The approval for the study was obtained from the Bioethical Committee of the Medical University of Białystok.

Statistical analyses. Statistical analyses were performed by use of Wilcoxon and Mann – Whitney U test. For correlation analyses the Spearman non-parametric correlation was used. A *P* value of < 0.05 was considered as a significant.

## RESULTS

The highest concentration of IL-22 was detected in HIV infected patients, with no HCV co-infection (33.5 pg/ml), and the lowest among those infected with HCV before the start of treated with pegylated interferon (10.9 pg/ml), Figure 1 and Table 1.

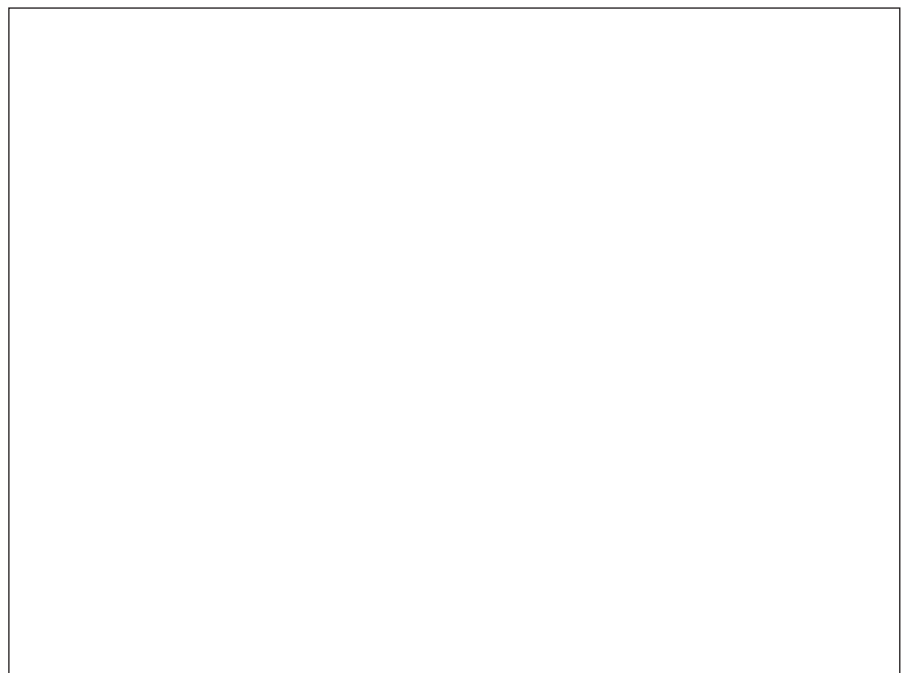


**Table 1.** The concentration of IL-22, sFas, sFasL and ALT activity in studies patients

Patients			IL-22 pg/ml	ALT U/L	sFas pg/ml	sFasL pg/ml
Norm	x		18,0	< 41	16,2	0
	S.D.		4,0	-	4,1	-
HIV infection	x		33,5	27	11,6	0,37
	S.D.		25,1	13	6,0	0,30
HCV/HIV infection	x		18,0	67	19,4	0,26
	S.D.		13,6	48	12,6	0,20
HCV infection	before the treatment	x	10,9*	111	23,3	0,03
		S.D.	7,4	69	13,8	0,03
	after 1 month therapy	x	15,5	52	29,8	0,03
		S.D.	9,3	30	14,4	0,02
	after 3 months therapy	x	15,2*	44	30,2	0,03
		S.D.	6,3	36	15,4	0,02

\* statistically significant differences,  $p < 0,03$

**Figure 1.**  
Serum IL-22 concentration  
in studied population



The concentration of sFas was the highest among HCV infected patients (23.3 pg/ml), lower among HCV and HIV co-infected patients (19.4 pg/ml), and the lowest in the group of HIV infected patients, with no HCV co-infection, Table 1. The concentration of sFasL was significantly higher among HIV infected patients in comparison to those infected with HCV (0.37 pg/ml vs. 0.03 pg/ml). Among HCV and HIV co-infected patients the concentration of sFasL was lower in comparison to those infected with HIV only (0.26 pg/ml vs. 0.37 pg/ml), Table 1

Antiviral treatment of patients with HVC resulted in a significant increase in the concentration of IL-22 (10.9 pg/ml vs. 15.2 pg/ml,  $z = -2.14$ ;  $p < 0.03$ ), Figure 2. In the same group of patients and in the same time period an increase in the concentration of sFas (23.3 pg/ml vs. 30.2 pg/ml) was observed; it was not, however, statistically significant.

No correlation between ALT and other studied parameters was detected.

A statistically significant correlation between the concentration of IL-22 and the concentration of sFasL was observed ( $r = 0.429$ ;  $p < 0.0006$ ).

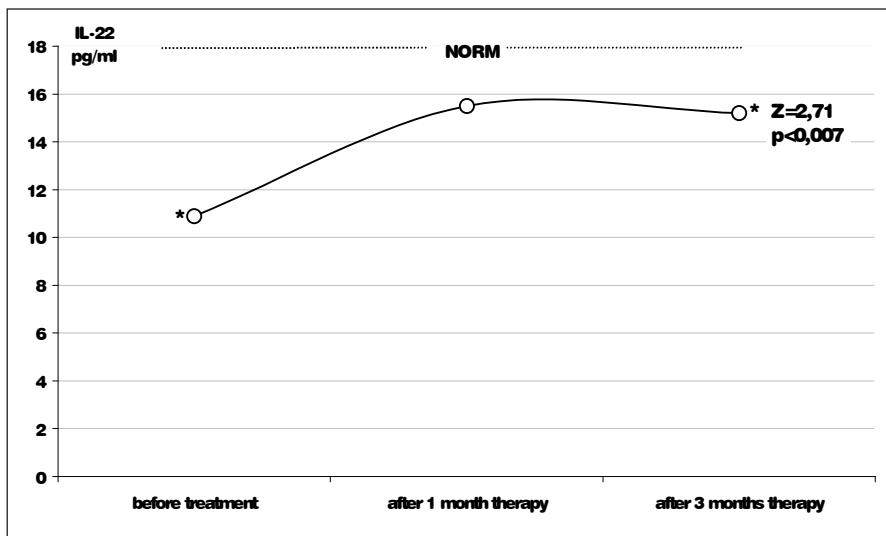
The concentrations of sFas and sFasL among HIV infected patients with the number of CD4 counts  $> 410$  cells/ul was higher in comparison to those with the number of CD4 counts  $< 410$  cells/ul (30.5 pg/ml vs. 13.5 pg/ml and 0.44 pg/ml vs. 0.25 pg/ml). The concentration of IL-22 was slightly higher in patients with the number of CD4 counts  $< 410$  cells/ul (22.3 pg/ml vs. 21.8 pg/ml).

No correlation between the concentrations of IL-2, sFas, sFasL, and HIV or HCV viraemia was detected.

## DISCUSSION

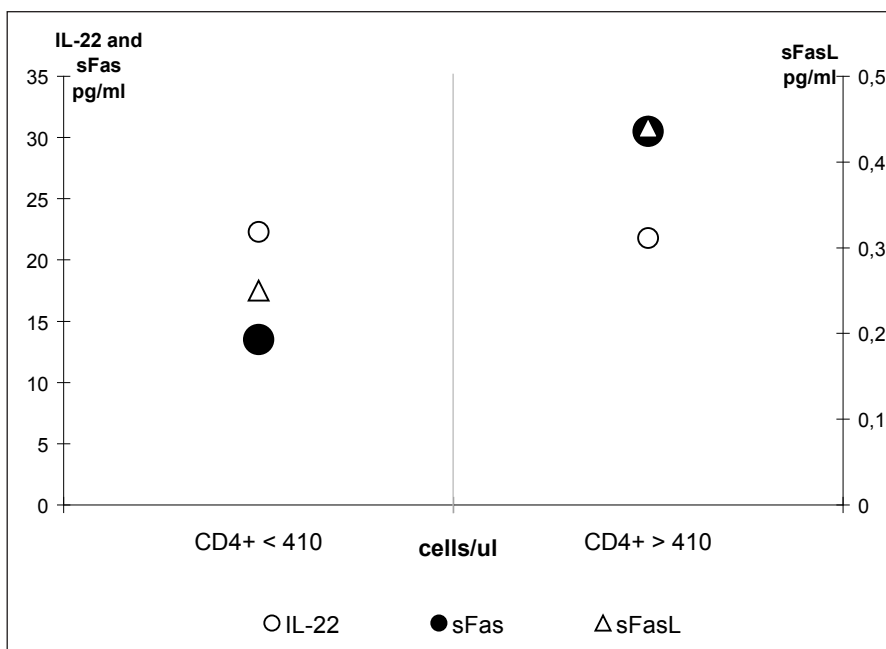
Among HIV infected patients a high activity of programmed cell death can be observed. The activation of the Fas/FasL system is the initiating factor of these processes.

