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title

The serum concentration of procalcitonin (PCT) in various infections in HIV positive patients

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summary

Procalcitonin (PCT) is a marker of severe bacterial and fungal infections. The value of PCT in immunocompromise patients with HIV infection is not well established. We present the usefulness of PCT in diagnosis of various bacterial or fungal infections among HIV infected individuals.

Procalcitonin (PCT) is released from the C-cells of the thyroid gland as a precursor of the hormone calcitonin. PCT has been proposed as a marker of severe bacterial and fungal infections. We analysed the last results of various trials with PCT as a marker of infections in patients with HIV.

key words

HIV infection, Procalcitonin (PCT), CRP C – reactive protein, SIRS (Systemic Inflammatory Response Syndrome), markers of inflammation, opportunistic infections.

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Introduction

The amino acid sequence of human PCT was described in 1984 **(1)**. In 1992 O`Neill WJ et al. **(2)** confirmed the significance of elevation of calcitonin precursors in patients with burn respiratory tract.

The significance of PCT and the first publication about it`s role in identification of bacterial sepsis was in 1993. High concentrations of PCT have been found in the blood of the most of 79 children suspected with infections. In this group 19 patients with severe bacterial infections had high serum PCT concentrations **(3).**

In many other publications the value of PCT was confirmed in patients with sepsis **(4, 5, 6)** , SIRS **(7)** and neoplastic diseases **(8)**. There is only a few publications about the role of PCT in sepsis diagnose in HIV infected patients or among other diseases in patients with immunodeficiency.

PCT synthesis

Procalcitonin (PCT) is a 116 amino acid protein (Figure 1) with a molecular weight of approximately 13 kDa. This is a prohormone of calcitonin (32 amino acids). In healthy individuals, PCT is released from the C-cells of the thyroid gland after specific intracellular proteolytic procession of the Pre-PCT contains 141 amino acids. In severe bacterial or fungal infections and sepsis, C-cells of the thyroid are not the source of PCT.

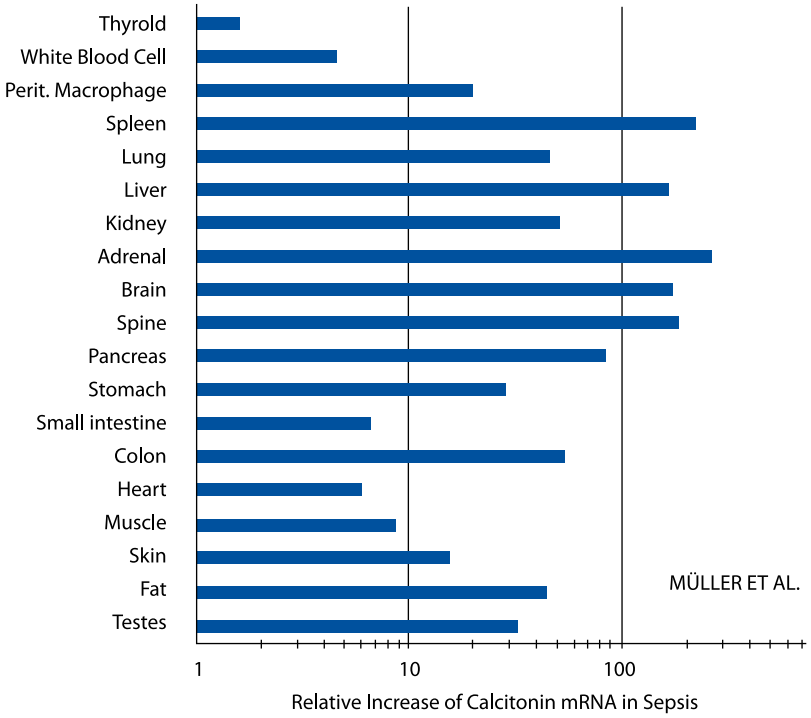
Figure 1. The Procalcitonin (PCT) amino acids sequence (1)

Other cells including: leucocytes, macrophages and monocytic cells of various organs are involved in releasing of PCT (**9, 10**). The biological function and origin is not yet defined but mRNA is localized in many organs.

