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Contents

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title

Gynecologic infections in HIV-infected women

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summary

The number of women infected with HIV and diagnosed of AIDS is increases all over the world. The predominant mode of HIV transmission in this population is sexual intercourse. Women are at risk of other infections sharing the route of transmission. The presence of sexually transmitted diseases increased the risk of acquire and transmission HIV. The compressive gynecologic evaluation should be an important and standard component of medical care for HIV infected women.

key words

HIV infection, women, gynecologic infections

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Women comprise about half of all people living with HIV worldwide and the estimated number of them in the end of 2007 was 15.4 million. Heterosexual intercourse is still responsible for the most HIV transmission to women. Although many of manifestations of HIV infection are gender independent, but there are same gender-based differences. Women infected with human immunodeficiency virus are at increased risk for gynecologic co-morbidity. Most gynecological infections are sexually transmitted and the presence of a STD in known to increase of both acquiring and transmitting HIV. Studies in women established that genital ulcers and cervical infections are risk factors for the detection of genital HIV-RNA (5, 8, 17, 24, 28, 30). Vertical transmission studies provide the best available evidence that shedding of HIV-1 in the female genital tract is associated with increased transmission risk. Several studies have demonstrated that the detection of HIV-1 in the birth canal is associated with infant infection (4, 7, 30, 42). Given the high prevalence of *Candida* vulvovaginitis and *Trichomonas* vaginitis among HIV-1-infected women, even a modest association with potential infectivity may lead to a substantial attributable risk for HIV-1 transmission (38, 42)

Herpes Simplex Virus

Infection with herpes simplex virus (HSV) causes genital ulcer disease. Both clinical and subclinical infection lead to enhance HIV replication at mucosal surfaces and increased transmission of virus (30, 35). High levels of HIV RNA have been detected in genital ulcers caused by HSV-2 in men, and it was demonstrated that higher levels of HSV-DNA were associated with higher levels of cervical HIV-RNA and the more frequent detection of HIV infected cells in cervical secretions (29, 30, 44),. HIV also alters the clinical presentation of HSV infection. Reactivations occur at all stages of HIV infection, most of them are subclinical. HIV infected women have more frequent reactivation rate compared with general population (9). The increased levels of HIV RNA in genital ulcers are probably related to local enhancement of virus replication or to recruitment of activated CD4 lymphocytes into mucosal lesions (44).

Treatment of acute episodes with acyclovir or other available antiviral agents limits the duration and severity of symptoms and reduces the risk of subsequent recurrence. HIV infected women with more advanced immunosuppression often need higher doses and longer treatment. At least 10% women is infected with acyclovir resistant HSV. Most of this isolates are cross resistance to valacyclovir and famiciclovir and susceptable to topical cidofovir or intravenous foscarnet. Chronic suppressive therapy should be considered for patients with more then six recurrences per year. Daily suppressive therapy with acyclovir reduces frequency of recurrences by more then 75% patients (9,30).

Bacterial Vaginosis (BV)

Bacterial vaginosis is a common gynecologic condition characterized by an overgrowth of mixed flora including aerobic bacteria including Gardnerella vaginalis and Mycoplasma hominis and a reducing lactobacilli that predominate in normal vaginal environment. Bacterial vaginosis and intermediate flora have also been associated with substantial local increases in proinflammatory cytokine levels produced by activated immune cells (16, 23,45).

BV is the leading cause of vaginal discharge and has been associated with adverse obstetric and gynecologic outcomes such as pelvic inflammatory disease, endometritis, preterm delivery, postpartum fever and with an increase in the development of an infection following surgical procedures such as a hysterectomy or an abortion (19,23, 24, 43). Up to 50% of women with BV are asymptomatic. The prevalence rate of bacterial vaginosis is similar in HIV infected and HIV uninfected women and HIV does not alter the clinical presentation or treatment efficacy (5, 8, 9,16, 43). Several studies demonstrated the association between BV and HIV infection. There are several mechanisms by which alterations in vaginal flora and other causes of vaginitis might enhance HIV acquisition (8,9, 45). BV may enhance acquisition of herpes simplex virus type 2 (HSV-2), a major cofactor in both the acquisition and secondary transmission of HIV, and BV may also increase susceptibility to other STIs, such as *T. vaginalis* infection, *Neisseria gonorrhoeae* infection, and *Chlamydia trachomatis* infection. Antiretroviral treatment has been associated with lower prevalence of BV(3, 9, 20,42,43).

Vulvovaginal Candidiasis (VVC)

Vaginal candidiasis occurs frequently in all women regardless of sexual activity. *Candida albicans* colonizes the genital tract of 10-55% women in the childbearing age group. Most studies show increased rates of vaginal colonization in women infected with HIV. Symptomatic infections occurs in presence of predisposing factors such as immunodeficiency, pregnancy, uncontrolled diabetes mellitus, use of corticosteroids, antibiotics or high-oestrogen oral contraceptive pills (37,38,). Infection is common and as estimated 75% of all women will have at least one episode of VVC, and 40%-45% will have two or more episodes. Recurrent VVC (RVVC), usually defined as four or more episodes of symptomatic VVC in 1 year, affects a small percentage of women (< 5%). The most frequent causative agent of VVC is *C. albicans* but occasionally disease is caused by other non albicans *Candida* sp (16, 38, 42). Typical symptoms of VVC include abnormal vaginal discharge, purities, vaginal soreness, dyspareunia, external dysuria, None of these symptoms is specific for VVC. Approximately 10%-20% of women will have complicated VVC, suggesting diagnostic and therapeutic considerations. The pathogenesis of RVVC is unknown, and the majority of women with RVVC have no apparent predisposing or underlying conditions (16,38, 37.) Vaginal cultures should be obtained from all patients with RVVC to confirm the clinical diagnosis and to identify unusual species, including nonalbicans species. *C. glabrata* and other nonalbicans *Candidia* species are observed in 10%-20% of patients with RVVC. Conventional antimycotic therapies are not as effective against these species as against *C. albicans*. The incidence of VVC in HIV-infected women is unknown. HIV Epidemiology Research Study (HERS) found a trend towards more azole resistance in non-albicans species in women with CD4 < 300/μl, and rare resistance among *Candida albicans* isolates (9, 12, 20). Wang et al in the recent prospective study reported that treatment of *Candida*

vulvovaginitis was connected with 3.2-fold decrease in the quantity of cell-free HIV-1 and an ~3-fold decrease in the likelihood of detecting HIV-1-infected cells in the vaginal fluid of HIV-1-seropositive women (8,9).

Trichomoniasis

Trichomoniasis is a common infection caused by *T. vaginalis* the protozoan parasite transmitted principally through vaginal intercourse. T. vaginalis parasite infects > 200 million people worldwide annually. The reported rates of Trichomoniasis prevalence are 5%-13% among women in the general population and 40%-60% among women who are commercial sex workers (5, 9). The clinical presentation and the response to therapy are not altered by HIV infection. Trichomoniasis is frequently asymptomatic or symptoms are minimal. Some infected women have symptoms characterized by a diffuse, malodorous, yellow-green vaginal discharge with vulvar irritation. Since most patients with *Trichomonas* infection are asymptomatic or mildly symptomatic, they are likely to continue to remain sexually active in spite of infection. The pathogenesis of infection with TV is still unclear. Lower genital tract infections results in an inflammatory response with punctate mucosal hemorrhages, thus increased effective transmission and acquisition of HIV infection. In addition the pathology induced by T. vaginalis infection can increase HIV shedding, by an aggressive local cellular immune response with inflammation of the vaginal epithelium and cervix in women (9,10,16,18). Studies from Africa have suggested that *T. vaginalis* infection may increase the rate of HIV transmission by approximately twofold (17). Infection with this protozoan is increasingly recognized to be associated with gynecological complications including sepsis that occurs after abortion or after cesarean section. Diagnosis of vaginal trichomoniasis is usually performed by microscopy of vaginal secretions, but this method has a sensitivity of only approximately 60%-70% and requires immediate evaluation of wet preparation slide for optimal results. Vaginal trichomoniasis has been associated with adverse pregnancy outcomes, particularly premature rupture of membranes, preterm delivery, and low birth weight (7,19). Trichomonas vaginalis is one of the most important cofactors in amplifying MTC HIV transmission. Wang et al demonstrated that Treatment of *Trichomonas* vaginitis resulted in a 4.2-fold reduction in the quantity of cell-free HIV-1 but did not affect the prevalence of HIV-1-infected cells. High rates of treatment failure among both HIV-positive and HIV-negative women indicate that a 2-g dose of metronidazole may not be adequate for treatment of some women and that rescreening should be considered (26,42).

Pelvic Inflammatory Disease (PID)

PID comprises a spectrum of inflammatory disorders of the upper female genital tract, including endometritis, salpingitis, tubo-ovarian abscess, and pelvic peritonitis or a combination of these. When diagnosed early, PID can be successfully treated with antibiotics (3, 41). Most cases of pelvic inflammatory disease (PID) are due to the sexually transmitted disease caused by *Neisseria gonorrhoeae* or *Chlamydia trachomatis*. Pathogens that comprise the vaginal flora (*G. vaginalis*, *Haemophilus influenzae*, enteric Gram-negative species, and *Streptococcus agalactiae*) also have been associated with PID. In addition, cytomegalovirus (CMV), *M. hominis*, *U. urealyticum*, and *M. genitalium* might be associated with some cases of PID. All women who are diagnosed with acute PID should be tested for *N. gonorrhoeae* and *C. trachomatis* and should be screened for HIV infection (16, 20, 27). Differences in the clinical manifestations of PID between HIV-infected women and HIV-negative women have not been well-delineated. The microbiologic findings for HIV-positive and HIV-negative women were similar, except HIV-infected women had higher rates of concomitant *M. hominis*, candida, streptococcal, and HPV infections and HPV-related abnormalities (20, 27). Some of observational studies have demonstrated that PID had similar clinical presentation in HIV infected and uninfected women. Although clinical course of acute PID in women infected with HIV is more difficult, there are no significant differences in rates of infectious morbidity compared with uninfected population. In previous observational studies, HIV-infected women with PID presented with higher temperatures in time of hospitalization, lower leukocyte counts and are were more likely to require a change of antibiotics treatment (3, 20, 21,25, 27). Recently *Irvin et al* reported that HIV infected women might be predisposed to endometrial colonization by mycoplasmas and streptococci. Explanation of this findings are greater use of antibiotics by HIV infected patients and reduced colonization by pathogens other then mycoplasmas. Both streptococci and mycoplasmas can affect PID pathogenesis and optimal therapy against them should be required (21). Most of researches have demonstrated that HIV infection increases the prevalence tubo-ovarian abscess and the need for surgical intervention (16, 25, 20,21, 27). The CDC recommends treating HIV-infected women with standard antibiotic regimens (41). Most studies have shown that HIV infected women generally respond as well as HIV-negative women, but some demonstrated that they are more likely to remain febrile 48 hours after initiation of therapy (3, 16,20,25,27)/ *Irvin et al* noted that appropriate antibiotics reduce treatment failure, cervicovaginal HIV shedding, drug resistance and toxicities (21).