In studies, an increase in FasL synthesis on lymphocytes neurophils and in Fas synthesis on CD4 counts is thus observed<sup>10-11</sup>. High concentrations of sFasL protein among



**Figure 2.**  
The concentration of IL-22 in HCV infected, before treatment, and after 1 and 3 months therapy

\* –  $p < 0,007$  – Wilcoxon test;  $p < 0,03$  Manna - Whitney test



**Figure 3.**  
The concentration of IL-22, sFas i sFasL in HIV infected patients in relation to number of CD4 counts

HIV infected patients corroborate the high activity of apoptosis in this group of patients. CD4 counts are responsible for both FasL synthesis and Fas membrane receptors presentation. These results are similar to the previously discussed concentrations of sFas, sFasL, and CD4 counts among patients infected with HCV and those co-infected with HIV/HCV<sup>12</sup>. Such state leads to CD4 counts apoptosis. High concentration of sFasL among HIV infected patients correlated with high IL-22 concentration. The concentration of this cytokine did not, however, depend on a change in sFasL concentration resulting from the number of CD4 counts. The showed higher concentrations of sFas and sFasL among patients with the normal number of CD4 counts > 410 cells/ul, in comparison to patients with CD4 counts < 410 cells/ul, suggests that apoptosis processes in HIV infection become reduced together with the loss of lymphocytes. IL-22 concentration seems to correlate with apoptosis; however, it also seems to be conditioned by the number of CD4 counts, which is one of the places of synthesis of the cytokine in question. High concentration of IL-22 among patients infected only with HIV, slightly lower among HCV/HIV co-infected patients and below the norm in HCV infected patients corroborates the correla-

tion between apoptosis and IL-22 concentration. Apoptosis stimulates IL-22 synthesis while the cytokine itself plays the role of a factor that inhibits the process. This inhibition depends on the stimulation of proteins (Bcl-2) blocking programmed cell death processes by IL-22. Misse et al.<sup>13</sup>, in studies on innate immunity to HIV infection suggest an engagement of IL-22 and acute phase proteins that correlate with it. It seems that these studies, as well as the studies of Wolk et al.<sup>4</sup> related to the influence of IL-22 on non-specific immunity of tissues, are a brand new direction of immunological studies.

Proteins comprising HCV affect apoptosis processes in different ways: structural E1 protein and non-structural NS5A protein stimulate whereas E2 and NS5B proteins inhibit apoptosis. Insufficient apoptosis activity in HCV infection has influence on the development of a chronic disease process<sup>14</sup>. The concentration of sFas before the start of interferon therapy is higher in comparison to the concentration of this protein in healthy persons. Antiviral treatment affects the increase of sFas concentration. The stimulation of programmed hepatocyte death among these patients is a consequence of interferon activity<sup>15</sup>. In the same time period an increase in IL-22 concentration occurs. Be-

fore interferon treatment of HCV infected patients, the concentration of IL-22 is very low, below the norm. An increase in the concentration of this cytokine during treatment with pegylated interferon indicates an improvement in hepatocyte function. It does not seem that in these states an increase in IL-22 concentration inhibits apoptosis processes. Brand et al. 16 who, in the studies of epithelial cell apoptosis in Crohn disease, did not observe an inhibiting influence of IL-22 on Fas receptors present such suggestions.

The fact that the lack of influence of HIV and HCV viraemias on the concentrations of IL-22, sFas and sFasL was shown is also of great importance in the undertaken studies. These observations suggest that there is no correlation between viraemia and programmed cell death processes.

## CONCLUSIONS

High activity of apoptosis processes among HIV infected patient's correlates with high concentration of IL-22. An increase in the concentration of IL-22 during treatment with pegylated interferon of HCV infected patients suggests that the cytokine in question could be considered as one of the indicators of hepatocytes function improvement.

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title

# Genetic background of the cardiovascular complications among HIV positive patients – preliminary report

authors

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summary

**Background.** An association between genetic factors and arteriosclerosis exists for chemokine receptor polymorphisms, CCR2 and two tightly linked SNPs of the CX3CR1, coding for positions 249 and 290 of the receptor.

**Aim of the study.** To describe frequency of I249V and M290T alleles in HIV-1 infected patients in the context of cardiovascular diseases (CVDs).

**Material and methods.** 168 HIV-1 infected Caucasians aged 23–66 ys (28.0% women and 72.0% men) were included into the study. QIAamp DNA Blood mini kit and was used to extract genomic DNA from blood. To assess the distribution of single nucleotide polymorphisms PCR–RFLP protocols were used. For all reaction products electrophoresis in the 2,5 % agarose gel stained with ethidium bromide was performed. Results were visualized in the UV light under transilluminator and recorded with polaroid camera.

**Results.** For the 249 site the I/I allele of CX3CR1 was present in 17(10,1 %) patients. For the second assessed variant in position 290, 102 individuals (60,7%) were carrying a T/T homozygous genotype, and 11 (6,6%) patients homozygous for the M/M genotype. Patients homozygous for both I/I and M/M genotypes had no history of CVDs.

Cardiovascular events were diagnosed in 8 patients. No protective homozygous I/I and M/M genotypes were found among these patients.

**Conclusions.** No research on the influence of these polymorphisms on the CVDs incidence among HIV patients was published – this is a preliminary report on this matter. As protective I/I and M/M genotypes were not present in a group with these complications, further study has to be performed to obtain conclusive results. Basing on fraktalkine polymorphism genotyping, patients may be treated with wider spectrum of antiretrovirals, so such analyses may become a useful tool for clinical practice.

key words

fraktalkine, cardiovascular complications, HIV infection

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## BACKGROUND

Leukocyte migration and trafficking plays a vital role in a pathogenesis of arteriosclerosis therefore factors influencing inflammatory responses – chemokines and chemokine receptors – remain to be perceived as “the key players” in this process<sup>1</sup>. Many factors influence the inflammatory responses and processes related to the endothelial inflammation, both in infectious disease and patients with the immunological response disturbances. Genetic background may influence atherosclerotic processes. It is also widely known that immunological dysfunction among HIV positive individuals disturbs inflammatory responses, strongly influencing the risk of arteriosclerosis.

Previous research indicates, that an association between genetic factors and arteriosclerosis exists for chemokine receptor polymorphisms, CCR2 and two tightly linked SNPs of the CX3CR1 coding for positions 249 and 290 of the receptor<sup>2,3</sup>. The first one, CCR2, being a second-line HIV coreceptor, is involved into monocyte cell migration and disposition in the arterial wall, with its V64I coding frame polymorphism related to the altered formation of the atherosclerotic lesions<sup>4</sup>. The second receptor, fraktalkine, being a ligand for a transmembrane chemokine receptor mediating distribution of the CX3CR1 (+) leukocytes with both chemoattractant and adhesive properties, seems to be also strongly involved in inflammatory process. Fraktalkine receptor polymorphisms have been linked to incidence of the coronary artery disease, dysfunction of the coronary endothelium and presence of the atherosclerotic lesions.

Introduction of combined, highly active antiretroviral therapy (HAART) has had a significant impact on morbidity and mortality among patients living with HIV and AIDS. Its use is associated with proatherogenic lipid changes, lipodystrophy syndrome, insulin resistance and other co-morbidities responsible for increased risk of cardiovascular diseases<sup>5-7</sup>.

It is important to consider these factors in HIV + individuals, especially with coexistent of the cardiovascular disease risk factors. With contemporarily observed decline in the HIV-associated mortality, other causes of death and possible co-morbidities have become increasingly important<sup>8,9</sup>.

Here we would like to present a study describing frequency of I249V and M290T alleles in HIV-1 infected patients in the context of cardiovascular diseases.