It has been known that synthesis of calcitonin precursor mRNA (CT-mRNA) takes place in the liver, spleen, lungs and brain (Figure 2). These are the most important organs in the process of synthesis and release of PCT in response to bacterial infections. **(11, 12, 13)**

One of the highest sensitivities and specificities of PCT were 85% and 91% respectively. PCT is the best marker in differentiating SIRS from sepsis, when compared with IL-2, IL-6, IL-8, CRP and TNF-alpha. **(3, 15).**

Figure 2. Analysis of CT-mRNA induction in all septic tissues (14)



A meta-analysis PCT mean reported sensitivity was 76% and specificity 70% **(16).** In the result of one of the clinical trials the comparison PCT vs. CRP shown that PCT is more sensitive and less specific alone than together with CRP – see Table 1**.**

Reference ranges

Normal plasma and serum concentration of PCT in healthy individuals is below 0,1 ng/ml measured with high sensitive methods. In clinical practice the most common is immunoluminoscence method (ILMA) with the normal PCT range below 0,5 ng/ml.

Values of approximately 2 ng/ml to 5 ng/ml are considered as moderately but exceeding 5 ng/ml are considered as very high PCT values.

Particularly high PCT values have been reported in patients with severe bacterial infections and septic inflammation, severe sepsis or septic shock scoring to ACCP/SCCM criteria (18). Very high values of PCT over 10 ng/ml have been observed during acute bacterial diseases and some isolated reports of over 100,0 even to 1000,0 ng/ml.

PCT (procalcitonin) is proposed for use as a diagnostic parameter for bacterial or fungal infections triggering a systemic inflammation in the body (sepsis, severe sepsis and septic shock). Local limited bacterial or organ – related infections induce a slight increase in PCT if at all **(3).**

Immunosuppression and neutropenia do not significantly affect PCT formation but in some patients with leucopenia (leucocytes count below 1,0 x 10^3 cells/ml) using PCT for diagnose of bacterial infection should be cautious. Sensitivity and specificity of PCT measurement is very low and even in severe sepsis in these patients were below 2,0 ng/ml **(20).**

Table 1. PCT sensitivity and specificity in comparison with C-reactive protein (CRP) (17)

	ProCT (cut-off 0,6)	CRP (cut-off 7,9)	ProCT+ CRP	ProCT and/or CRP
Sensitivity	67,6	71,8	60,0	81,8
Specificity	61,3	66,6	82,3	48,1
Positive predictive value	71,0	75,2	82,5	68,7
Negative predictive value	57,5	62,6	59,6	65,5

Table 2. Ranges of PCT in various diseases in immunocompromised patients (19)

PCT ng/ml	Interpretation	Options for futher action Investigations, treatment
< 0,5	• Sepsis, severe sepsis or septic shock unlikely • However, localized infections can not be exclused	• Observe patient • Concentrate diagnostic investigations on clinical findings
0,5 – 2,0	• Result needs further investigation, infection or sepsis possible • Severe sepsis or septic shock unlikely	• Search for the local focus of infections • Evaluate aetiologies for elevated PCT other than infection • Consider antibiotic therapy
2,0 – 10,0	• Bacterial infection complicated by systemic inflammation most likely • In some patients other causes are possible, e.g. major trauma or cardiogenic shock	• Intensify search for focus of infection • Evaluate aetiology of elevated PCT • Initiate specific and supportive therapy • Antibiotic therapy recommended, if indicated
> 10,0	• Severe sepsis or septic shock most likely • High risk to develop multiple organ dysfunction	• Search for focus of infection • Initiate specific and supportive therapy • Intensive care strongly recommended

This problem was the issue of analysis in Shah et al. publication when the serum PCT concentration was analysed among non-immunocompromised and immunocompromised patients from the beginning of infection . In the first and second day of observation the serum level of PCT was the same in both groups, but in third and fifth day was significantly lower among non-immunocompromised patients (with leucocytes count below 4,5x10^3 cells/ml) than in immunocompromised patients **(21).**

PCT AND CYTOKINES

Similar correlation in the time of course of PCT, IL-6 and TNF-alfa during the acute course of clinical infections as demonstrated below in 169 in patients presenting with severe sepsis and septic shock. If the inflammation rapidly wanes, PCT values start to decline and in blood serum, where procalcitonin half-life is from 25 to 30 hours after this time PCT results go back to normal range.