Human Papilloma Virus (HPV)

Most of HPV infections are asymptomatic and resolve without treatment. Approximately 70% of women with HPV infections become negative within one year and 91% within two years, 10% of all women infected with HPV develop persistent infection (1, 6). Genital infections with low-risk types of HPV are associated with genital warts. Clinical manifestation of persistent high-risk HPV infections include cellular abnormalities known as cervical intraepithelial neoplasia (CIN) or anogenital squamous cancer. HIV infected women should have a complete gynecologic examination including a cervical cytology as part of initial evaluation. A Pap smear should be obtain twice in the first year after diagnosis of HIV infection. A single Pap smear is associated with false-negative rates of 10-25%. Newer techniques using liquid-based media increased sensitivity and appear to reduce false-negative results. Those techniques are more expensive but they offer the possibility of direct HPV testing. The sensitivity of a single Pap smear for diagnosis CIN 2,3 or cancer managed from 33-94%. Colposkopy is more sensitive than HPV testing or repeat cytology for detection cervical precursor lesions, and HPV testing is more sensitive than repeat cytology (13,40, 32,

34). Abnormal cytology is associated the degree of immunosuppression. It was also found an association between the high plasma HIV-RNA and progression of abnormal cytology (11, 32,34, 36, 39). Women infected with HIV are more likely to be infected with high-risk oncogenic types of HPV are at greatest risk for developing CIN, lesion that may lead to invasive cervical cancer *(ICC).* Infection with HIV and immunosuppression has been shown to alter the clinical course of HPV and CIN by increasing the likelihood of viral persistence and lesion progression. HIV-positive women were shown to be at five-fold and seven-fold risk for cervical and anal cancer respectively, compared with general population. Several studies support the strong relationship between co-infection with HIV and HPV and CIN (1-2, 14,). Analysis performed in Womens's Interagency HIV Study (WIHS) demonstrated that high risk HPV types were three time more common among women with CD4 cell count less then 200/µl compared with women with CD4 cell count ≥ 500/µl (100. Similar to the WIHS study results were demonstrated by *Ahdieh et al.* It was noted that probability of subsequent HPV positivity among HIV negative and HIV positive with CD4 cell count ≥ 200 and < 200/µl was 47.5, 78.7 and 92.9% ($p < 0{,}001$). The relative incidence of HPV clearance was 0.29 among HIV negative women and 0.1% among HIV positive women (1,2). *Strickler et al* was demonstrated that plasma HIVRNA and CD4 count in combination have a strong and statistically interactive association with detection of HPV, which my also reflect HPV reactivation in sexually inactive women. The rate of incident HPV detection in women who have been in celibate for at least 18 month, was strongly associated with grade of immunosuppression. This results suggest that there are sources of HPV unrelated to recent sexual activity. *Strickler et al.* have shown that 80% of women with HPV reactivation were infected with HIV and had CD4 cell count > 500/ µl. In this study it was shown that the prevalence of CIN was significantly associated with CD4/HIV-RNA stratum, even in women with CD4 cell count > 500/µl (39). Invasive cervical cancer was included as an AIDS-defining condition by the Centers for Disease Control in 1993. Other epidemiologic factors associated with cervical cancer include cigarette smoking, long-term use of oral contraceptives and co-infection such as infection with Chlamydia trachomatis (1, 32). HPV also can be detected in the absence of sexual risk factors. Infection is transmitted through skin-to-skin contact and it can by passed by penetrative genital contact and by oral-genital, manual-genital contact (39). Cervical cytology is the most commonly used screening method to detect pathological lesions. The accuracy of this examination in HIV infected women was evaluated in several studies. The UK National Health Service Cancer Screening Programme recommends annual cytology, with an initial colposcopy in HIV infected women. More frequent Pap smear should be obtained in women with CD4 count < 200/µl or with symptomatic HIV infection. Those recommendations are connected with high rate of false negative results among HIV-infected women compared with general population. In HIV infected women invasive cervical cancer has been recognized in significantly younger age of between 30-40 years and a more advanced stage compared with HIV seronegative women (2,11, 13,14, 22). Treatment of CIN lesions has high recurrence rates of up 80% in HIV infected women. The American College of Obstetricians and Gynecologists recommends Pap smears every 3-4 month for the first year after treatment of pre-invasive cervical lesions, follow by cytology every six month (11, 2, 33, 39).

SUMMARY

The compressive gynecologic evaluation should be an important and standard component of medical care for HIV infected women. The prevalence of gynecologic infection is higher among those women, they have unusual clinical manifestation. Full screening for cervical lesions remains necessary in HIV positive women to prevent invasive cervical cancer in the era of antiretroviral therapy. Transmission of HIV is enhanced by co-infection with sexually transmitted infection. The relative decrease in genital HIV RNA after treatment is comparable to that observed after initiation of antiretroviral therapy. Appreciate treatment of STDs is particularly important in prevention HIV transmission. In the other hand HIV testing should be routinely recommended in women presented with gynecologic infections such as recurrent vulvovaginal candidiasis, oncogenic *human papilloma virus* (HPV), *herpes simplex virus* (HSV), *Treponema palladium*, genital warts or abnormal (Pap) smear. Infections increase susceptibility to HIV infection by resulting disruption of the epithelial barrier, recruitment and stimulation of susceptible cells or loss of protective lactobacilli. Efforts to reduce the incidence and duration of gynecological infections may substantially reduce the infectivity of HIV-1-infected women and could have a measurable impact on the HIV-1 epidemic.

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title

Integrase inhibitors as a new class of ARV treatment

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summary

Retroviral enzyme integrase is absolutely indispensable to HIV-1 replication by catalysing proviral HIV DNA integration into host genome. Its inhibition becomes a new therapeutic target for antiretroviral therapy. For ten last years many integrase inhibitors molecules have been discovered. The most promising are raltegravir (RAL) and elvitegravir (EVG). Raltegravir (Isentress®) is the first oral integrase inhibitor which received FDA and EMEA approval, respectively in October and December 2007. This ARV drug is indicated in treatment-experienced adult HIV-1 infected patients with multidrug resistances, who have already been exposed to simply three ARV classes.

key words

HIV replication, integrase, integrase inhibitor, ARV treatment, multidrug resistance

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HIV-1 (human immunodeficiency virus type 1) is a plus-sense RNA virus that, for its retroviral replication, involves absolutely three different enzymes: reverse transcriptase, integrase and protease. HIV enzymes activity inhibition by small-molecules is one of the best strategy for suppressing the HIV-1 life cycle in the host cells. Until recently during HAART (highly active antiretroviral therapy) three main viral enzyme inhibitors have been used: nucleoside/non-nucleoside reverse transcriptase inhibitors (NRTIs/NNRTIs) and protease inhibitors (PIs). Fortunately, since the end of 2007, the physicians have already an access to the absolutely new ARV drug class – integrase inhibitors (Raltegravir, MK-O518, Isentress®), which is indicated in treatment-experienced adult HIV-1 infected patients with multidrug resistances [1].

Mechanisms of HIV-1 life cycle

HIV-1 penetrates into host cytoplasm after binding with the CD4 receptor and CCR5 or CXCR4 co-receptor. 1-2 hours later, reverse transcriptase leads to production of proviral cDNA from viral RNA. Then, cDNA is integrated with host DNA by integrase (IN). This process is essential for HIV-1 replication and consists numerous steps. Firstly, two nucleotides are removed from 3'-end of cDNA in the process called 3'-processing. Then, in the cytoplasm, the preintegration complex (PIC) is formed from four IN and two cDNA molecules associated with many viral and cellular co-factors (Table 1). PIC enters into nucleus where the stand transfer reaction (integration of proviral cDNA with host genome) and viral DNA transcription take place followed by viral RNA translation, maturation and new viral particles formation in cytoplasm (Figure 1). In vitro, 3'-processing as well as stand transfer reaction require the presence of di-cations such as Mn^{2+} or Mg^{2+} [2].

HIV-1 integrase

Retroviral enzyme integrase, encoded by POL gene, is absolutely indispensable to HIV-1 replication. IN belongs to the nucleotidyl transferases superfamily. It is a 32kDa protein, composed from 288 amino acids (aa) and contained three main domains: the N-terminal domain (NTD), catalytic core domain (CCD) and C-terminal domain (CTD) (Figure 2).

The NTD (1-50 aa) is highly conserved part of IN, binds with zinc atom, interacts with INI1 and LEDGF/p75, and takes part in IN multimerisation and catalytic function. The CCD (51-211 aa) binds with Mn^{2+} or Mg^{2+}, interacts with LEDGF/p75 and contains active site of IN. The CTD (212-288 aa) is the least conserved IN domain, interacts with RT and EED, and binds viral DNA as well as viral and host genome [2, 3, 4].

Table 1. Viral and cellular co-factors associated with PIC

Viral integration co-factors	Cellular integration co-factors
Matrix (MA) Viral protein R (Vpr) Nucleocapsid (NC) Reverse transcriptase (RT)	Barrier to autointegration factor (BAF) High mobility group protein A1 (HMGA1) Integrase interactor 1 (INI1) Lens epithelium-derived growth factor/p75 (LEDGF/p75) Hepatoma-derived growth factor related protein 2 (HRP2) p300 acetyltransferase Heat shock protein 60 (HSP 60) Polycomb group embryonic ectoderm development protein (Polycomb group EED)

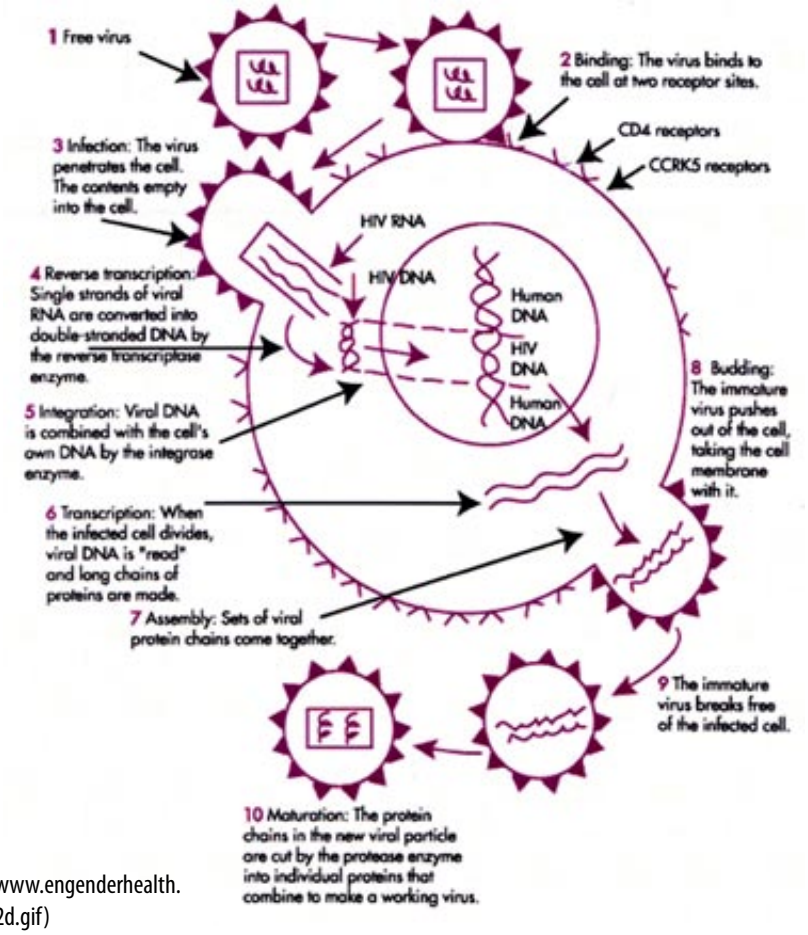

Figure 1. HIV-1 life cycle (available from: URL: http://www.engenderhealth.org/res/onc/hiv/understanding/images/hiv2d.gif)