## MATERIAL AND METHODS

The studied group comprised of the 168 HIV-1 infected Caucasians aged 23-66 ys (mean 36±9 years; median – 35 years) recruited randomly from the patients of the Department of Infectious Diseases and Hepatology, Pomeranian Medical University, Szczecin, Poland. Only adult subjects of both genders (28.0% women and 72.0% men), with confirmed HIV-1 infection (western-blot) were included into the study. Sexual transmission of HIV-1 was more prevalent route of the infection (94 patients – 56.0%) than injection drug use (74 individuals – 44.0%). The following HIV infection categories were represented (CDC/WHO 1993): A – 24 (14.3%), B – 97 (57.7%), C – 47 (28.0%).

## DNA EXTRACTION AND GENOTYPING

QIAamp DNA Blood mini kit and (QIAGEN, Hilden, Germany) was used to extract genomic DNA from full blood samples, previously collected to tubes containing EDTA anticoagulant. The extraction was performed according to the manufacturer's protocol, DNA was re-suspended in the 200 µL of AE buffer (QIAGEN, Hilden, Germany) and stored in 4°C for further analyses. To assess the distribution of single nucleotide polymorphisms previously described PCR-RFLP protocols were used<sup>10</sup>, with the following primer pair: CCgAggTCCTTCAggAAATCT (forward primer) and TCAGCATCAGgTTCAggAACTC (reverse primer). The reaction products were subsequently digested with the Pst 1406I enzyme. Allele I has remained undigested with a product of the 594 b.p. while V allele was cleaved into 207 and 387 b.p. fragments. For all reaction products electrophoresis in the 2,5% agarose gel (SIGMA, Saint Louis, USA) stained with ethidium bromide was performed. Results were visualized in the UV light under transilluminator (Transilluminator 4000, Stratagene, La Jolla, USA) and recorded with DS-34 Polaroid Direct Screen Camera.

## RESULTS

For the 249 site the I/I allele of CX3CR1 was present in 17(10,1 %) patients and the following genotype frequencies were noted: I/V – 61(36,1%), V/V – 91(53,8%). For the second assessed variant in position 290, one hundred and two individuals (60,7%) were carrying a T/T homozygous genotype, with 55 (32,7%) T/M heterozygotes and 11 (6,6%) patients homozygous for the M/M genotype. Allelic frequency for the I249 variant was 28,2% while the other analysed allele, M290 was observed in 22,9%.

Patients homozygous for both I/I and M/M genotypes had no history of cardiovascular disease.

In the analysed group cardiovascular events were diagnosed in 8 patients, aged 40-66 years (mean 51,5±7,8) Three patients developed myocardial infarction, four suffered from unstable coronary heart disease, and one person was diagnosed with the ischaemic stroke.

In this group five were carriers of the I249 allele while M290 allele was observed in four cases. No protective homozygous I/I and M/M genotypes were found in this group, while among patients with no cardiovascular complications 17 (11,3%) I/I homozygotes and 10 (6,0%) M/M genotypes were observed. In total the I249 and M290 allele was carried by 73 (45,6%) and 62 (38,8%) patients with no history of cardiovascular complications respectively with a frequency of 30% for I249 and 25% for M290 alleles.

## DISCUSSION

Genetic factors remain to be perceived as a means influencing progression of HIV disease, but also an important clinical factor related to the opportunistic infections and adverse events during the treatment. Fraktalkine polymorphisms seem to be a novel, important factor in the patho-

genesis of HIV disease, despite the controversy which has arisen in the research on CX3CR1 polymorphisms, indicating both protective and adverse effect of the fractalkine receptor polymorphisms on HIV progression<sup>11-15</sup>. Future study on this receptor may prove beneficial especially for development of the new treatment immunomodulating strategies, as its role in leukocyte trafficking, and its influence on the HIV-infected cell spread and entrapment within various tissues is extensive<sup>16</sup>.

Divergent data exist on the relationship between fractalkine polymorphisms and cardiovascular disease. I249 allele is related to the reduced number of fractalkine binding sites on polymononuclear blood cells and therefore potentially impaired monocyte adhesion during the formation of atherosclerotic lesions. In a case-control study Moatti et al. have shown that I249 allele, reduces the risk of the coronary artery events in patients with acute coronary disease – both myocardial infarction and unstable angina. Despite tight linkage between I249 and M290 alleles, no such correlation was found for the second allele in this study<sup>10</sup>. Protective effect of the I249 allele was confirmed in the study by McDermott et al., investigating individuals with diagnosed coronary artery disease. In I249 allele carriers coronary artery stenoses occurred significantly less frequently and endothelial function was better, independently on the risk factor<sup>17</sup>. This was confirmed in a study on animal models with CX3CR1 knock-out mice being less susceptible to the atherosclerotic plaque formation<sup>3,18</sup>. On the other hand Neissner et al., have found that I249 in the absence of the other potentially protective variant – M290, may be related to the increased risk of acute coronary syndromes. Authors suggest that both variant may act antagonistically and that M290 stabilises influence of the I249 on the enhanced inflammatory responses<sup>19</sup>. Similarly, carrying the I249 allele increased the risk of restenosis after coronary stenting<sup>20</sup>.

Genetic research in HIV is focused mainly on the disease progression and viral resistance to the treatment, with some pharmacogenomics being currently implemented into practice. Data on the background for cardiovascular diseases in HIV are based mainly on traditional risk factor assessments<sup>21</sup>.

Among HIV infected patients cardiovascular complications were analysed both prospectively and retrospectively. The largest prospective study to date is the D:A:D study, which includes cohorts from Europe, Australia, and the United States. During the D:A:D study period, the incidence of MI was increasing with longer exposure to HAART. The study reported that treated or untreated patients infected with HIV-1 had a high prevalence of CV risk factors. However, the increased risk of MI was independent of the known risk factors for CAD<sup>21</sup>.

A major retrospective study, analysing French data have found a link between protease inhibitors use and myocardial infarction<sup>22</sup>. This is in accordance to the data obtained from D:A:D where similar findings were reported.

Despite the benefits of cART, its use is often associated with adverse effects related to lipid abnormalities and associated cardiovascular risk, and these factors are important to consider when treating patients. However, it must be strongly stated here, that a patient always benefits from antiretroviral therapy, therefore introduction of the effective antiretroviral therapy should not be delayed or discontinued solely due to the cardiovascular disease risk.

## CONCLUSIONS

So far, no research on the influence of these polymorphisms on the incidence and outcome of the complications among HIV patients was published – this is a preliminary report on this matter. If protective influence of the I/I and M/M genotype on cardiovascular complications is to be considered, and no such genotype was present in a group with these complications, further study has to be performed to obtain conclusive results. Basing on genetic data on fractalkine polymorphism, clinicians may treat patients with wider spectrum of antiretrovirals, so such analyses may become a useful tool for clinical practice.

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title

# HIV-1 drug resistance patterns among treatment-naïve and therapy-experienced patients in Poland

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summary

**Background.** HIV-1 drug resistance (DR) is becoming a growing concern. It is estimated that about one out of ten newly HIV-infected persons in Europe acquires HIV-1 drug resistant strain. DR testing is now believed to play a crucial role in proper and successful treatment.

The aim of the study was a retrospective analysis of the HIV-1 drug resistance patterns among Polish naïve and therapy-experienced patients.

**Materials and methods.** In all cases before drug resistance testing viral load was determined as well as CD4/CD8 counts. The sequencing assay was carried out using commercially available assays according to manufacturer's protocol. Electrophoretic analysis data were verified and manually corrected. Interpretation was performed using Stanford's Genotypic Resistance Interpretation Algorithm or by a built-in algorithm included in ViroSeq System.

**Results.** In the treatment-naïve group 14,1% of tested patients have acquired HIV-1 strains harbouring resistance-associated mutations. In the therapy-experienced group among 75 analyzed patients 58 (77%) were found to be carrying drug resistant mutants. In this group 14 persons (19%) had at least one resistance-associated mutation in all three drug classes. Among therapy-experienced patients the prevalence of K65R was very low (1,3%).