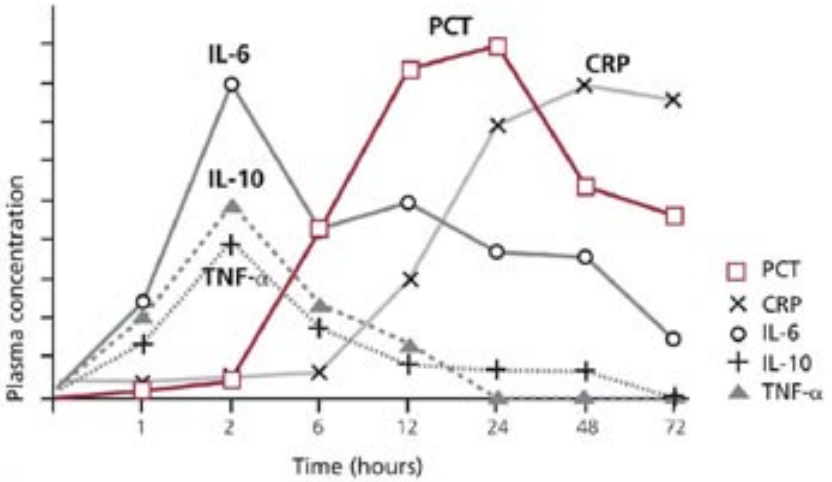
During severe bacterial or fungal infection in immunocompromised patients PCT level can be over normal range even 27 days from the first onset and as one of all used markers has the best correlation with the stage of the disease than cytokines or CRP. Decrease of IL-6 is earlier than PCT despite presence of infection. CRP concentration is still over normal range when PCT and IL-6 are in normal range. See figure 3.

Community acquired pneumonia (CAP) – Prognosis of pneumonia

In acute lower respiratory infections significantly elevated levels of PCT were found in comparison to the control group, but below the usual cut-off level. No differences in concentrations were observed between tuberculosis and the control group. The common used PCT range below 0,5 ng/ml concentration is not a useful tool for diagnosis of lower respiratory tract infections.

In comparison to the control group PCT levels in patients with hospital-acquired pneumonia, community-acquired pneumonia and acute exacerbation of chronic bronchitis were significantly elevated but all results were below 0,5 ng/ml **(23)**

Figure 3. PCT, CRP and cytokines correlation in acute infection (22)



PCT CONCENTRATION IN HIV INFECTION

We know there is no influence of HIV infection on releasing PCT and we can not differentiate stages of HIV infection using PCT measurement. **(24).**
However in the presence of bacterial or fungal sepsis concentrations over the normal ranges of PCT were observed in all HIV and AIDS patients.

Using of PCT in diagnosis of bacterial or fungal infection in HIV and AIDS patients need more advance investigations and trials to describe the role of PCT in early phase of bacterial or fungal diseases.

Mycobacterium tuberculosis pneumonia

Mycobacterium tuberculosis do not trigger an increase of PCT concentrations in HIV-infected patients, but more investigations about this coinfection are necessary. Despite some suggestions assessment of PCT is very important, because among non-immunocompromised patients *Mycobacterium tuberculosis* infection is more than 120 times often than among immunocompromised patients. This is bacterial infection with unknown correlation to the PCT. In one of analysis the serum concentration of PCT was the same (no statistical differences) like in control group **(25).**

Pneumocystis jiroveci pneumonia

In Nyamande K and Lalloo U.G. **(25)** observations serum PCT distinguishes CAP (*CAP – Comunity Acquired Pneumonia*) due to bacteria from patients with *Mycobacterium tuberculosis* or *Pneumocystis jiroveci* pneumonia.
PCT as a marker can be very useful and important in distinguishing bacterial from *Mycobacterium tuberculosis* and *Pneumocystis jiroveci* pneumonia in HIV infected patients. More of clinical symptoms had atypical presentations so it can be difficult to confirm useful of measurement of PCT in these diagnosis**.**

Toxoplasma gondii

The cerebral infection of *Toxoplasma gondii* do not trigger an increase in PCT concentrations in HIV-infected patients but more observations are needed.