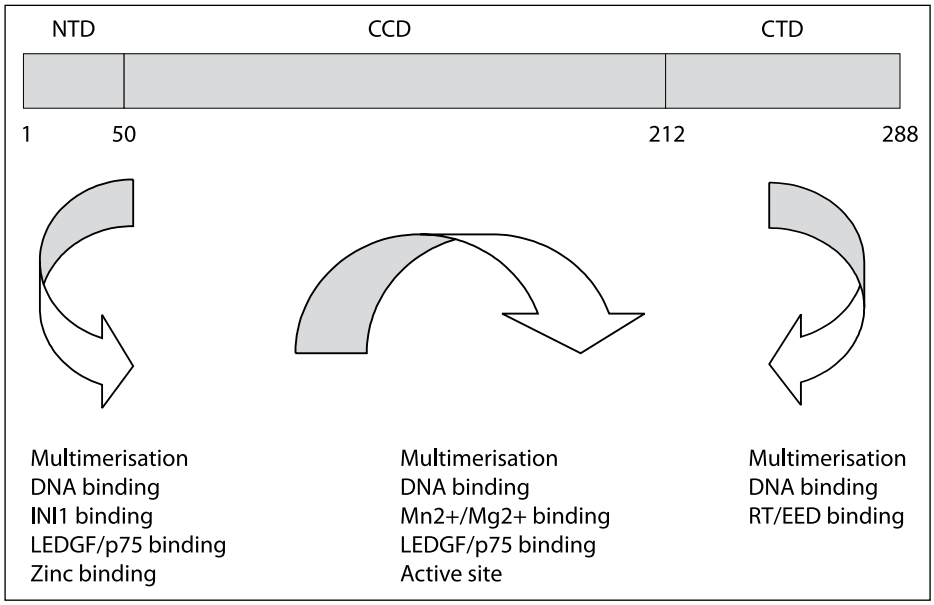

Figure 2.
Schematic diagram of HIV-1 integrase structure

INTEGRASE INHIBITORS: NEW CLASS OF ARV TREATMENT

HIV DNA integration into host genome is essential process for HIV replication in human cells. Moreover, only one enzyme – integrase (IN) – catalyses this reaction and this is why its inhibition becomes a new therapeutic target for antiretroviral therapy. For ten last years many IN inhibitors molecules have been discovered. Generally, IN inhibitors could be divided into two main groups: INBI (Integrase Binding Inhibitor) that restrain IN and viral DNA binding, and INSTI (Integrase Strand Transfer Inhibitor) that restrain PIC and host DNA binding. Furthermore, IN inhibitors are classified under their chemical structure for catechol-containing hydroxylated aromatics, diketoacid-containg aromatics, quinolines and non-catechol containing [4]. Despite many IN inhibitors molecules have been designed, only six of them are currently observed during phase II or phase III clinical trials: MK-0518 (Raltegravir), L-870810 and L-870812 from Merck Research Laboratories, GS-9137 (Elvitegravir) from Gilead Sciences, Inc., S-1360 from Shionogi-GSK and JKT-303 from Japan Tobacco [4, 5, 6, 7]. The most promising are raltegravir (RAL) and elvitegravir (EVG) which belong to the INSTI family, inhibit HIV-1 as well as HIV-2 replication, are more potent and give less side effects than other already known IN inhibitors [8].

Raltegravir (Isentress®) (Figure 3) is the first oral IN inhibitor which received FDA (Food and Drug Administration) and EMEA (European Medicines Agency) approval, respectively in October and December 2007 [1, 9]. This ARV drug is indicated in treatment-experienced adult HIV-1 infected patients (above 16 years old) with multidrug resistances, who have already been exposed to simply three ARV classes (especially new IP like tipranavir or darunavir) and should be used with at least one other active ARV molecule to decrease the risk of virologic failure and development of resistance. Its recommended dosage is 400mg twice daily with or without food. Its efficacy in this group of patients and recommended dose were confirmed in many clinical trials. The first MK-0518 phase II study was presented in San Francisco during ICAAC 2006 [10]. In this study 178 treatment-experienced patients with triple class resistant virus-infection were randomized. Three raltegravir dosages (200, 400 and 600mg bid) plus optimized background regimen (OBT) versus placebo were compared. After 24 week therapy 57-67% of the patients in the raltegravir arm versus 14% in the placebo arm had undetectable viral load. In the arm where enfuvirtide as OBT was used, nearly 90% of patients had viral load <400 copies/ml. The next two multi-centre, randomized, double

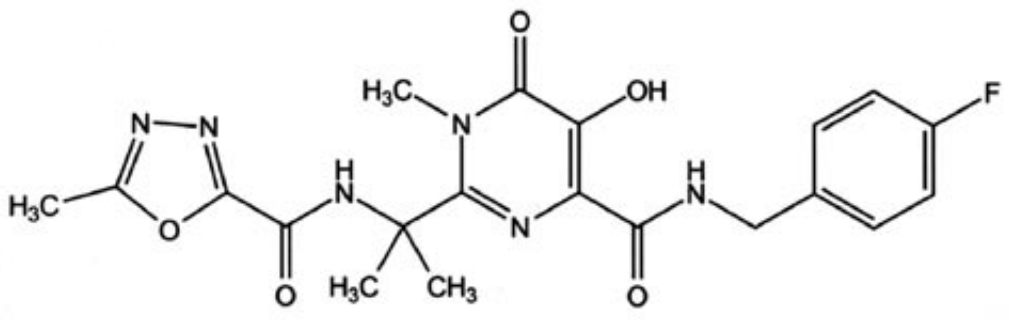

Figure 3. Chemical structure of raltegravir (5-hydroxy-1-methyl-2-{1-methyl-1[(5-methyl-[1,3,4]-oxadiazole-2-carbonyl)-amino]-ethyl}-6-oxo-1,6-dihydro-pyrimidine-4-carboxylic acid 4-fluoro-benzylamide)

blind, placebo-controlled phase III trials BENCHMRK-1 and BENCHMARK-2, where 400mg raltegravir administered twice daily was compared with placebo in a combination with OBT, confirmed the results from phase II studies. The results after 24-week outcome, were presented during CROI 2007 and EACS 2007 [11, 12, 13] and after 48-week outcome during CROI 2008 [14, 15]. The other study, presented during IAS 2007, confirmed virologic and immunological efficacy of raltegravir versus efavirenz in HIV-infected antiretroviral-naïve patients [16]. Four dosage of raltegravir were evaluated (100, 200, 400 and 600 mg bid). For all of them, raltegravir efficacy was verified (>2.2 log10 decline of viral load and augment of CD4+ from 144 to 221 copies/ml). Moreover, undetectable viral load was achieved earlier in all the raltegravir groups. Generally, 83 to 88% of raltegravir-treated subjects had viral load <50 copies/mL and this response was comparable in Week 24 and Week 48.

Recently, during CROI 2008, Arponen S et al [17] presented poster in which they confirmed the hypothesis that raltegravir thankfulness its unique molecular action mechanism could decrease proviral DNA in peripheral blood mononuclear cells (PBMC) in patients achieving undetectable viremia. Despite the virological and immunological response was achieved in both studied arms (OBT with raltegravir versus OBT alone), the significant decline of proviral DNA was observed at Week 12 merely in raltegravir arm.

Raltegravir is eliminated mainly by UGT1A1-mediated-glucuronidation in the liver and is not a substrate for cytochrome P450 enzymes. It could be used in combination with any other active anti-retroviral drugs without change of posology, even if they are potential UGT1A1 (uridine diphosphate glucuronosyltransferase 1A1) inducers or inhibitors like efavirenz, nevirapine or atazanavir. Actually, no more than co-utilization of rifampicine and drugs increased gastric pH avoids a double dose of raltegravir if their administration is inevitable. Moreover, there are any dosage change in case of renal or hepatic failure. However, the risk of fatal hepatic events increases in patients with preexisting liver disease such as hepatitis B or C. During raltegravir therapy, the most frequently observed clinical undesirable effects are diarrhea, nausea, headache, dizziness, vertigo, pruritus, athralgia, fatigue and asthenia. Biochemically, hypertriglyceridaemia, hypercholesterolemia as well as aminotransferases and bilirubin augmentation are occasionally viewed [18].

The potent and durable antiretroviral activity of elvitegravir (EVG) were presented by Zolopa A et al. during CROI 2007 and ICCAC 2007 [19, 20]. The efficacy of EVG boosted ritonavir was confirmed: in the arm of EVG/r 125/100 and 50/100 40% and 38% of patients, respectively, had an undetectable viral load in Week 16. Moreover, the addition of enfuvirtide to OBT increases this response to 74%. However, in Week 24, only in the arm 125/100 the significant decline of viral load was observed.

Unfortunately, raltegravir and the other IN inhibitors have a very feeble genetic barrier. Besides, extensive cross-resistance for all integrase inhibitors develops very rapidly and this is why it is fundamental to use raltegravir in combination with at least one other active ARV molecule and, the best, in patients with lower baseline viral load. The resistance mutations are associated with enzyme active site. The most common mutations are Q148K/R/H/E, N155H, E92Q and E157Q [5, 21]. The identified factors which increase the risk of viral mutant selection during raltegravir therapy are viral load above 100 000 copies/ml, Genotypic or Phenotypic Sensitivity Score (GSS/PSS)=0 and absence of enfuvirtide in ART [22].

Conclusion

It seems that integrase inhibitors such as raltegravir (Isentress®) are a new promising class of potent ARV drugs, which is extremely useful in treatment of patients with three-class resistance virus. Moreover, already accessible raltegravir is not only well tolerated but also has minimal drug interactions and easy posology and administration. This is why integrase inhibitors could became a base of new ARV therapy guidelines in the next few years.

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title

Validity of VIDAS-HIV DUO tests in the screening of sperm donors and women undergoing facilitated reproduction

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summary

The aim of the study was to assess the validity of the IVth generation tests in the screening diagnosis of HIV infection among sperm donors and women undergoing facilitated fertilization. The studied group included 1000 volunteers who underwent voluntary HIV infection tests. Among the studied subjects there were 531 men-sperm donors and 469 women prepared for the process of artificial insemination. The negative results of the screening VIDAS HIV DUO and VIDAS HIV DUO QUICK tests were observed in 983 cases. Positive results were reported in 9 subjects (3 women and 6 men) in VIDAS HIV DUO test and in 8 subjects (2 women and 6 men) in VIDAS HIV DUO QUICK test. The specificity of the VIDAS HIV DUO test was 98,5%. This test has been replaced by the VIDAS HIV DUO QUICK test, with a specificity of 99,3%. In assessing the validity of the test, 100% negative predictive value and a low positive predictive value – 33,3% for VIDAS HIV DUO test and 50% for VIDAS HIV DUO QUICK test was observed. False positive results were observed in 10 subjects (1%) studied. The VIDAS HIV DUO QUICK test has a high sensitivity and fairly good specificity. Reduced time of performance and full automatization assuring the safety of medical staff strongly influence the recommendation for its daily use in medical practice. HIV infection incidence among sperm donors and women who undergo aided reproduction is not high but single positive cases require complex diagnosis of this population.

key words

HIV, diagnosis, VIDAS HIV DUO

address

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HIV infection diagnosis is based on detection of special anti-HIV antibodies using EIA IIIrd generation tests. In the last few years IVth generation tests, which simultaneously detect anti-HIV antibodies and/or the p24 antigen have been introduced. Antigen p24 detection was implemented in order to shorten the serological window by 5-7 days in comparison to the effectiveness of HIV detection using IIIrd generation tests. At present five screening IVth generation commercial tests are applied: VIDAS HIV DUO (HIV4) (bioMérieux, Lyon, France), Vironostika HIV Uni-Form II Ag/Ab (Organon Teknika, Boxtel, Netherlands), Enzymun-Test ® HIV Combi (Roche Boehringer Mannheim, Penzberg, Germany), Enzygnost HIV Integral (Dade Behring, Mannheim, Germany) and Genscreen plus HIV Ag-Ab (Biorad, Hercules, USA). The introduction of IVth generation tests was a great step forward in HIV infection diagnosis and significantly improved its quality [26, 27, 28, 29]. Due to the wide variability of the HIV-1 and HIV-2 genome, each new method should be tested in the target population prior to its introduction into wider, everyday use [13, 14].