**Conclusion.** The results of both analysed groups indicate the necessity of resistance testing in Poland. In the group of patients never exposed to ART in 14,1% cases viral variants resistant to at least one of the drugs were identified. The problem of resistance intensifies with consecutive regimen failures limiting the remaining treatment options. A shift has been observed in the route of HIV-1 infection in Poland. The obtained data also documents a change in HIV subtype pattern in Poland. The low prevalence of K65R mutation may result in very potent salvage therapy options based on TDF. Presented data documents high prevalence of drug resistance in Poland and justifies the necessity of DR testing.

key words

HIV-1 drug resistance, therapy naïve, therapy experienced

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## INTRODUCTION

Although the primary reason of highly active antiretroviral therapy (HAART) failure and the change of current regimen is still the drug toxicity and severe adverse effects associated with it, HIV-1 drug resistance (DR) is becoming a growing concern [1]. It is estimated that about one out of ten newly HIV-infected persons in Europe acquires HIV-1 strain resistant to available drugs [2]. In countries with prolonged history of HIV-1 treatment this proportion is either on the same level: 10% in USA [3] or even higher: 15% in Mexico [4] and above 13% in Australia [5]. This world-wide observed increase of drug resistant strains prevalence forced changes in the recommendations issued by EuroGuidelines HIV Resistance Group. Experts suggest considering resistance testing even in patients never exposed to antiretroviral therapy (ART) and strongly recommend testing for newly infected persons in countries where the prevalence of drug resistant strains exceeds 10% [6]. Drug resistance testing is now believed to play a crucial role in proper and successful treatment of HIV-positive persons.

In Poland drug resistance testing was first introduced in year 2000 by AIDS Diagnosis and Therapy Centre in Warsaw. For two years it has been performed using a commercially available assay based on reverse hybridization. Although simple, sensitive and relatively inexpensive this method was found to be of lower clinical and scientific utility compared to population sequencing. In 2002 automated DNA sequencing has been offered to clinicians involved in the treatment of HIV-1-positive patients providing complete information about DR mutations and, additionally, the subtype of the virus. Due to participation in numerous international trials sequencing is available for the vast majority of the patients either free or at affordable costs.

The aim of the study was retrospective analysis of the HIV-1 drug resistance patterns among Polish drug-naïve and drug-experienced patients.

## PATIENTS AND METHODS

The treatment-naïve group consisted of patients participating in SPREAD programme ([www.spread-europe.org](http://www.spread-europe.org)) and patients scheduled for the initiation of HAART. The main criteria for inclusion in the SPREAD study were: plasma level of HIV-1 RNA at least 1000 copies/mL, time since first HIV-positive result of maximum 3 months and no earlier history of ART. Seventy one plasma samples were collected in the years 2003-2004. The samples along with the epidemiological data were obtained from 4 clinical centers located in Warsaw, Białystok, Łódź and Szczecin. The epidemiological data included e.g. age, area of residence and reported by patients routes of infection (more than one answer was possible). Demographic and clinical data are shown in Table 1.

The treatment-experienced group consisted of patients admitting the HIV/AIDS Care Centre in Warsaw and clinical centers in 3 other cities. The cohort included 75 patients who needed to have their current regimen changed either because of drug toxicity and severe adverse events or virological failure. The plasma samples were collected from January 2004 to April 2005.

Differences in duration of treatment and therapeutic regimens used make it difficult to compile coherent epidemiological data

In all cases before drug resistance testing viral load was determined (Cobas Amplicor HIV-1 Monitor Test, Roche Diagnostic Systems, Branchburg, NJ, USA) as well as CD4/CD8 counts (TriTest, Becton Dickinson, Franklin Lakes, NJ, USA). The declared detection limit of the used genotyping assay was 1000 copies/mL; samples of lower viral loads were not analyzed.

Upon receiving the blood samples the plasma was separated and stored until further testing in -20°C. The extraction of HIV-1 RNA was performed using lysis buffer (Roche) and modified Chomczynski method. The sequencing assay was carried out using either The ViroSeq™ HIV-1 Genotyping System (Applied Biosystems, Foster City, CA, USA) or Trugene® HIV-1 Genotyping Kit (Bayer HealthCare, Tarrytown, NY, USA) according to manufacturer's protocol. After obtaining the raw data of electrophoretic analysis all the resistance-associated codons were verified and, where necessary, manually corrected.

The interpretation of resistance in drug-naïve group was performed using freely available Stanford drug-resistance algorithm (beta test version 3.6; available at: <http://hivdb.stanford.edu>).

The interpretation of clinical relevance of mutations present in therapy-experienced group has been performed by a built-in algorithm included in ViroSeq System and a drug resistance report was generated.

## RESULTS

### Characteristics of the patients

The drug-naïve group consisted of 71 persons (48 males and 23 females) diagnosed HIV-positive in one of the participating clinical centers.

The majority of the patients (45,1%) was between 25 and 40 years old; the median age was 31 years; range: 18-65 years. Fifteen persons (21,1%) were older than 40 years. Nearly half of the patients (46,5%) lived in areas described as rural (< 100000 inhabitants), 16,9% in urban (100000-500000) and 35,2% in metropolitan areas. The most common route of infection was heterosexual contact (40%), followed by intravenous drug usage (37,5%) and homo/bisexual contact (12,5%). Six persons were either not willing to answer this question (1 patient) or stated the main route of infection as unknown (5 patients). The epidemiological characteristics of treatment-naïve group are shown in Table 1.

The therapy-experienced group consisted of 75 patients (54 males and 21 females). The age ranged from 18 to 63 years. The youngest patient has initiated therapy in the age of 12 years. The longest treated patient included in the analysis started ART in 1994, beginning with AZT monotherapy.

### Resistance-associated mutations among drug-naïve patients

The frequency of occurrence of nucleoside reverse-transcriptase inhibitor (NRTI) resistance-associated mutations

**Table 1.** Characteristics of treatment-naïve group

<b>Age of patients</b>	range: 18-65 years median age: 31 years 24 pts. (33,80%) < 25 years old 32 pts. (45,07%) 25-40 years old 15 pts. (21,12%) > 40 years old
<b>Gender of patients</b>	23 (32,39%) females 48 (67,61%) males
<b>Area of residence</b>	25 pts. (35,21%) metropolitan areas ( > 500000 inhabitants) 12 pts. (16,90%) urban areas (100000-500000 inhabitants) 33 pts. (46,47%) rural areas (< 100000 inhabitants) 1 pt. (1,40%) missing data
<b>The most common route of infection (more than one option could have been indicated)</b>	32 pts. (40,00%) heterosexual contact 30 pts. (37,50%) intravenous drug usage 12 pts. (15,00%) homo/bisexual contact 5 pts. (6,25%) unknown 1 pt. (1,25%) not willing to answer
<b>CD4 count</b>	15 pts. (21,12%) – CD4 > 500 cells/mm <sup>3</sup> 17 pts. (23,94%) – CD4 350-500 cells/mm <sup>3</sup> 14 pts. (19,71%) – CD4 200-350 cells/mm <sup>3</sup> 23 pts. (32,39%) – CD4 < 200 cells/mm <sup>3</sup> 2 pts. (2,81%) – CD4 count not performed
<b>HIV-1 viral load</b>	5 pts. (7,04%) < 10000 copies/mL 25 pts. (35,21%) 10000-100000 copies/mL 26 pts. (36,61%) > 100000 copies/mL 15 pts. (21,12%) – viral load testing not performed
<b>HIV-1 subtypes recognised</b>	67 cases (94,40%) of subtype B 1 case (1,40%) of subtype C 1 case (1,40%) of subtype D 1 case (1,40%) of subtype CRF03_AB 1 case (1,40%) of subtype CRF01_AE

was highest for K70E/R, T69S/N and T215D/E, each of them present in two cases (2,8%), followed by one case of G333E (1,4%). Among nonnucleoside reverse-transcriptase inhibitor (NNRTI) resistance-related mutations V108I, A98G, M230L, K101E, K103N were detected, each in one case (1,4%). For protease inhibitor (PI) resistance-related mutations V77I was the most common – 4 cases (5,6%). Subsequently, L10I and M36I were determined in two cases each (2,8%), followed by N88D, F53L and M46I, each in one case (1,4%).

### Resistance-associated mutations among therapy-experienced patients

Among therapy-failing patients 14 persons out of 75 analysed (19%) had at least one resistance-associated mutation in all three drug classes.

The frequency of occurrence of NRTI resistance-related mutations was highest for M184V – 17 cases (23%), followed by M41L in 9 cases (12%) and E44D, D67L, L210W – each in 8 cases (11%). The prevalence of K65R was very low – it was detected only in one sample (1,3%). For NNRTI resistance-related mutations the prevalence of K103N, Y181C/I and Y188L/C was the most common, ranging from 8 – 11%. In protease region the highest frequency of occurrence was as follows: L10I in 13 cases (17%), M36I in 12 cases (16%) and V77I in 11 cases (15%). The prevalence of any other DR mutation in that region was below 8%.