HEPATITIS B, HEPATITIS C AND CMV INFECTIONS

PCT concentrations were within normal or slightly above the normal range in cases of acute hepatitis B, C and CMV infections.

In one study the serum procalcitonin (PCT) concentration was measured in fifty-two consecutive patients with chronic hepatitis C during pegylated interferon-α (PEG-IFN-α) plus ribavirin (RIB) treatment and to correlate them with clinical and virological outcomes.

In conclusion serum PCT levels decline in chronic hepatitis C patients during PEG-IFN-α plus RIB treatment, especially in the sustained virological responder group, while they elevate only when bacterial infections complicate the treatment course **(26).**

Fungal infections – Candidiasis or Aspergillosis

PCT elevated values have been reported also in systemic fungal infections like *Candidiasis* or *Aspergillosis* **(27, 28).** It can suggest that PCT is also induced from many sites in these infections.

Reference is made to case reports relating to four patients with *Aspergillosis* and *Candidiasis* in whom only minor PCT induction was observed **(29).** But we know that fungal infections need more investigations for better assessment the place of PCT in diagnosis of these infections.

Viral infections or local bacterial or fungal infections

No viral infection increase PCT concentrations in HIV infected patients. We can suggest that also more of the local bacterial infections are without influence on PCR serum concentration and most of them are without SIRS criteria. Only slight increase is observed in some of them but there is no correlation with pathogen and can not be used as a diagnostic method. PCT serum concentration can be over the normal range but it is difficult to distinguish this arise from other infections.

CONCLUSION

From the first publication in 1992 considering the value of PCT many analysis were performed and it is hard to assess the value of this marker in bacterial infections in non-immunocompromised persons like HIV and AIDS patients.

In some infections of other etiology (viral or fungal) we can suggest the significance of PCT in diagnosis, but there is no data to confirm it.

Especially in HIV/AIDS patients the role of this marker is not enough explain particularly in *Mycobacterium tuberculosis* or other bacterial and fungal infections like *Candidiasis* or *Aspergillosis*.

We know that diagnostic usefulness of CRP or other markers in non-immunocompromised patients is not quite clear. Can we be sure that this marker is enough sensitive and specific. We have no trustable marker of bacterial or fungal infection but not only in HIV/AIDS patients. This is problem in ICU`s (ICU – Intensive Care Units) and in some infections both of these markers can be normal in non-immunocompromised patients with infection.

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title

Primary HIV infection

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summary

The article describes clinical image of the primary HIV, as well as diagnosis opportunities at this stage of the infection. It presents the data concerning indications for initiation of antiretroviral therapy and the meaning of diagnosis the infection at this stage both for the patient and the public health.

key words

HIV, primary infection, symptoms, diagnosis, treatment

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Regardless of the infection route, the early stage of the HIV infection is the primary infection, called also the acute HIV infection, acute retroviral syndrome or mononucleosis-like syndrome. Its symptoms occur between the infection and the moment when HIV antibodies become detectable, usually between 2 and 4-6 (sometimes up to 12) weeks after exposure, they last several days to several weeks and recede within 14 days, although some of them may last longer, even up to several months.

Clinical symptoms related to primary HIV infection were first observed in 1984 (1), and in 1985 they were defined as a clinical syndrome, which precedes seroconversion (2,3).

It is estimated that 40 to 90% of HIV-infected persons suffer from clinical symptoms related to the primary HIV infection (4).

Signs and symptoms of primary HIV infection

The most common symptoms of the primary infection are: fever, fatigue, enlargement of lymph nodes, myalgia, nausea and diarrhoea. The symptoms may resemble influenza infection (5). Laboratory examination reveals quite often leukopenia, thrombocytopenia or slightly increased transaminase activity. Fever is the most common symptom of the primary infection does not usually exceed 38.9°C, although it may be as high as 39-40.4°C. In the cases of leukopenia with thrombocytopenia and associated fever, the differential diagnosis should include primary HIV infection (6).

Acute HIV infection may be associated with rash as well: reddish, without itching, similar to measles or rubella, sometimes maculopapular, first affecting the trunk, then extremities, including hands and feet, it may occur on the face as well. In the literature there are reports of maculopapular rash with necrosis of the epidermis in the course of the primary HIV infection (7). Severity of the rash ranges from single eruptions, easily missed both by the patient and the doctor to multiple cutaneous lesions, it may be also generalised, which may lead to misdiagnosis (8).