The aim of the study

The aim of the study was to assess the validity of the IVth generation tests in the screening diagnosis of HIV infection among sperm donors and women undergoing facilitated fertilization. A second aspect of the study was the retrospective assessment of the sensitivity, specificity, positive and negative predictive value of the VIDAS HIV DUO and VIDAS HIV DUO QUICK tests. The IVth generation tests studies are intended to improve the effectiveness of HIV infection detection in order to facilitate primary prevention of HIV transmission, prevention of unplanned pregnancies among HIV infected women, prophylaxis of HIV mother-to-child-transmission and early implementation of treatment, care and support for the infected mothers.

Materials and methods

The studied group included 1000 volunteers who underwent voluntary HIV infection tests. Among the studied subjects there were 531 men-sperm donors and 469 women prepared for the process of artificial insemination. The centre which conducted the study was the Centre for Marital Infertility Treatment "Kriobank". The subjects who were qualified for the tests were questioned before the test including risky behaviour (sexual contacts with a person of unknown serological status or intravenous drugs injections) in the last three months. The studied material was human serum which was achieved from full blood taken for clot in a "Sarstedt" closed system in a 2 ml volume. In 407 patients VIDAS HIV DUO test was done. It is an automatic, qualitative, screening test VIDAS HIV DUO (bioMerieux, Lyon, France), operating in a VIDAS system. It is based on total detection of p24 antigen HIV-1 virus, G immunoglobulin (IgG) anti-HIV-1 and anti-HIV-2 in the human serum or plasma using ELFA technique (enzymoimmunofluorescence method). This method limits the risk of laboratory error because the whole kit is prepared by the producer. 593 persons were subjected to testing with the VIDAS HIV DUO QUICK tests for which SPR modified probes were used. The inside of the probe was opsonised with gp 160 protein and synthetic peptides of O HIV-1 i HIV-2 group (in a lower part of the probe) and monoclonal antibodies anti-p-24 (in the upper part of the probe). The time of measurements was shortened to about 80 minutes.

In the participants of the study in whom positive results were achieved third generation ELISA tests were done after 6 weeks. Positive ELISA results were confirmed by Western blot.

The results were considered false positive when proved positive in the VIDAS HIV DUO and VIDAS HIV DUO QUICK tests, and negative in the screening IIIrd generation ELISA tests at the time of the study and three weeks afterwards.

For the assessment of the tests credibility, the sensitivity, specificity, positive and negative predictive value and diagnostic preciseness were used according to the definitions and patterns given below:

The sensitivity of the test – the percentage of the subjects in whom the test showed true positive results:

Diagnostic sensitivity = PD / PD+FU ×100%

Diagnostic specificity of the test – the percentage of the studied volunteers in whom the results of the tests were true negative.

Diagnostic specificity = PU / PU+FD ×100%

Predictive positive value – the cognitive value of the positive test. This parameter determines the percentage of the positive results among all the examined volunteers. (HIV infected and non-infected persons). This is a percentage of the true positive values among all achieved positive results.

The predictive positive value = PD / PD+FD ×100%

The predictive negative value – the cognitive value of the negative test result. This parameter determines the percentage of all true negative results among all negative results. Predictive negative value = PU / PU+FU ×100%

Diagnostic exactness – the percentage of people in whom the result of the test was true.

PD – true positive results
FD – false positive results
PU – true negative results
FU – false negative results

Results

The negative results of the screening VIDAS HIV DUO and VIDAS HIV DUO QUICK tests were observed in 983 cases. Positive results were reported in 9 subjects (3 women and 6 men) in VIDAS HIV DUO test and in 8 subjects (2 women and 6 men) in VIDAS HIV DUO QUICK test. The subjects aged from 19 to 43 years, among whom there were 7 HIV positive cases (1 woman and 6 men). In 10 persons the results of the test were false positive in VIDAS HIV DUO test (2 women and 4 men), and in VIDAS HIV DUO QUICK test (2 women and 2 men). These data are presented in table 1.

In the present paper the specificity of the VIDAS HIV DUO test was 98,5%. This test has been replaced by the VIDAS HIV DUO QUICK test, with a specificity of 99,3%. In assessing the validity of the test, 100% negative predictive value and a low positive predictive value – 33,3% for VIDAS HIV DUO test and 50% for VIDAS HIV DUO QUICK test was observed. False positive results were observed in 10 subjects (1%) studied.

False negative results were seen in 4 men co-infected with HCV, while 3 women in whom false negative results

were observed had been receiving hormonal therapy, which may have affected the results of the tests. The advancement of the IVth generation tests results from the improvement of diagnostic parameters and reduction of the time to carry out the procedure to 80 min. Earlier possibilities of detecting free p24 antigen required minimal concentrations of 20 pg/ml. VIDAS HIV DUO QUICK tests are able to detect p24 antigen in concentrations of 4,52 pg/ml, which substantially influences the sensitivity of the test and brings about shortening of the serological window.

Table 1. The results of the HIV infection screening test in the volunteers using VIDAS HIV-DUO and VIDAS HIV DUO QUICK tests

	Serological status of the examinee		
	Negative	Positive	All
VIDAS HIV DUO RESULTS			
Negative	398	0	398
Positive	6	3	9
All	404	3	407
VIDAS HIV DUO QUICK RESULTS			
Negative	585	0	585
Positive	4	4	8
All	589	4	593

Table 2. Diagnostic value of VIDAS HIV DUO and VIDAS HIV DUO QUICK tests

	All cases (n = 1000)	VIDAS HIV DUO	VIDAS HIV DUO QUICK
Sensitivity (%; 95% CI)	100%	100%	100%
Specificity (%; 95% CI)	98,9%	98,5%	99,3%
PPV (%; 95% CI)	41,1%	33,3%	50%
NPV (%; 95% CI)	100%	100%	100%
Diagnostic exactness (%; 95% CI)	99%	98,5%	99,3%

Discussion

To date a number of studies assessing the validity of various IVth generation tests for HIV detection have been carried out. All published papers have shown a greater validity of the IVth generation tests since their ability to detect the p24 antigen allows shortening of the serological widow by 7 days on average. This fact is especially important in the screening of blood and sperm donors [3, 12, 15]. Presently each blood, sperm, or tissue donor has to be subjected to a full diagnosis of any viral infection transmitted through blood. This is especially important in the case of HIV, HCV, HBV or CMV. According to world standards, specimens of frozen sperm should undergo quarantine for at least 6 months and may be used only after achieving negative control tests from the donors. At present it is not possible to use fresh sperm for facilitated reproduction [1, 19]. So far, HIV infection during artificial fertilization has not been noted in Poland. However such incidents have been reported worldwide. One of the first reports comes from a case study of 4 women from New South Wales in Australia, in whom HIV infection was contracted during artificial fertilization from the HIV infected donor. These infections occurred before the introduction of obligatory sperm donor testing for anti-HIV antibodies [25]. Similar cases of infections have been reported in the USA [8, 9]. Moreover one HIV infection was observed in one woman during aided reproduction in India [2]. However to date, even the obligatory testing of sperm donors did not assure full safety from the risk of infection. In the 90-ties acute HIV infection symptoms were observed in a 35 year old German health care worker, three weeks after artificial fertilization with fresh sperm. Three weeks later the presence of anti-HIV antibodies was confirmed in her blood (EIA and Western blot). In a consequent sperm donor examination seroconversion was observed and the analysis of viral RNA of the donor and the infected women showed almost 100% identity of the viral strain [24]. There have also been reports of effective post exposition prophylaxis in a woman who received sperm from a donor at the stage of seroconversion. After 10 days from sperm administration, antiretroviral treatment was started, the fertilization was successful and the women conceived a healthy boy and was not infected with HIV herself [4]. Thus the improvement of diagnostic methods facilitating earlier HIV infection detection is important in many medical fields.

The analysis of the validity of VIDAS HIV DUO and VIDAS HIV DUO QUICK tests has shown very high sensitivity for the tests from these two generations (100%). The specificity of the tests increased with the introduction of the QUICK test. Similar results have been obtained by other authors. Poljak et al [22] have carried out a retrospective analysis assessing the sensitivity and specificity of VIDAS HIV-DUO test. The results of the studies of these authors showed 100% sensitivity and high specificity (99,27%) of this test and corresponded to observations from other authors [13, 14, 16, 23, 26, 27]. In other studies the sensitivity of VIDAS HIV-DUO test was 99,5-100% and its specificity 98-100% [15, 18]. Only one study showed a specificity of 88,2% [26, 27]. The majority of studies which assessed the sensitivity and specificity of the VIDAS HIV DUO test reported 100% sensitivity, when the samples were taken from subjects with chronic HIV infection. The only study which showed a sensitivity of 91,4% was based on 17 panels of seroconversion. In that test the analytical sensitivity, that is an ability to detect an infection, and not the epidemiological sensitivity was taken into account. These authors also showed low specificity of the test at the level of 88,2% [20]. Other studies in which 29,657 cases were tested showed 99,5% specificity for this test [6, 7, 16, 17]. Moreover in these tests, 453 infected samples identified, among which there were 17 infected with positive p24 antigen, previously negative in the ELISA test. Also in the Poljak et al. study [22] the high specificity of the VIDAS HIV DUO test has been shown. The incidence of false positive results in the studies from these authors was 6,3% in 94 samples which showed a positive reaction and were lower than when using third generation tests. According to these authors the false positive results in the third generation tests ranged from 8.1%

to 16.2%, of the studied samples. Bourlet T. et al. [5] carried out a prospective study of serum samples from screening VIDAS HIV-DUO tests. 1443 blood samples were tested showing 100% sensitivity and 99,86% specificity. In the present paper the specificity of the VIDAS HIV DUO test was 98,5%. At present, this test has been replaced with the VIDAS HIV DUO QUICK test for which the specificity was 99,3%. False negative results were observed in 4 men co-infected with HCV, while 3 women in whom false negative results were obtained, had been receiving hormonal therapy, which may have influenced the results of the test. The advancement of the IVth generation tests rests on the improvement of their diagnostic parameters and the reduction of the performance time to 80 minutes. The earlier possibilities of free p24 antigen testing required minimal concentrations of 20 pg/ml [10, 11]. VIDAS HIV DUO QUICK tests detect p24 antigen at concentrations as low as 4,52 pg/ml, which markedly influences the sensitivity of the test and reduces the serological window. At an early stage of the infection before the seroconversion, apart from the tests which detect p24 antigen, the diagnosis of HIV infection is possible through detection of genetic material HIV-RNA or cDNA. However these tests are costly and not practical in mass diagnosis, for example, among blood donors and may also show false positive results irrespective of HIV-1 subtype. Apart from the above, tests which are based on genetic material detection may also show false negative results in persons with very low viraemia, which may require usage of ultra sensitive methods of detection [21]. The most widely used third generation tests – ELISA have shown very high specificity at a level of 99,86% [21].