### Predicted HIV-1 drug resistance

In the treatment-naïve group 14,1% of tested patients have acquired drug resistant virus and further 9,9% were infected with HIV-1 variants with possibly lowered susceptibility. In the therapy-experienced group among 75 analyzed patients 58 (77%) were found to be carrying drug resistant strain.

Among the newly diagnosed patients infected with resistant virus in all cases resistance to single class of antiretroviral (ARV) drugs was identified – 30% in PIs, 30% in NNRTIs and 40% in NRTIs.

In the treatment-experienced group forty eight pts (64%) were infected with HIV-1 strain resistant or with lowered susceptibility to at least one drug in PI class, 34 pts (45%) to at least one drug in NRTI group and 22 pts (29%) in the NNRTI class.

In the treatment-naïve group in the PI class the lowered susceptibility to NFV was the most common (2,8%); in the group of NNRTIs resistance was distributed almost equally between available drugs; in the NRTIs lowered susceptibility to AZT and d4T was most frequent (7% and 4,2%, respectively).

Also in the HAART-experienced cohort the majority of DR cases in the PI class was associated with resistance to NFV – 11 samples (15%), followed by resistance to IDV and RTV – 9 samples in each case. In the NNRTI class the resistance was distributed almost evenly among all three drugs – 20 cases (27%) for NVP, 19 (25%) for DLV and 16 (21%) for EFV. As for the NRTIs most frequent DR cases were related with resistance to 3TC and ddC – 20 samples (27%) for each drug, followed by AZT – 12 samples (16%).

### HIV subtypes among newly diagnosed HIV-positive persons

Additionally, sequences obtained from the group of naïve patients were exported to SPREAD database allowing the determination of HIV-1 subtype. The most common HIV-1 subtype detected was subtype B – in 67 (94%) cases. The other subtypes included C, D, CRF03\_AB and CRF01\_AE, each identified in one case (manuscript in preparation).

## DISCUSSION

HIV-1 drug resistance becomes a more and more frequent reason of HAART failure worldwide. One of the major causes of this phenomenon is the transmission of drug resistant variants to the group of therapy-naïve patients. Numerous observations indicate this problem [7] [8]. Available up-to-date HAART regimens do not eradicate the virus forcing lifelong administration of the drugs. This mechanism effects in permanent selective pressure leading to the emergence and propagation of resistance. Blower et al. suggests that some of the reasons causing the rise of drug-resistant HIV might be the increase in treatment rates and the increase in relative fitness of ART-resistant strains [9]. The rising prevalence of resistance might be also caused by either insufficiently suppressive treatment allowing HIV replication or the selection of resistant virus already existing in population of HIV quasi-species. Recently the attention was focused on a New York patient in-

fectured with a HIV strain resistant to all drugs except efavirenz and enfuvirtide. This case very rapidly progressed to AIDS [10].

Latest findings suggest also that in case of acquiring HIV through superinfection the likelihood of successful ART along with the value of patient's prior drug resistance testing and lack of prior antiretroviral use is significantly lowered [11].

Since the year 1985, when HIV epidemic in Poland began, until now 9594 cases of HIV-1 infection were diagnosed among Polish citizens; 1647 persons developed AIDS and 700 died. In 5260 cases infection was acquired via intravenous drug usage [12].

Clinical centers involved in the presented study admit about 65% of Polish HIV-positive patients. Such percentage and the localization of participating clinics ensure a fair representativeness of the enrollment, although southern regions of Poland are underrepresented.

Analysis of the results obtained in the therapy-naïve group shows the prevalence of HIV-1 DR in Poland to be 14,1%. This value is higher than median reported for Europe – 10,9% [2]. According to the European Guidelines prevalence of the DR above 10% indicates the necessity of resistance testing before the initiation of therapy.

The problem of resistance intensifies with consecutive regimen failures limiting the remaining treatment options. As expected, drug resistance-related mutations are much more frequent in the therapy-experienced cohort when compared to treatment-naïve group. Among the therapy-experienced patients of Warsaw cohort 77% were found to carry HIV strain with lowered susceptibility to ART and in 19% of the samples at least one resistance-associated mutation in each drug class was identified. The determined ratios are very similar to those reported by other studies [13], [14], [15].

In the therapy-experienced cohort the drug resistance patterns in the RT region are concordant with common usage of 3TC, AZT and d4T as the NRTI backbone of therapeutic regimens in Poland and AZT monotherapy in early 90's. These drugs also have a lower genetic barrier when compared to protease inhibitors. This mechanism explains higher frequency of resistance to NRTIs detected in analysed samples. The low prevalence of K65R mutation is probably caused by the relatively rare usage of tenofovir. Along with the fact that the sensitivity to TDF is increased by a common in this group M184V mutation [16] this finding may result in very potent salvage therapy options.

As proofed in earlier clinical studies i.e. Viradapt and GART [17], [18] genotype testing along with expert advice provides better outcomes compared to standard clinical management and might be a significant help in choosing a potent regimen.

The use of DNA sequencing for genotyping has many advantages over line probe assays and two significant disadvantages: much higher cost and lower sensitivity [19]. It is estimated that sequencing-based methods measure minority populations down to 20-30% while assays based on reverse hybridisation can detect down to 4-5%. This fact explains the higher ratios of drug resistance in NRTI class among treatment-naïve persons reported in previous study [20] and makes it difficult to compare with the two presented cohorts.

Additional analysis of the obtained sequences indicates a shift in the HIV-1 subtype pattern in Poland. The study performed in 1996 revealed subtype B in all of the tested samples [21]. In the present study we have detected presence of non-B subtypes. The recognised subtypes are com-

monly observed among East-European sexual workers and IVDUs [22]. Similar changes were documented in our studies concerning the genotypes of HCV in Poland: between years 1992-2003 the prevalence of HCV 1b genotype decreased from 83,3% to 71,8% and the prevalence of 3a genotype increased from 4,2% to 20,7% [23], [24]. This trend will probably intensify. We suggest that this phenomenon, observed throughout Europe [25], is mainly the result of intensification of tourism. In Poland it might be also the effect of political liberalisation and growing incomes.

Presented data supports the results of numerous earlier reports documenting increasing prevalence of HIV-1 drug resistance and justifies the necessity of drug resistance testing in good clinical practice.

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## title

# Tuberculin Skin Test reactivity rates among adults with Human Immunodeficiency Virus in relation to age, transmission mode and lymphocyte CD4 count

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## summary

Tuberculosis (TB) remains an emerging health problem in Poland, with incidence rate almost twice higher than in E.U. Nationwide screening is insufficient and only 4% of all cases is diagnosed by contact examination. HIV-positive patients are considered as vulnerable population being at higher risk of acquiring and developing tuberculosis. TST sensitivity and specificity is unsatisfactory in this group of patients. Here we investigate factors related to TST reactivity rates in HIV-positive adults group.

A total of 535 patients with TST result were identified. 36 (6,7%) were TST positive and eight (22,2%) were diagnosed with active tuberculosis (ATB) during the follow-up. Higher rate of positive TST was observed in injecting drug users (IDU) and in patients with CD4 > 300 cells/ml ( $p = 0,04$  and  $p = 0.026$ , respectively). In multivariate logistic regression higher risk of having a positive TST result was significantly associated with being IDU (OR 1,22 [1,03-1,46]) and with CD4 count above 300 cells/ml (OR 2,74 [1,25-5,99]).

Our study shows significantly decreased rate of TST reactivity at CD4 lymphocyte count below 300 cells/ml and indicates strong need for new screening tools.

## key words

HIV, TST, tuberculosis, CD4 count

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## BACKGROUND

Tuberculosis (TB) remains an emerging health problem in Poland. Since 1994 incidence of TB is decreasing, yet only in 2003 10.125 new TB cases were registered. The incidence rate (24.9 for 2004 and 24.3 for 2006) is approximately twice as high as in E.U. countries (1,2).

Pulmonary TB represents 90,9% of overall TB cases and it is suspected that repeatedly low proportion of extrapulmonary TB (3.8%) reflects insufficient diagnosis of this form of disease (3). Despite many efforts mortality from TB was 2,4 in 2004 (37,4 in 1965 and 5,6 in 1990) and only in 2006 over eight hundred people died from TB in Poland.

Nationwide screening provides control chest X-ray in high-risk groups (ex. medical students) and every two years for employed adults. In 2003 as much as 82% of all TB pulmonary cases were detected by symptoms and only 4% by contact examination (4).