Histological examination of biopsy specimens of these skin lesions have revealed perivascular infiltration including CD4 cells (9).

Rash and fever are the most characteristic symptoms of the acute primary infection (10).

The patients may also suffer: pharyngitis, painful joints, thinning (loss of weight up to 10 kg), gastrointestinal discomfort (nausea, vomiting diarrhoea, pain at swallowing because of oesophagus ulceration (11) or candidiasis), night sweating, orthostatic hypotonia. The most common clinical symptoms are listed in Table 1.

During the primary infection symptoms related to the central nervous system may occur as well. Apart from aseptic meningoencephalitis (with such symptoms as fever, headaches, photophobia and stiff neck), there may also occur strong, long-lasting headaches, peripheral neuropathy, facial nerve palsy (12), Guillain-Barré syndrome (13), as well as psychoses and the neurological syndrome may affect the patient several weeks after recession of other primary infection-related symptoms (14). There were reports of symptoms indicating multiple sclerosis (15), leukoencephalopathy (16) and acute diffuse encephalomyelitis (17).

The primary HIV infection may be associated with opportunist infection (this concerns persons with transient reduction of CD4 lymphocytes level below 200/µl), such as hepatitis caused by *Pneumocystis carinii* (18), oral cavity or oesophagus candidiasis (19), brain toxoplasmosis, pulmonary tuberculosis. A case was reported of occurrence of lymphocytic *alveolitis*, with fever < 39°C, cough and night sweating, diagnosed based on BAL in a patient who was also HCV-positive (20) and also a case of severe haemorrhagic inflammation of colon related to HCV infection in the course of acute HIV infection (21). The primary HIV infection may also be clinically manifested by acute rabdomyolysis (22), as well as acute liver failure with associated sepsis, myocarditis, rabdomyolysis and marked leukopenia, which affected a 16-year-old woman (23). In another patient, primary HIV infection was manifested with acute pancreatitis (24).

Both number and severity of primary HIV infection symptoms are variable, most of patients suffer more than one symptom.

Table 1. The most common symptoms of the primary HIV infection

Symptom	Frequency (%), (12)	Frequency (%), (13)	Frequency (%), (4)
Fever	96	98	> 80-90
Fatigue	74	86	> 70-90
Rash	70	45	> 40-80
Headache	32	34,5	32-70
Enlarged lymph glands	74	55	40-70
Pharyngitis	70	53	50-70
Myalgia and /or painful joints	54	65,5	50-70
Nausea, vomiting or diarrhoea	27-32	24	30-60
Night sweating	–	48	50
Aseptic meningoencephalitis	–	Neurological symptoms – 9	24
Oral cavity ulceration	12	15,5	10-20
Ulceration of the sex organs' area	–	5	5-15
Thrombocytopenia	45	–	45
Leukopenia	38	–	40
Increase transaminase activity	21	–	21

Clinical symptoms of acute HIV infection recede, regardless of their duration and severity, without any treatment or despite it.

Clinical course of acute HIV infection is the same regardless of infection route (25), although it has been reported lately that persons infected with HIV through intravenous application of stupefacient agents are not so frequently affected by primary infection symptoms as persons infected through sexual intercourses (26).

Diagnosis of primary HIV infection

It is very difficult to diagnose primary HIV infection based on its clinical symptoms. The symptoms are so unspecific that they are diagnosed in less

than 1 – 3% of HIV-infected patients at this stage, although majority of patients with symptomatic infection seek doctor's help and some of them are hospitalised because of marked intensification of complaints (27, 28). Out of 19 patients, participants of the programme of monitoring of HIV-infection frequency in population with higher frequency of risky behaviours, in whom HIV antibodies tests were routinely performed every 4 – 6 months, HIV infection in the primary infection stage was diagnosed in five persons (29). Also a study in patients of a cohort prepared for research on prophylactic vaccination showed that acute HIV infection was diagnosed only in 8 out of 50 patients who sought medical help at this time, while in other cases, diagnoses included upper respiratory tract infection, cold, viral infection, sinusitis and streptococcal pharyngitis (30).