In determining the validity of the test, 100% negative predictive value was observed, with low positive predictive value – 33,3% for the VIDAS HIV DUO test and 50% for the VIDAS HIV DUO QUICK test. Different results come from the Poljak et al. study. [22]. These authors have shown a very high 94,69% positive predictive value. Such discrepancies may be connected to a small number of HIV positive cases in the studied population, and a relatively high number of false positive results. In Poland the number of HIV infected people is not very high so the probability of diagnosing an HIV infected person in a non-specialist centre is low. It is also known that the number of false positive cases decreases with the increase in the incidence of HIV infections, which also triggers an elevation of positive predictive value. Summing up, the two screening tests of the IVth generation are easy to apply, safe for the medical staff, because of the automatization of the procedure which makes direct contact with potentially infective material virtually impossible. There has also been a marked improvement of analytical parameters in the advanced version of the VIDAS HIV DUO test – VIDAS HIV DUO QUICK.

Conclusions

1. The VIDAS HIV DUO QUICK test has a high sensitivity and fairly good specificity.
2. Reduced time of performance and full automatization assuring the safety of medical staff strongly influence the recommendation for its daily use in medical practice.
3. HIV infection incidence among sperm donors and women who undergo aided reproduction is not high but single positive cases require complex diagnosis of this population.
4. The assessment of diagnostic value of the VIDAS HIV DUO QUICK test in Poland requires further studies on big population.

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title

Serum concentrations of α-defensins in patients with different stages of HIV-infection

authors

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summary

In our study we demonstrated significantly higher serum concentrations of HNP, IL-22 and hsCRP in the HIV-infected group, compared to the control group. The levels of HNP in the HIV-infected group differed significantly according to the stage of the disease as determined by CDC classification (Kruskal-Wallis ANOVA test, $H = 15.80084$, $p = 0.0004$).

key words

α-defensins, Il-22, HIVCorresponding

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Introduction

In HIV-1 transmission and disease progression, relevant innate mechanisms of immunity have been described, including various host-derived soluble factors with antiviral activity. They act by both specific and non-specific mechanisms, and include chemokines, cytokines, interferons and transforming growth factor-β, as well as, more recently, defensins and IL-22.

α-defensins 1-3 are 3-4 kDa cationic peptides (29-30 aa)secreted by NK, B cells and T lymphocytes, and possibly bymonocytes/macrophages [1-4]. The anti-HIV activity of α-defensins was first described in 1993 [5], and recently several authors confirmed that α-defensins are active against HIV-1 [3, 6-8]. The mechanism of α-defensin-mediated HIV-1 inhibition is stillunclear. Recently, Furci et al [9] demonstrated in an experimental study that α-defensins specifically block the initial phase of the HIV infectious cycle, and modulate the expression of CD4, a critical receptor in the HIV-1 entry step.

IL-22 is a newly-discovered cytokine in the IL-10 family that functions to promote tissues' innate immunity against infection. The major sources of IL-22 are activated T-cells, especially CD4+ memory lymphocytes and NK cells. Neither lymphocytes B nor monocytes/macrophages show any ability to secrete IL-22. In the Furci study, [9] it was shown to exert its proinflammatory effect by regulatingthe production of acute phase proteins that may play an important role in innate immune response. Interestingly, the Misse study [10] demonstrated significantly heightenedexpression of IL-22 in individuals who had been repeatedly exposed to HIV-1 but had not been infected. This suggests that IL-22 may play a crucial role in hosts'innate resistance to HIV infection.

Therefore the aim of our study was to evaluate the serum concentrations of HNP 1-3 and IL-22 and their possible relationship to immunologic parameters, as well as to disease advancement in HIV infected patients.

Materials and Methods

A cross-sectional study was performed of 33 HIV-1-infected patients (27 males and 6 females, aged from 22 to 54 years, with amean age of 33.8). 24 patients were not receivinghigh activity antiretroviral treatment (HAART) and had no history of antiretroviral treatment; nine patientswere receiving HAART. The subjects were followedup for a mean period of 96.7 ±0.4 months, during whichα-defensins, IL-22 and hsCRP levels were analyzed according to subjects' CDC classification [11]. CD4 and CD8 cell counts and HIV-1 viral load were assessed. None of the subjects had any signs of concomitant acute opportunistic infection.

The percentages and absolute counts of peripheral CD4 and CD8 cells were determined by means of three-colorflow cytometric analysis (Beckton-Dickinson, Franklin Lakes, NJ USA). Plasma HIV-1 RNA was evaluated using the Amplicor system (Roche Diagnostics, Basel, Switzerland), with the sensitivity range between 50 and 75000 RNA copies per ml.

α-defensin (HNP 1-3), IL-22 and hsCRP were measured usingthe ELISA test (HyCult biotechnology b.v Netherlands; Human IL-22 Quantikine ELISA Kit,R&D Systems, MI, USA; Imuclone CRP ELISA, CT, USA, respectively)according to the manufacturers' instructions.

The control group consisted of 20 healthy, HIV-negative volunteers aged 45.7 ± 11.7 years. The study was approved by the Bioethical Committee of the Medical University of Bialystok. Informed consent was obtained from each subject.

Statistical analysis-measured values were expressed as means,taking into account thestandard mean error (±SE). The significant differences between the studied groups werecalculated using the non-parametric Mann-Whitney U test and the Kruskal-Wallis ANOVA test. For correlation analyses the Spearmann non-parametric correlation was used. A p value of < 0.05 was considered significant. Statistical analyses were performed using Statistica 5.0 for Windows (Statsoft Inc., Tulsa, USA).

Results

In our study we demonstrated significantly higher serum concentrations of HNP 1-3, IL-22 and hsCRP in the HIV-infected group,compared to the control group. These findings are shown in Table 1.

Table 1. Mean serum concentration HNP, IL-22 and hsCRP in HIV individuals and control group

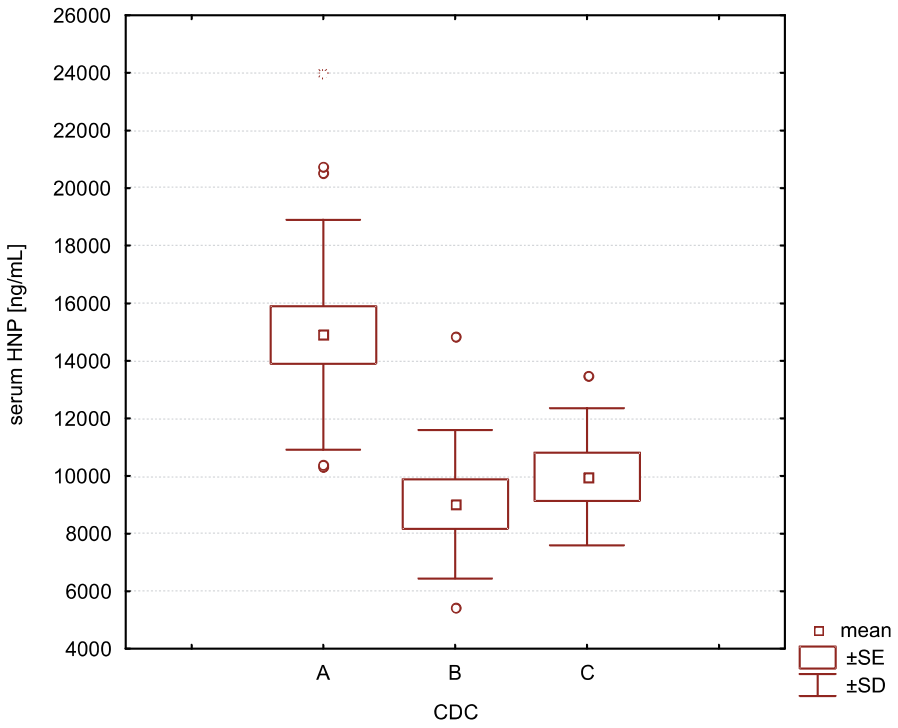
	HIV(+)	Control group	p
HNP [ng/mL]	18546.44±740.57	8739.404±425.17	0.0167
IL-22 [pg/mL]	49.77±5.23	15.492±5.29	0.04313
hsCRP [pg/mL]	31.53±5.99	7.45±3.32	0.02

The levels of HNP in the HIV-infected groupdiffered significantly according to the stage of the disease as determined by CDC classification (Kruskal-Wallis ANOVA test, $H = 15.80084$, $p = 0.0004$). Thesedifferences were established by the Mann Whitney U test,which confirmed that the concentration of HNP 1-3 in stage A was significantly elevated in comparison to its concentrations in stages B and C respectively. This result isillustrated in Figure 1. We did not find any association between the serum concentrations of HNP,IL-22 andhsCRP. Moreover, there wasno relationship between these parameters and CD4+, CD8+, and nadir CD4+ count, and no connection with HIV viral load,although we observed a decreasing level of serum HNP 1-3 concentration relative to the duration of the infection. The ARV treatment seemed to have no influence on the concentration of theanalyzed parameters.

Discussion

Disease progression in HIV infection is highly variable, with some individuals progressing rapidly to AIDS and others remaining entirely well and maintaining normal CD4 cell counts for more than 15 years after infection, in the absence of antiretroviral treatment. Innate defenses are responsible for the most rapid responses to microbial inva-

Figure 1. Mean (±SE) serum concentration HNP in relation to CDC stage



sion. HIV's interactionwith various elements of innate immunity is still under investigation.

The evidence from the most recent study showsthat α-defensins inhibit the earliest stages of the HIV-1 infectious cycle. From different experimental models, it appears that α-defensins act at two different levels, inhibiting the viral replication cycle, and blocking HIV-1 entry into target cells [7, 8]. Recently Furci et al [9] demonstrated the ability of α-defensins to specifically bind both to the primary HIV-1 cellular receptor, CD4, and to the viral envelope glycoprotein-gp120. Moreover, treatment of CD4+ T cells using α-defensins caused a dramatic downmodulation of CD4+ expression. The ability of α-defensins to downmodulate the cell-surface expression of CD4+ may have important implications for the potency and duration of the antiviral effects of α-defensins secreted in vivo. These results suggest that these peptides may influenceHIV-infected patients during infection.

In our study ofHIV-infected patients, the serum concentrations of α-defensins, IL-22 and hsCRP were significantly higher in HIV-infectedsubjects compared touninfected individuals. Our results concerning concentrations of IL-22 are at variance with the conclusions of Misse et al [10], who found overexpressionof this protein in the PBMC of HIV-1-exposed, uninfected individuals only. They did not observe any differences betweenthe expressions of IL-22 in the PBMC ofthe healthy control groupand of the HIV-infected group respectively.