This situation poses a serious treat for vulnerable populations like children, elderly and immunocompromised patients (5).

Data collected for 2000 – 2002 shows that, in HIV-positive patients, fungal infections and tuberculosis are the most common opportunistic infections in Poland (6). Recent analysis shows that within last years the incidence rate was 1.62/100 patients/year (7). In addition the rate of extrapulmonary TB, which is more difficult to diagnose, is higher in HIV-positive population (8). The need for proper screening approach is an issue emerging not only in Poland (3,12), especially in HIV – positive patients with severe immunodeficiency (9).

TST was considered as the gold standard for TB screening for a century, yet its sensitivity and specificity is insufficient for HIV-infected patients with immunodepression (10,11). Moreover, it is still unclear which level of lymphocyte CD4 count would be high enough to ensure unchanged reactivity to purified protein derivative (PPD).

Therefore we decided to evaluate response to PPD in relation to current lymphocyte CD4 count and other factors in Warsaw Cohort of HIV-positive patients.

## MATERIAL AND METHODS

Information on TST results, CD4 lymphocyte count, infection mode and demographic characteristics were obtained from Warsaw Out-Patient Clinic's database, prospectively designed to collect medical information on HIV-positive patients followed up in routine care. Information is available since 1996. We analyzed the data from the 1<sup>st</sup> of January 1996 to the 31<sup>st</sup> of December 2005.

Patients with available CD4 lymphocyte count measured within 6 months prior to or after the date of TST reading were included in the main analysis. Reaction to TST was analyzed with purified protein derivative (Tuberculin PPD RT 23 SSI), dosage 0.1 ml, using Mantoux technique. Positive TST was defined as > 5 mm induration diameter. Only the first TST result for each patient was included into the analysis.

In statistical analysis unpaired t- test was used for normally distributed variables and Chi-squared or Kruskal-Wallis tests for variables with skewed distribution. All vari-

ables available were examined in univariate models, and those significant ( $p < 0.5$ ) were included in the final multivariate logistic regression model.

Confidence interval (CI) of 95% was accepted.

## RESULTS

There were 535 patients screened with at least one TST during the analyzed period of time. 478 of them had CD4 lymphocyte count measured within half a year prior or after TST reading date. Data about infection mode were available for 482 patients, among them 295 were injecting drug users (IDU) and 187 represented other risk group. 53 patients didn't indicate infection mode on first visit. Mean age was 33,3 years (range 16,0-68,3), median CD4 count was 282 cells/ml (range 7 – 1264). Baseline characteristics for TST positive and negative groups are shown in Table 1.

Table 1. Baseline characteristics

	N	TST (+)	TST (-)	p value
All	535	36	499	-
Infection mode		n(%)		
IDU	482	25 (8.47)	270 (91.53)	0.04
Others		7 (3.72)	180 (96.28)	
		Mean (SD)		
Age	535	33.36 (9.05)	31.63 (8.78)	0.26
		Median (IQR)		
CD4	478	30	448	0.004
		389 (201-568)	313 (143-449)	

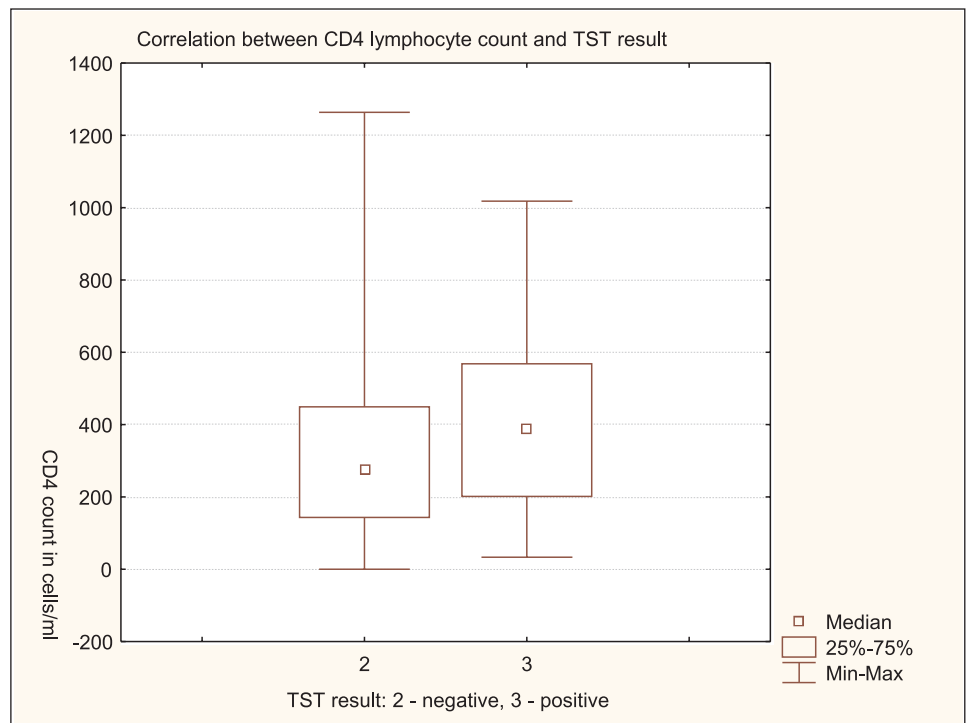
36 patients (6,7%) were TST positive, among them eight were diagnosed and treated for active tuberculosis (ATB) during the follow-up (22,2%). CD4 count was significantly higher for TST positive group, with median 389 cells/ml, than for TST negative one, median 278 cells/ml ( $p = 0,004$ ). Figure 1. Higher rate of positive TST results was observed in injecting drug users (IDU) in comparison to all other infection modes ( $p = 0,04$ ). There was no correlation between patient's age at the date on TST reading and TST result (Figure 2).

While the group was stratified by CD4 lymphocyte count only the 300 cells/ml cut-off level was statistically significant, showing increased rate of positive TST results in > 300 group ( $p = 0.026$ ).

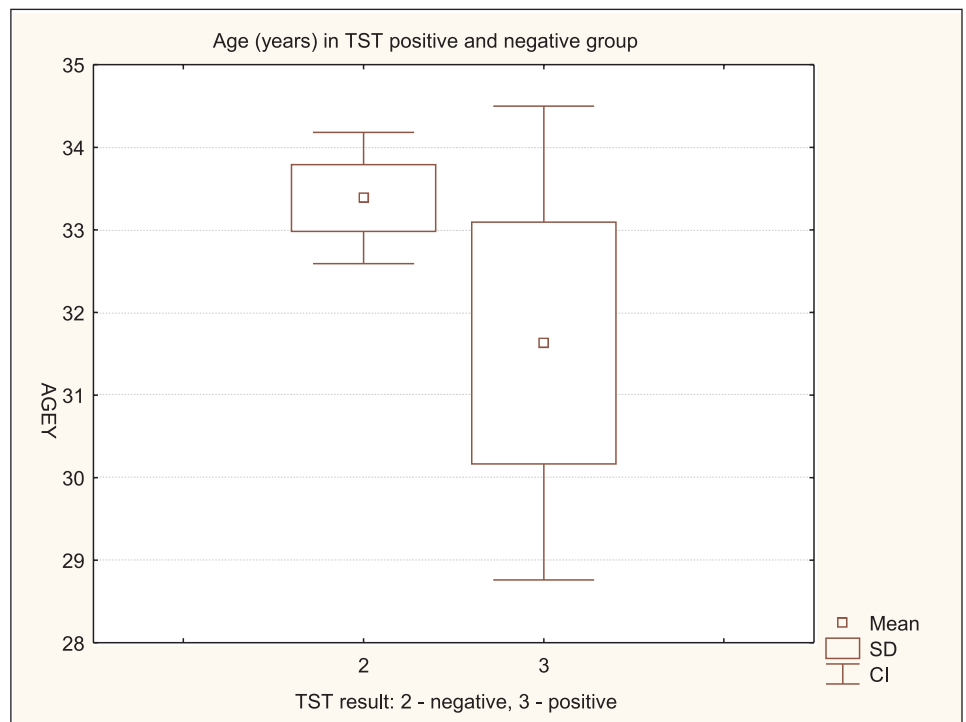
In multivariate logistic regression higher risk of having a positive TST result was significantly associated with being IDU (OR 1,22 [1,03-1,46]) and CD4 count above 300 cells/ml (OR 2,74 [1,25-5,99]).