Early diagnosis of HIV infection is difficult also because it is necessary to obtain specific information including the data about the patient's sexual behaviour. Not always doctors are prepared to ask such questions and the patients themselves are no willing to mention their risky behaviours. Sometimes patients were involved in sexual intercourse which they are convinced do not bear risk of HIV infection (e.g. oral or anal sex to avoid the risk of unwanted pregnancy), and they do not expect it to be an important subject to discuss with their doctors. Quite often only after being asked for consent for a HIV-infection test do the patients say that they have had unsafe sexual intercourse lately. And yet everybody who does have a sex life (or uses injected drugs) is at risk of HIV infection.

It is also important not to exclude HIV infection based on age. Aggarwal and Rein reported a case of primary infections in a 15-year-old boy who told – when not accompanied by his mother – that he had had sex with five girls in his life (31).

HIV infection through sexual intercourse may occur in elder patients, above 50 years of age as well. In those patients it is usually diagnosed in a very advanced, symptomatic stage of infection because doctors do not find such infection probable in elderly patients (32).

Diagnosis of primary HIV infection is possible only if the doctor considers this possibility. As mentioned before, fever and associated rash should raise suspicion of primary HIV infection.

During the primary infection phase, tests for anti-HIV antibodies are negative yet. Western blot test may be negative or equivocal as well in a patient with typical clinical symptoms of acute infection. This is why the diagnosis should be based on quantitative indication of RNA HIV virus in plasma or qualitative in the case of DNA HIV. Acute disease is usually associated with high viremia, exceeding even several millions of copies of RNA HIV/ml, antigen p24 is usually presence, but its absence does not exclude primary infection (in general it is detectable in a very short period). Interpretation of results indicating number of copies of RNA HIV in the analysed serum one should take into account the possibility of false positive results (occurring in 2 – 3% of patients), especially if the revealed number of copies is below 3 000 – 10 000/ml (33, 34). Tests for HIV RNA are very specific, but they give also false positive results the number of which may be reduced by repeated test and results < 5 000 copies of RNA/ml should be treated as equivocal, requiring additional examination. Searching for antigen p24 is a more specific but less sensitive test (10).

Tests which search both for anti-HIV antibodies and antigen p24, so-called 4th generation tests are less sensitive than those addressed only at antibodies or only at antigen p24 (35). However – regardless of the result – they cause necessity to test for presence of antigen p23and anti-HIV antibodies separately which may postpone diagnosis of HIV infection (36).

Diagnosis of HIV infection based on HIV RNA level should be confirmed with Western blot test 2-4 months after the negative or equivocal result of this test.

Initiation of antiretroviral therapy during primary HIV infection

First reports about beneficial influence of early treatment of HIV infection on its clinical course were published in 1997 (37) and concerned macaque monkeys which had been infected with pathogenic HIV-2 strain causing usually fast course of the disease and death. Monkeys which were not treated died 6 months later and out of the 6 which received ddI for 16 weeks – 5 were fine after 3 years after the infection. One of the first control studies in HIV-infected people in which ZDV was applied at 250 mg twice a day suggested that using antiretroviral drugs during the primary infection may slow down the progress of HIV infection and increase the number of CD4 cells (39).

However, it was soon proven that application of a single drug is incomparably less efficient than a combined therapy, including two nucleoside reverse transcriptase inhibitors and a protease inhibitor. Berrey at al. performed a study where patients during primary infection received ZDV, 3TC and indinavir proving than as compared to non-treated patients during 78 weeks of follow-up in the treated patients opportunist infections were observed less frequently (5% versus 21%), less frequently progress to AIDS was observed (0% in treated patients, 13% in non-treated patients). In the patients who received antiretroviral drugs viremia was maintained below 50 copies of RNA/ml, but 9 – 12 months later HIV became detectable in latently infected CD4 cells and in lymph node mononuclear cells (40).