In our study, the concentration of α-defensinsdifferedsignificantly relative to the stage of the disease. The highest concentration was in patients with stage A, independent of CD4 count or HIV viral load. We observed a decreasing level of serum α-defensins concentration according to the duration of the infection. This may be due to the shrinkingof the pool of granulocytes, the most important source of defensins. The highest serum concentrations wereobserved in 3 patients without disease progression for at least 14 years (data not shown). In patients withsymptomatic HIV infection, the concentrationof α-defensins was significantly decreased. We did not observe any correlation with IL-22.

These results may suggest that α-defensins play animportant role in immunity and disease progression in HIV infection.Further study is needed to evaluate the significanceof α-defensins in disease progression in HIV-infected patients.

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title

Panuveitis as syphilis manifestation in HIV-1 infected patients

authors

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summary

Syphilis prevalence has been on the rise worldwide, including Poland, for the last 5 years, most often affecting young individuals who have unprotected intercourse. Currently most syphilitic patients, if diagnosed, are treated early and more advanced disease is rarely seen. We present a case of two individuals with AIDS and advanced syphilis with ocular in-volvement which was the reason to test for syphilis. It is worth reminding that syphilis and HIV share the same route of infection and in the light of recent epidemiological data it is wor-th considering if universal screening for syphilis is needed once again just like universal scre-ening for HIV has become the standard of care in many countries.

key words

syphilis, lues, uveitis, HIV, AIDS

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Case 1

33-year-old woman (IDU, SW), HIV-positive (CD4 T-cells 176 cells/µl, HIV-RNA 67 100 copies/mL) was admitted to hospital with headache, vertigo, temporal alopecia, intraocular pain, flashes and vision deterioration lasting 6 months. Bilateral panuveitis and vitritis were diagnosed on ophthalmic exam. Cerebrospinal fluid examination revealed elevated protein concentration of 156 mg%, glucose level of 32 mg%, positive Pandy and non-Appelt reactions and pleocytosis of 254 cells (mostly lymphocytes – 87%, neutrophils 8% and monocytes 5%). Additionally serological tests were strongly positive both from blood and cerebrospinal fluid (VDRL – Veneral Disease Research Laboratory, FTA-ABS – Fluorescent Treponemal Antibody Absorbed, TPHA – Treponema pallidum Hemaglutination Assay) hence symptomless neurosyphilis with ocular involvement was diagnosed.

Treatment: crystalline penicillin (6 weeks), systemic plus local steroids, mydriatics and cART. Panuveitis treatment in the right eye was successful and syphilis seronegativisation was achieved after 6 weeks together with gradual improvement and final resolution of inflammatory response in cerebrospinal fluid. However, retinal detachment had occurred in the left eye leading to blindness.

Case 2

41-yer-old man (IDU), HIV-positive (CD4 T-cells 180 cells/µl, HIV-RNA 64 396 copies/mL) known to have had regular sexual contacts (lasting 2 years) with the above mentioned patient was admitted to hospital with cachexia, fever, diarrhea, photophobia, pain and excess lacrimation in the right eye (panuveitis on ophthalmic exam). The patient presented with patchy alopecia, partial eyelash loss and eyebrow thinning, red maculopapular desquamatous syphilids on head, neck, face, trunk, palms and soles (typically teeming with *Treponema pallidum*), hyperkeratotic inflammatory lower leg lesions and mucous patch lesion on tongue. Cerebrospinal fluid examination was negative with protein and glucose concentrations of 34 mg% and 37 mg% respectively, negative Appelt and Pandy reactions and pleocytosis of 3 cells. Serology for syphilis was highly positive in peripheral blood but negative in CSF hence secondary syphilis was diagnosed.

Treatment: Even though the patient was diagnosed with secondary syphilis, he received treatment for neurosyphilis because of the underlying HIV infection: crystalline penicillin (for 3 weeks) and local ophthalmic steroids with mydriatics. Full recovery was achieved. Later, however, the patient was lost to follow-up.

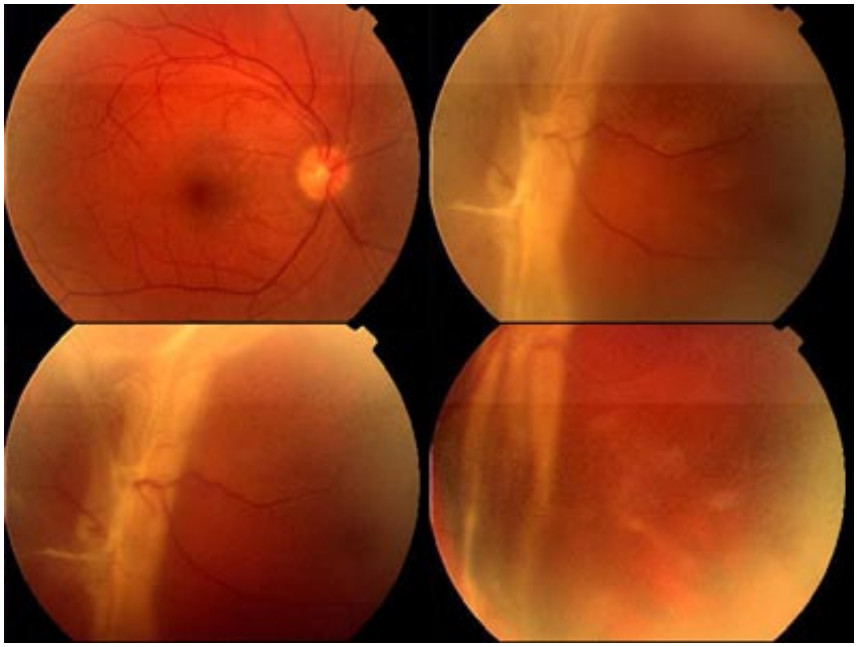
We present these cases because currently most syphilitic patients, if diagnosed, are treated early as primary syphilis and more advanced disease is rarely seen. It is worth reminding that syphilis prevalence has been on the rise worldwide, including Poland, for the last 5 years, most often affecting young individuals who have unprotected intercourse. Syphilis and HIV share the same risky behavior responsible for rising numbers of both infections so much effort is needed to convince everyone to use condoms if having an intercourse with a non-tested individual.

Other than primary syphilis or when time of infection remains unclear necessitates in depth diagnosis to rule out neurological and ophthalmic complications, especially among patients co-infected with HIV. Indications for lumbar puncture are neurological, ocular or auditory signs, treatment failure or late latent syphilis. In the case of neurosyphilis, cerebrospinal fluid examination usually reveals elevated protein concentration and mononuclear pleocytosis (> 5 WBC/mL) with positive serology. One should bear in mind that HIV infection can cause pleocytosis (> 5-15 mononuclear cells/mL) itself which has to be taken into account in differential diagnosis. Ruling out neurosyphilis in HIV patients may sometimes be very demanding or even impossible and they often need prolonged intravenous treatment with aqueous penicillin which raises strain on public health sector.

On the other hand, patients diagnosed early in the course of disease have great difficulty accessing best possible treatment as many out-patient clinics refuse to administer penicillin for fear of side effects often with little relevance to facts. As result, doxycyclin used for 30 days has become the drug of choice in ambulatory setting.

No matter if primary or late, syphilis has once again become an important epidemiological factor, especially in the HIV/AIDS era. Joint efforts are needed to address the matter. It is worth considering if universal screening for syphilis is needed again (as has been adopted for HIV positive patients) just like universal screening for HIV has become the standard of care in many countries.

Figure 1. Lues retinal detachment in the female patient



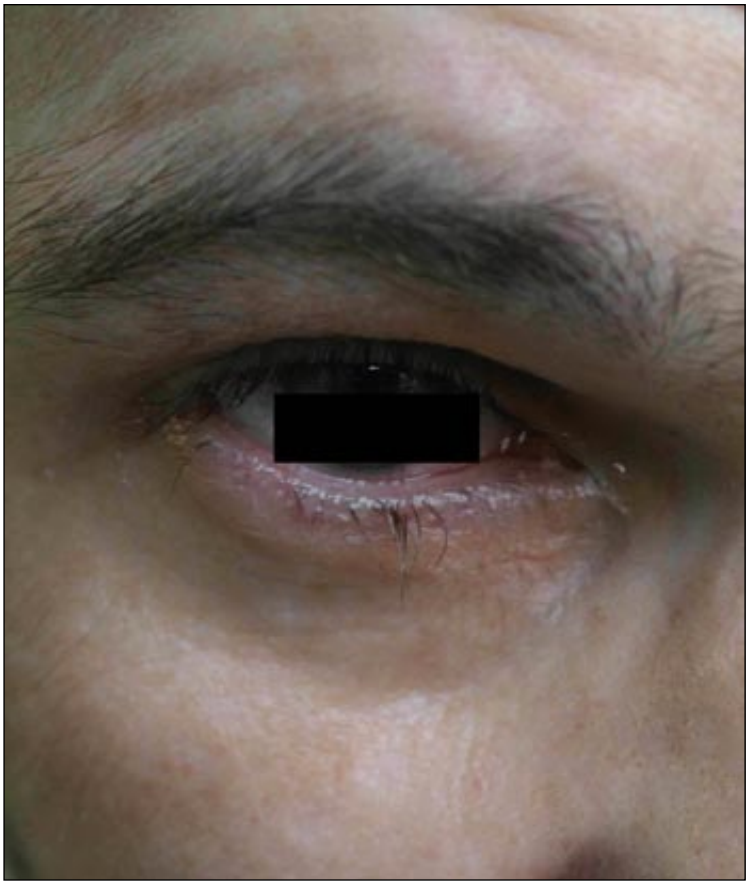


Figure 2. Eyelashes loss in the male patient

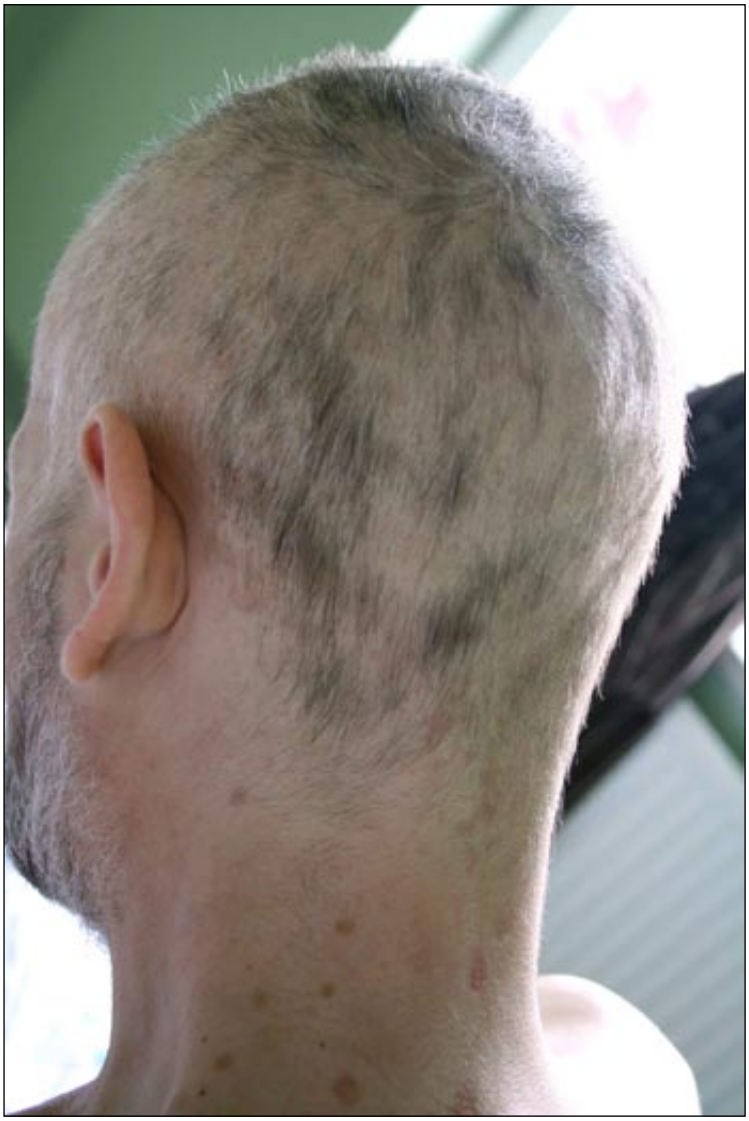


Figure 3. Alopecia areata in the male patient

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title

Noteworthy Features of HIV-associated T-cell Non-Hodgkin lymphoma

authors

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summary

The incidence of NK/T-cell lymphomas is increased in patients infected with HIV. Their pathogenesis is undefined, but appears to be related to immunosuppression and concomitant oncoviral (EBV, HTLV-I, HTLV-II, HHV8) coinfection. Experience related to the manifestation and management of these aggressive NK/T-cell malignancies in afflicted HIV-positive individuals is limited. We present three varying cases of HIV-associated T-cell lymphoma. The heterogeneity of their clinicopathological features and outcome are discussed in light of the emerging literature on this HIV-related subject.