We have additionally performed Spearman's correlation analysis for positive TST diameter and CD4 lymphocyte count (Figure 3), and TST diameter and age in years (Figure 4). In both cases, there was no statistical significance.

**Figure 1.**  
Correlation between CD4 lymphocyte count and TST result (Kruskal-Wallis test)



**Figure 2.**  
Correlation between age in years and TST result (unpaired T-test)



## CONCLUSIONS

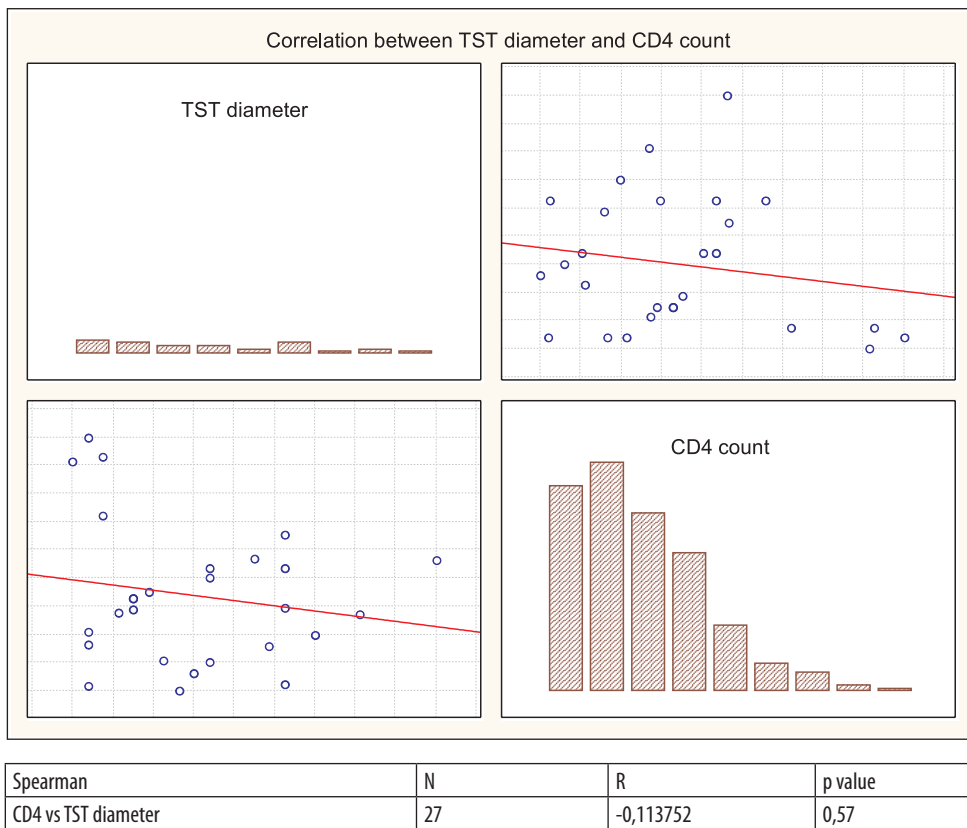
Our study shows significantly decreased rate of TST reactivity among patients with CD4 lymphocyte count below 300 cells/ml. Those findings are consistent with previous study from our center showing higher, and comparable to HIV negative control, prevalence of positive TST in the group of HIV positive patients whose CD4 cell count was above 350 cells/ml (12).

However this results varies from international studies showing that the difference remains significant while the lymphocyte CD4 boarder line is fixed at 200 cells/ml (13).

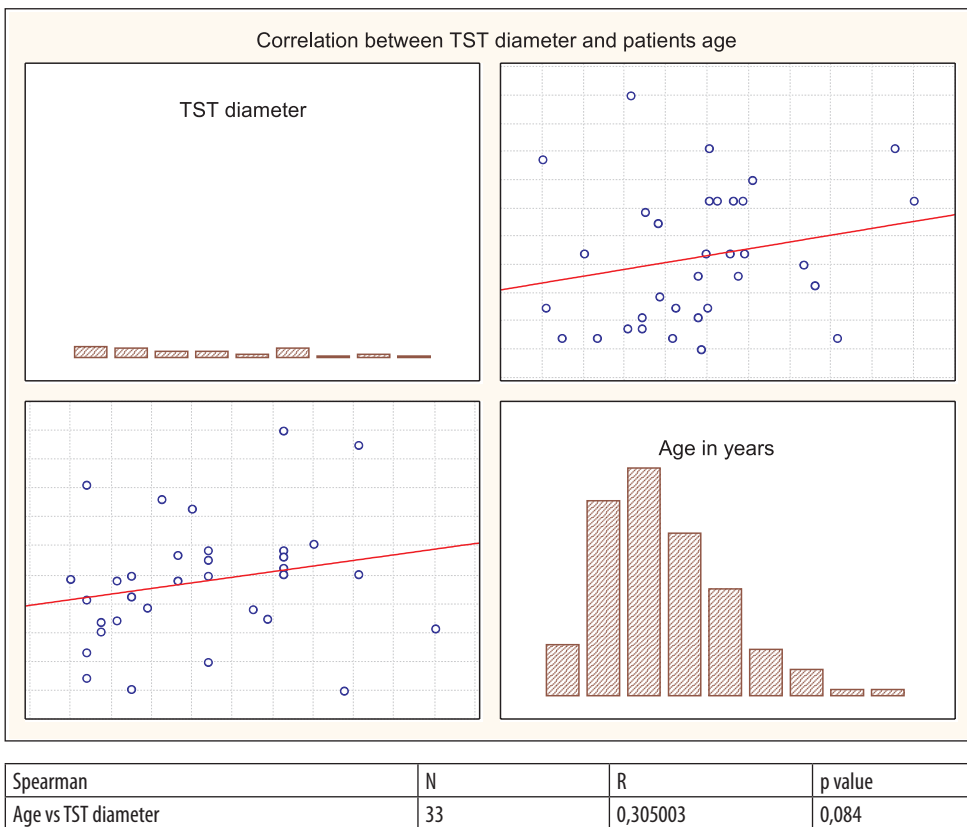
IDU status and CD4 count above 300 cells/ml were found to be independently associated with higher rate of

positive TST. It should be noted that our study has some potential limitations. Long period (six months) between available CD4 count and TST may affect understanding of correlation between those parameters, which are strongly dependent of actual cell immunity.

Lower rate of positive TST results in the group of patients with low CD4, who are in fact at higher risk of TB infection, indicates strong need for new screening tools and new screening approach. Although new tests based on interferon-gamma release shows higher sensitivity and specificity, their usefulness in the group of patients with CD4 count below 200-300 cells/ml needs to be closely investigated (14).



**Figure 3.**  
Spearman's correlation between TST diameter and CD4 lymphocyte count



**Figure 4.**  
Spearman's correlation between TST diameter and age in years



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title

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## Three different manifestations of immune reconstitution disease in a HIV-1 positive man receiving effective HAART

authors

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summary

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A patient with advanced immune deficiency due to HIV-1 infection had developed 3 different immune restoration diseases. Their manifestation depended on the stage of immune reconstitution. First pneumocystodosis was observed, then mycobacterial infection with enlargement of the abdominal lymph nodes and after seven months Burkitt's lymphoma.

key words

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Immune reconstitution disease, HIV-1 infection, PCP, Mycobacteriosis, Lymphoma

address

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## BACKGROUND

Effective antiretroviral therapy can lead to rapid decrease in plasma HIV-1 RNA often followed by an increase in the CD4(+) T cells count (1). The improvement of immune response has important impact on the occurrence and clinical course of AIDS-defining diseases and other disorders (1,2). However restoration of the mechanisms of innate and adaptive immunity is partial (3). Moreover quantitative and qualitative changes in the immune system can also result in paradoxical, atypical inflammatory reactions called IRIS (immune restoration inflammatory syndrome), IRS (immune reconstitution syndrome/ immune recovery syndrome) or IRD (immune restoration disease), especially in individuals with advanced immunodeficiency (3,4). The syndrome is often unexpected and may become more commonly encountered as more patients are treated with highly active antiretroviral therapy (HAART). Unusual clinical inflammatory syndromes associated with HAART are increasingly being noted after the initiation of the therapy (5,6,7,8). These disorders are important issues for physicians treating their patients with HAART.

Here we report an unusual case of multiple IRD.