Theoretical arguments in favour of initiating therapy so early are as follows:

- inhibition of the initial "eruption" of virus replication, which may reduce the number of long-lived HIV-infected cells, such as CD4 memory cells,
- reduction of severity of acute disease symptoms,
- possibility to change viremia level, which is established after the acute infection – this may affect the speed of disease progress,
- possibility to reduce frequency of virus mutation occurrence through reduction of HIV replication,
- possibility to reduce virus transmission risk,
- maintaining the function of immunological system, including HIV-specific CD4 cells and cytotoxic T lymphocytes: in patients in whom HAART allows for long-term viremia control, researchers have observed reduction of HIV-specific response of CD4 memory cells, which is visible in *long-term non-progressors* to a lesser extent (41), frequency of occurrence of HIV-specific CD8 cells decreases as well: after commencement of antiretroviral therapy the response of HIV-specific CD8+ cells is markedly variable during 1 – 2 weeks and then it is significantly reduced (average $T^1/_2$ about 45 days), and the effect is sustained despite undetectable viremia (42).

Risks related to initiating therapy during primary HIV infection include:

- adverse effect on quality of life due to the drugs' side-effects and difficulties in observing recommendations related to their application,
- if HIV replication is not sufficiently inhibited, drug resistance may occur, which may reduce treatment possibilities at further stage,
- likelihood of continuing the therapy until the patient's death.

In 2004 a review of previous research results was published and its authors found that there is no evidence that the patients who commenced anti-retroviral treatment so early were clinically at advantage and it would be necessary and ethically justified to performed randomised control study of short-term HAART application during primary infection as compared to later onset of therapy (43).

There are many questions related to treatment of primary HIV infection. It is not known how long such therapy should last. It is not known either whether the obtained virusological and immunological advantage will be sustained after withdrawal of drugs. But it is known that once initiated, management of chronic HIV infection with currently available drugs should be continued until the end of the patient's life. It is impossible to eradicate HIV from the organism. It is still necessary to carry out randomised studies which could answer these questions (44).

In recommendations on commencement of antiretroviral therapy in HIV-infected patients prepared by International AIDS Society – USA Panel and published in 2008, it was stressed that no definitive evidence has emerged that supports routine initiation of antiretroviral therapy (45). European AIDS Clinical Society recommends treatment of patients at primary infection stage within clinical studies and an indication to commence it would be AIDS-defining events, confirmed CD4 < 350/mm^3 at month 3 and beyond, and treatment should be considered if the patient suffers severe, prolonged symptoms, especially CNS symptoms (46).

In patients with positive results of anti-HIV antibodies tests who believe that they got infected recently, but there is no documentation confirming this fact, decisions on initiation of the therapy should be based on recommendations concerning persons at the asymptomatic stage of the chronic infection.

Under no circumstances should antiretroviral therapy be initiated in persons without documented diagnosis of HIV infection. In medical literature you can find reports of malingered HIV infection (47). The only exception to this rule is post-exposure prophylaxis.

Conclusion

Early diagnosis of HIV infection at the stage of primary infection has paramount meaning for the infected person, because thus the patient may take care of their health with an aid of a HIV-therapy specialist and protect their sexual partner. It is also very important for reduction of HIV infection transfer to other persons – HIV viremia level at this early stage of infection is the highest.

Very soon evidence may be obtained from clinical studies showing that initiation of anti-retroviral therapy during primary infection has positive effect on further course of HIV infection. But before it emerges, it is advisable to consider a balance of potential benefits of the antiretroviral therapy and the risk related to initiating treatment so early.

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title

JCV and BKV prevalence in people infected with HIV-1

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summary

Human pathogenic viruses of the polyoma type were discovered only in the 70s of the 20th century. So far seroepidemiologic researches concerning these infections have been carried out on small groups and probably are not representative of the entire of population. They showed a past polyomaviruses infection in 35-85% people in a healthy population. In two cases: JSV and BKV reactivation plays a role in a development of diseases in people with impaired immunological response, e.g. HIV infection history.

The aim of the study was to examine the frequency of prevalence of JCV and BKV in various body fluids and pathogenicity connected with these viruses in patients infected with HIV-1.

The tests included 108 people of both sexes, in whom HIV-1 infection was diagnosed. JCV/BKV infection was diagnosed in 27 (25%) HIV-1 infected patients, where JCV constituted 18 (16.7%) cases and 9 (8.3%) BKV. The infections were proven on the basis of a presence of virus genetic material in urine. In none of the patients was JCV and BKV detected in blood and cerebrospinal fluid.