key words

HIV, T-cell, Non-Hodgkin lymphoma, HAART

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Introduction

Lymphoma is a frequent complication of HIV infection. Typically, AIDS-defining lymphomas are aggressive B-cell non-Hodgkin lymphomas (NHL). Lymphomagenesis in the setting of HIV infection is believed to be secondary to oncoviral participation in concert with immunological derangements [1]. Although HIV-associated T-cell NHL was recognized relatively early in the AIDS epidemic [2-4], there are far fewer published reports of these neoplasms compared to B-cell NHL in the HIV infected population. In fact, the occurrence of HIV-associated T-cell lymphoma was previously believed to be exceptional [5]. Many cases of T-cell NHL were included with B-cell tumors in larger series of AIDS-related lymphoma. Since then, the number of published cases of AIDS-related T-cell NHL reports has increased considerably, as has our experience with diagnosing and managing these diverse lymphomas.

There is a growing body of literature that correlates HIV infection with T-cell abnormalities [6, 7], some of which are believed to predispose HIV infected patients to developing NK/T-cell lymphomas. For example, some authors believe that the preponderance of CD8+ lymphocytes seen with chronic HIV infection may facilitate their neoplastic transformation [8]. In addition, there is strong epidemiological data linking HIV infection with an increased risk of developing T-cell lymphoma, of different subtypes [9]. In general, a 15-fold increased risk of T-cell NHL has been estimated in HIV positive persons. T-cell lymphomas can be divided into three major categories: precursor T-cell neoplasms (e.g. precursor T-lymphoblastic lymphoma/leukemia); mature (peripheral) T-cell neoplasms (e.g. adult T-cell lymphoma/leukemia, nodal anaplastic large cell lymphoma, mycosis fungoides/Sézary syndrome, primary cutaneous anaplastic lymphoma, and peripheral T-cell lymphoma unspecified); and T-cell proliferations of uncertain malignant potential (e.g. blastic NK cell lymphoma). However, not all of these T-cell entities have yet been fully characterized in HIV infected patients.

In this paper, we present three different cases of T-cell lymphoma that occurred in HIV-positive individuals. Their varying clinicopathological findings and outcome are discussed in light of the emerging literature on this HIV-related subject.

Case reports

Case 1

A 35-year-old male with a history of intravenous drug use, AIDS, prior *Pneumocystis jirovecii* and *Mycobacterium avium* (MAI) infection, presented with a mass on his upper lip almost 4 years after his initial diagnosis of HIV infection. He was on lamivudine, stavudine and nelfinavir. His CD4+ cell count was 10 cells/mm^3 and HIV viral load 470,401 copies/mL. Initially, he reported the formation of a large blister on his lip that developed over a 2 week period into an inflamed, painful mass severely limiting his oral intake. Viral cultures submitted from the blister fluid were negative. An open excisional biopsy under general anesthesia was performed for diagnostic and palliative purposes. Microscopically, the subcutaneous tissue showed a diffuse infiltrate of malignant lymphocytes with irregular and convoluted nuclei (Figure 1). A diagnosis of T-cell CD30+ primary cutaneous anaplastic large cell lymphoma (ALCL) was made. Cytotoxic therapy with cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP) was started and highly active antiretroviral therapy (HAART) was continued as outlined above, along with azithromycin, itraconazole, ethambutol and trimethoprim/sulfamethoxazole for infection prophylaxis. The patient received only one cycle of combined chemotherapy. His course was complicated with multiple admissions for febrile neutropenia and refractory diarrhea, and shortly thereafter developed a new lymphoma lesion in the right groin. He died shortly after this progression.

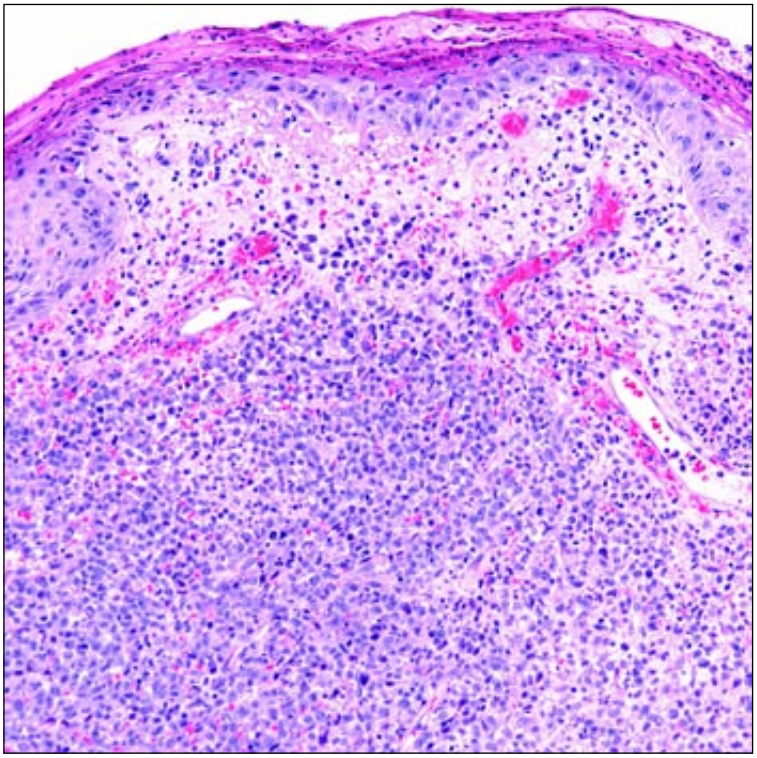


Figure 1A.
Subcutaneous primary cutaneous anaplastic large cell lymphoma with associated edema (H&E stain, magnification ×200)

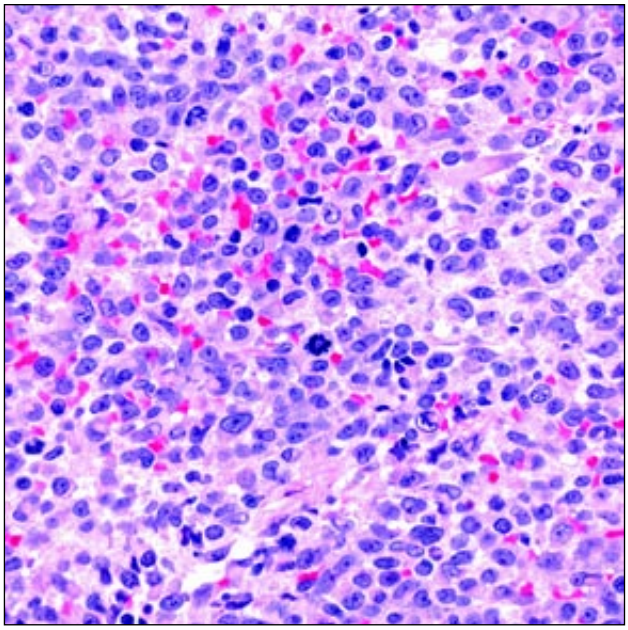


Figure 1B. Higher power shows a diffuse infiltrate of malignant lymphocytes with irregular and convoluted nuclei (H&E stain, magnification ×600)

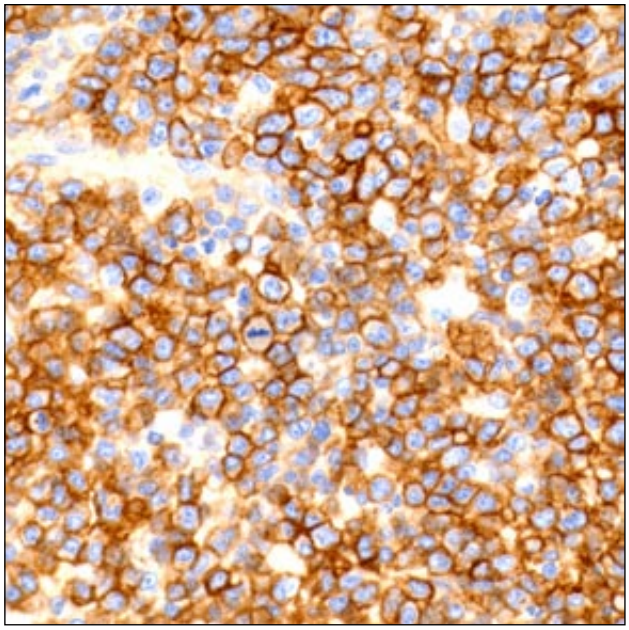


Figure 1C. Lymphocytes demonstrate strong CD30 membranous immunoreactivity (CD30 immunohistochemical stain)

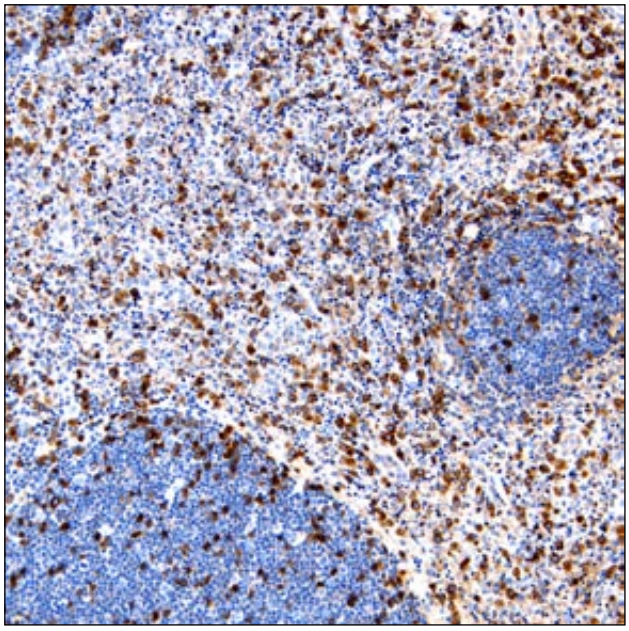
Case 2

A 35-year-old man with known HIV infection presented with fevers, pancytopenia and splenomegaly. His white blood cell count was 1400 cells/mL and his peripheral blood smear had a leukoerythroblastic picture (i.e. myelocytes, metamyelocytes and nucleated red blood cells were present). In addition, his hemoglobin level was 9.1 mg/dL and platelet count 10,000 cells/mL. A bone marrow biopsy was performed as an initial diagnostic procedure. The marrow cellularity was greater than 90%, and most of the marrow space was replaced by sheets of malignant immunoblasts. No granulomas were present. Lymphoma cells were CD45+, focally CD3+ and CD20 negative. A diagnosis of high-grade T-cell immunoblastic lymphoma was made. Unfortunately, no further history was available. This patient died shortly after his lymphoma diagnosis.