## CASE REPORT

In an 47 year-old man oral thrush was observed in February 2003 but it resolved rapidly after ketokonazole was used. In May 2003 HIV-1 infection was confirmed and his CD4(+) T cells count was 5 cells/mm<sup>3</sup> and plasma HIV RNA 535 000 copies/mL. He complained of malaise, low grade fever, and weight loss of 6 kg during previous 2 months. On the physical examination enlarged and freely movable cervical, axillar and inguinal lymph nodes 1-2 cm in size were seen. Only one right axillar lymph node was 4 cm in size. There were no findings of chest radiography. On the 13<sup>th</sup> of May 2003 HAART with stavudine, lamivudine, lopinavir/ritonavir was initiated. He got also cotrimoxazole 960 mg once daily as the prophylactic treatment of toxoplasmosis and pneumocystodosis. Since 18<sup>th</sup> of May the patient had suffered from extreme fatigue, high grade fever, cough, chest discomfort and dyspnea at the rest. Physical examination revealed high grade fever (40° C), respiratory rate of 30 breaths/minute, oral thrush, hepatosplenomegaly, normal breaths sounds, numerous enlarged peripheral lymph nodes of the same size as had been noted before. The right axillar lymph node was smaller then previously observed. Chest x-ray showed patchy opacities mainly in the two upper lobes. Ultrasonography of the abdomen revealed enlargement of the liver and spleen and no other abnormalities including lymph nodes. Bronchoscopy and bronchoalveolar lavage disclosed non specific inflammatory findings. The CD4(+) T cells count increased significantly to 95 cells/mm<sup>3</sup> (09 06 2003), plasma viral load was not measured).

The tests for toxoplasmosis, CMV, HBV and HCV and bacterial infections were negative. Based on the findings mentioned above, especially chest radiograph *Pneumocystis carinii* (*jiroveci*) pneumonia was suspected. He got cotrimoxazole and corticosteroids as well as ketokonazole because of oral thrush. Antiretroviral therapy was continued. After 22 days of treatment there was complete resolution of

signs and symptoms, chest radiograph and PaO<sub>2</sub> were normal. He felt well within the next fifteen days. On the 02<sup>th</sup> of July the patient worsened again. He suffered from asthenia, lack of appetite, high grade fever. Physical examination revealed waisting, hepatosplenomegaly, the right axillar lymph node was movable, 3 cm in size. Chest radiograph was normal but abdominal ultrasonography showed hepatosplenomegaly and numerous enlarged lymph nodes forming conglomerates localized retroperitoneally. Blood cultures for bacterial and mycobacterial infections again were negative. Despite the results mycobacteriosis and/or lymphoma was suspected. As a part of an evaluation he underwent excision of the retroperitoneal lymph node and bone marrow biopsy. Histological examination of both samples did not reveal any malignancy but granulomatous lesions containing mycobacteria were seen. These mycobacteria were not identified. On the 21<sup>st</sup> of July he got clarithromycine, isoniazid, etambutol and amikacine. Within 17 days of the treatment significant enlargement of the right axillar lymph node was observed to the size of 8 cm. Again lymphoma was suspected. The patient did not agree for the excision of this lymph node and asked for discharge from the hospital on the 08<sup>th</sup> of August 2003. His CD4(+) T cells count was 75 cells/mm<sup>3</sup>. He had improved during the next three months using antimycobacterial and antiretroviral therapy. All the peripheral lymph nodes were smaller. The patient felt well. In October CD4(+) T lymphocyte count was 87 cells/mm<sup>3</sup>, plasma HIV RNA was 2600 copies/mL, indicating significant suppression of viral replication. The right axillar lymph node was 4 cm in diameter. Because of the improvement and lack of patient's agreement there was still no histological examination of the right axillar lymph node. Again he visited outpatient department in the first week of December 2003 because of a tumor localized in the right axilla, about 30 cm in size, which had enlarged within previous 14 days. This tumor was removed surgically. Histological examination of it disclosed Burkitt's lymphoma CD20+, MIB1 +++. From January to March 2004 he got chemotherapy with CODOX/IVAC. The therapy was complicated by bacterial sepsis and agranulocytosis. After IVAC he required treatment with TPI. The patient gradually worsened because of lymphoma and died in May 2004.

## DISCUSSION

The case described here is unusual, because three different manifestations of IRD were observed in one patient: pneumocystodosis eight days after HAART was initiated, mycobacteriosis forty nine days from the beginning of antiretroviral therapy, and Burkitt's lymphoma after seven months. At the time of initial presentation profound immunodeficiency and high viral load was seen. The patient had only minimally symptomatic disorders such as low grade fever, fatigue, slightly enlarged peripheral lymph nodes. Shortly after HAART was started signs and symptoms suggesting PCP developed. The diagnosis was not definitive but we assume that it was PCP because chest radiograph showed picture resembling pneumocystodosis, there was rapid improvement after cotrimoxazole treatment, other reasons of pneumonia were excluded and significant re-emerging of the immunocompetence evaluated by 19-fold rise of CD4(+) T cells within twenty four days was observed. There was probably subclinical, latent infec-

tion not seen at the initial presentation. Immune restoration inflammatory syndrome was a sign of improving control of infection. Although the patient suffered from severe illness antiretroviral therapy was continued. This was based on some reports generally recommending continuation of HAART besides of a few exceptions (9,10,11). No relapse of PCP was seen, but only for a short period of time the patient felt well. Soon symptoms suggesting lymphonodular mycobacteriosis or lymphoma were observed. Histologic examination of the abdominal lymph nodes and bone marrow revealed granulomatous inflammation and mycobacteria by staining. Cultures were negative. The presence of granulomas and the temporal relationship to vigorous immunologic response to HAART indicated that the clinical presentation was due to the restored inflammatory response (12,13,14,15). The localized mycobacterial lymphadenitis suggested that patient had subclinical disease before HAART and was anergic to mycobacterial antigens that time. The disease developed within forty nine days with prominent inflammatory reactions. This confirms other reports that mycobacterial IRD usually occurs within two months of commencing HAART (16,17). Appropriate antimycobacterial treatment was used together with HAART. Over the next several weeks the symptoms resolved and no relapse was observed. This fact also confirms the diagnosis of IRD-mycobacteriosis (12-17).

Despite effective antiretroviral, antimycobacterial therapy and PCP prophylaxis after three months of improvement the patient worsened again. Rapid growing huge tumor of right axilla was diagnosed as Burkitt's lymphoma. Based on the symptoms of lymphomas (18) it was suspected from the initial observation, but was not confirmed by the histological examination of the lymph node and bone marrow samples. First the patient did not agree for the excision and then physicians decided to observe it because the lymph node was gradually smaller and the improvement seemed to be due to effective antimycobacterial treatment.

The IRD in our patient was complex. Different immunological mechanisms were probably involved in the development of the aberrant reactions dependent on the causative factor and stage of immune restoration. The occurrence of certain disease reflected probably a kind of immune reactions. We believe that genetic susceptibility could play an important role in the development of multiple IRDs in our patient and had an impact on their development.

The mechanisms involved in IRD are not similar for all the diseases (19,20,21). Different opportunistic infections require different mechanisms of immune control and different factors are involved in their manifestation (19,20,21). The clinical features of mycobacteriosis depend on the host cell-mediated immune responses and strong cell-mediated immune responses result in localized disease (2,4,15,17,22). Studies to assess immune-system reconstitution during HAART have shown that increase in the total CD4(+) T cells count results initially from an increase in the memory subtype of CD4(+) lymphocytes. This subset possesses T-cell receptors specific for mycobacterial antigens. Thus inflammatory response is mediated (2,4,6,19). The other mechanisms concerning IRD-PCP and IRD-NHL are not well recognized. High levels of CD8 T cells, IL-6 and IL-6 receptors, soluble CD30 and soluble CD26, cells producing interferon gamma, persistent polyclonal hypergammaglobulinaemia, increased expression of CCR3 and CCR5 on monocytes and granulocytes, genetic susceptibility and polymorphism in cytokine genes may play a role in the development of these IRDs (3,4,6,19,20,21).

## CONCLUSION

Clinicians need to be aware of the possibility of occurrence of multiple IRDs in HIV-1 infected patients with advanced immunodeficiency receiving potent HAART, followed by a rapid immune restoration. The unexpected worsening of the clinical course after temporal improvement can result in the development of new IRD. Continuation of HAART may subsequently result in protective immunity and clearance of one infection but does not protect against other IRDs. The case raise the question of what clinical course can we expect during IRD.

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