Case 3

A 16-year-old male with HIV infection he acquired during the perinatal period had a history of multiple opportunistic infections including CMV, MAI and pulmonary aspergillosis. He presented with tender unilateral, posterior occipital lymphadenopathy. An excisional biopsy showed partially effaced lymph node architecture due to lymphoma involvement (Figure 2). Immunohistochemical studies confirmed a diagnosis of nodal peripheral T-cell lymphoma (PTCL) that was CD3+, CD43+ and weakly CD45RO+. Staging CT scans found no additional evidence of lymphoma. At that time of his presentation, the patient was on lamivudine, stavudine and nelfinavir, as well as ethambutol and clarithromycin for prophylaxis. Chemotherapy was initiated to treat PTCL using a combination of cyclophosphamide, doxorubicin, etoposide, methotrexate, cytarabine and hydrocortisone with G-CSF support. A complete response was achieved with six cycles of this chemotherapy regimen. As of today, he has no further evidence of lymphoma by CT scan assessment. His last CD4 count was 81 cells/mm^3.

Figure 2. An effaced lymph node due to CD3 positive peripheral T-cell lymphoma cells (CD3 immunohistochemical stain)



Discussion

The three cases presented here illustrate several key features of HIV-associated T-cell lymphoma; viz. T-cell lymphomas usually present with advanced HIV disease and frequent prior opportunistic infections, a broad morphological spectrum with a heterogeneous clinical presentation (extranodal and nodal) is likely to be encountered, and in general such lymphomas are likely to exhibit an aggressive clinical course with a poor prognosis. While case 1 (treated) and case 2 (untreated) died soon after presentation, case 3 was successfully treated with HAART and chemotherapy. In a review of 25 cases collected during 8 years in San Francisco, researchers described two forms of

HIV-associated T-cell lymphoma [10]: (I) Epstein-Barr virus (EBV)-negative epidermotropic T-cell NHL with an indolent course arising in patients with high (600 cells/mm^3) CD4 counts and (II) CD30+ large cell lymphomas that harbored EBV, had epidermotropism, and were associated with severe immunosuppression (CD4 count of 50 cells/mm^3) and a poor prognosis.

In a recent review of 93 published cases of NK/T-cell lymphoma in HIV-positive individuals, it was shown that afflicted patients were of median age 38 years (range, 1-63 years) at presentation, exhibited a 4:1 male predominance, and had a median CD4 count of 184 cells/mm^3 [11]. Up to 54% of these reviewed cases had a prior AIDS-defining illness. In our small series, all individuals were relatively young males with advanced immunosuppression and opportunistic infections. The male predominance, as seen with other lymphomas such as Burkitt lymphoma, is interesting, but may simply reflect a Western HIV-infected population. NK/T-cell neoplasms are uncommon, even in the non-HIV population. They bear significant differences in incidence in different parts of the world. In general, T-cell lymphomas are more common in Asia. Adult T-cell leukemia/lymphoma (ATLL) is more common in regions with a relatively high prevalence of HTLV-1, such as the Caribbean basin and South Japan. However, reports of HIV-associated NK/T-cell neoplasms have been documented worldwide including the USA, Europe, South America, and Asia [11].

The most common clinical findings in HIV-associated NK/T-cell lymphomas include lymphadenopathy, B symptoms, erythroderma and pruritus [11]. Mature NK/T-cell lymphomas infrequently involve lymph nodes, even with recurrences. Frequent spread to other extranodal sites, especially the skin, is commonly seen. Indeed, most (74%) HIV-related T-cell lymphomas are extranodal, with at least 50% involving skin [11]. Apart from T-cell NHL, other causes of erythroderma to consider in patients with HIV infection are drug-related eruptions, infections, and photodermatitis. Additional dermatoses in HIV+ persons characterized by lymphocyte-rich infiltrates include (seborrheic, contact, atopic and interface) dermatitis and psoriatic erythroderma. Many of the clinical manifestations of T-cell NHL can be related to cytokine expression from lymphoma cells. A cytokine profile was not available for review in our cases. Staging in 60 reported HIV-infected patients was found to be stage IV (53%) > I (27%) > III (17%) > II (3%) [11].

While many T-cell subtypes have been recorded in HIV+ patients, their exact frequency is difficult to ascertain as several lymphomas were not classified according to the current WHO classification. Moreover, some atypical cases (so-called pseudo-Sezary or cutaneous T-cell lymphoma stimulant) proved difficult to categorize [12]. Nevertheless, the NK/T-cell lymphoma subtypes presently documented in HIV-infected patients include, from most to least frequently reported [11], PTCL (n = 36), cutaneous T-cell lymphomas (CTCL) including Mycosis fungoides (n = 25), ALCL (n = 13), ATLL (n = 8), NK-cell neoplasms (n = 4), and various others (enteropathy-associated, primary effusion and intravascular lymphoma of T-cell phenotype). A case of HIV-associated T-cell lymphoblastic lymphoma has also been documented [13].

In case 1 the patient was diagnosed with primary cutaneous CD30+ ALCL. In non-HIV individuals, this particular lymphoma usually presents in individuals over 50 years of age, has an indolent course, and in up to 25% of cases may regress spontaneously. Our patient with ALCL was considerably younger (35-years-old), did not tolerate CHOP, and consequently died soon after his lymphoma diagnosis. In another series involving four patients with CD30+ ALCL, investigators similarly reported aggressive disease with patients dying at a median of 3 months [14]. Severe immunosuppression, more than any other factor, seems to result in a similar clinical course in both CD30+ and CD30- ALCL patients [15]. Case 3 had a nodal PTCL, unspecified. Contrary to our case, this wastebasket (unspecified) category presents usually with high stage disease and an overall aggressive course. Case 2, a high grade immunoblastic lymphoma, was not easily classified according to the proposed WHO scheme, most likely due to the limited studies performed at the time of diagnosis.

In contrast to B-cell lymphomas, cytologic grade is not useful in predicting the clinical course of NK/T-cell neoplasms. Moreover, unlike B-cell NHL, there are no convenient immunophenotypic markers to readily demonstrate monoclonality. The presence of an aberrant immunophenotype is often used to indicate a NK/T-cell malignancy. Most NK/T-cell NHL to date have expressed CD45RO and CD3 antigens, CD4 > CD8, and CD30 in a subset of cases [11]. CD45RO and CD3 are highly sensitive lineage markers for NK/T-cell neoplasms. Clinicians should be aware that in patients with a CD4+ Sezary syndrome, an increased peripheral CD4+ count may cause problems in following HIV-related immunodeficiency [16]. Patients with advanced AIDS and a low CD4+ T-cell count have been shown to present with large reactive skin infiltrates comprised largely of CD8+ T lymphocytes, a process that may mimic CTCL [17]. While CD30 expression is required for the diagnosis of ALCL, it may be found in several extranodal T-cell lymphomas. In cutaneous lymphomas, CD30 expression is prognostically important; since CD30+ cases are generally associated with a favorable prognosis [18].

The actual pathogenesis of T-cell lymphomas in HIV is incompletely understood [19]. EBV is usually associated with extranodal NK/T-cell lymphomas and NK-cell leukemias. However, EBV has only been detected in around 20% of HIV-associated NK/T-cell cases [11, 20, 21]. Human T-cell leukemia virus (HTLV-1), etiologically linked to ATLL, has rarely been reported with HIV coinfection [2, 22]. HTLV-II, which is especially prevalent among intravenous drug abusers, has been noted with CTCL in a patient with HIV-1 infection [23]. Unfortunately, the EBV and HTLV status in all three of our cases was unknown. A case of human herpesvirus 8 (HHV8) associated T-cell lymphoma has been reported in a HIV-positive man in a lymph node with concurrent peritoneal effusion [24]. The HIV genome has also been detected in malignant T-cells, leading researchers to believe that HIV infection itself may have a central role in lymphocyte transformation [25, 26].

No standard therapeutic approach has been proposed for the management of HIV-associated NK/T-cell NHL. Therapy in published cases to date constituted both single-modality (46%) and multimodal (17%) treatment [11]. A minority of cases (12%) were also untreated [11]. Treatment of these malignancies is challenging. Based largely upon the collective experience in the treatment of HIV-related B-cell lymphomas, HAART and combination chemotherapy should be offered to patients who present with advanced stage NK/T-cell NHL [27]. The extranodal nature of HIV T-cell lymphomas requires additional creative ways of managing earlier stage disease, such as surgery, phototherapy, radiation therapy, chemotherapy and/or immunotherapy. Local irradiation has been used to successfully manage AIDS-related CTCL [28]. Autologous stem cell transplantation has been shown to be effective and safe in the treatment of HIV B-cell lymphomas [29]; the experi-

ence has not been the same for non-HIV T-cell lymphomas [30, 31]. There is insufficient data supporting the use of autologous stem cell transplantation in HIV T-cell lymphomas. The role of HAART needs to be further explored.

Clinically, NK/T-cell neoplasms are among the most aggressive of all hematopoietic and lymphoid neoplasms. Therefore, it is not surprising that death occurred in up to 55% of reviewed patients, with a median overall survival in 83 patients of 1.1 years from the time of HIV-related NK/T-cell lymphoma diagnosis [11]. Factors that likely contribute to the aggressive clinical behavior of this particular subgroup of malignancies include advanced clinical lymphoma stage at presentation, marked HIV-related immunosuppression, and chemotherapeutic resistance. There is published evidence that the prognosis of HIV-related lymphomas treated with HAART and combination chemotherapy depends more upon tumor-related factors (such as achievement of complete response, International Prognostic Index score and lymphoma histological subtype) than on HIV-related factors [32]. Unfortunately, in patients with T-cell lymphomas, who accounted for a minority (3.8%) of these studied cases, the complete response rate was poorer than B-cell counterparts resulting in a worse prognosis.

In conclusion, a NK/T-cell neoplasm should always be entertained in patients manifesting with an AIDS-defining NHL. Explicit immunohistochemical markers (e.g. CD56 or N-CAM, granzyme B, TIA-1, perforin), molecular studies (e.g. T-cell receptor gene rearrangement) and the demonstration of EBV positivity (e.g. EBER) in malignant cells along with clinical follow up should be employed to help establish the diagnosis. HIV-infected persons diagnosed with a NK/T-cell lymphoma are likely to be young males with AIDS who have a CD4 count under 200 cells/mm^3 at presentation [11]. They are also prone to present with extranodal disease, frequently skin involvement, and at an advanced stage. A standard treatment approach is required, as the incidence of these neoplasms appears to be increasing and their prognosis even in the HAART era remains poor.

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