In the patient group with TCD4 lymphocyte count < 200 cell/ µL JCV DNA was detected in urine in 5 (11.4%), and BKV in 7 (15.9%) cases, in turn in the group of people with neurological conditions JCV in 3 (15%) and BKV also in 3 (15%) cases, (together in 6 (40%) people). Among the tested people with lymphocyte TCD4 lymphocyte count > 500 cell/ µL JCV DNA was detected in urine in 9 (20.5%) cases and BKV DNA in 1 (2,3%) case.

key words

HIV, JCV, BKV

address

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Introduction

Human pathogenic viruses of the polyoma kind were discovered only in the 70s of the 20th century however population studies prove that the viruses and particularly JCV have accompanied humans since prehistoric times. Nowadays among polyoma type viruses we distinguish 4 pathogenic types: KIV, WUV- discovered in 2007 and not entirely studied (1, 2) and JCV and BKV, where reactivation of an infection is particularly significant in pathogenicity in patients with an impaired immunity, e.g.: HIV infected or with organ transplants (3, 4).

Seroepidemiological studies of the occurrence of those viruses in people have been carried out on small groups and probably are not representative of the entire population. They showed a past polyomaviruses infection in 35-85 % people among healthy population (5, 6, 7, 8).

The presence of JC virus as a result of its reactivation was found in HIV infected patients with symptoms of Progressive Multifocal Leukoencephalopathy (PML) as well as in asymptomatic HIV carriers. BKV infection in patients HIV-positive is rarely described (9,10, 11). In singular cases JCV and BKV were found in brain tissue in HIV infected patients suffering from PML.

In Poland so far there has been no seroepidemiologic study on the occurrence of these viruses in patients infected with HIV-1. Studies among populations noninfected with HIV have been rare, too.

Aim of the study

The aim of the study was to examine the prevalence rate of JCV and BKV in various body fluids and related pathogenicity in HIV-1 infected patients.

Materials and methods

One hundred eight HIV positive patients entered the study, before antiretroviral therapy was introduced. They were divided in 3 groups, respectively of T CD4 lymphocyte count and found symptoms:

1. < 200 cells/ μL – 44 patients with advanced immune deficiency which correlates with increased risk of any opportunistic infection (JCV/BKV infection).
2. > 500 cells/ μL – 44 patients with good immunological status decreased risk of any opportunistic infection (JCV/BKV infection).
3. With symptoms from central nervous system (CNS) – 20 patients
4. The control group consisted of 23 healthy individuals, with no HIV infection history.
5. The following laboratory tests were carried out on all patients:
   - JCV and BKV: in blood and urine and in justified cases in cerebrospinal fluid
     – Isolation of DNA from fluids by the use of kits: Qiuamp DNA Blood Mini Kit- in plasma and cerebrospinal fluid, Quiamp Viral RNA Mini Kit – in urine),
     – Multiplication by PCR method with the use of common primers both for JCV virus JCV as well as BKV one i.e. PEP1 5' AGT CTT TAG GGT CTT CTA CC 3' and PEP2 5' GGT GCC AAC CTA TGG AAC AG 3',
     – polymerase Hot Star Taq (QIAGEN),
     – differentiation towards BKV and JCV by RFLP method, the product was digested with BamH1 enzyme (Fermetas);
   - CD4 T cell count: Absolute CD4 T cell count was determined by the flow cytometry on a FACScan (Becton-Dickinson);
   - Quantification of HIV-1 RNA in plasma - Plasma HIV-1 RNA was measured in -70^{0}C stored plasma prepared from blood using the Roche Amplicor 1.0 standard assay (lower limit of detection, 40 copies/ mL).

Statistical analysis

The data were analyzed with the use of SPSS 12.0 for Windows. Test t was used to calculate if the differences between tested groups were statistically significant. Pearson correlation and Friedman ANOVA test were also performed. A P value of less than 0.05 was considered to indicate statistical differences.

Results

The tests included 108 people of both sexes, in whom HIV-1 infection was recorded, 80(61%) men and 51 (39%) women. The average age of the test group was 33.3 years old. 63 (58.3%) people were infected by intravenous use of intoxicants. The control group consisted of 23 people not infected with HIV-1.

Characteristics of the tested group are in table 1.

HIV RNA was tested in most patients' plasma, where in over 65% with T CD4 lymphocyte < 200 cell/μL count, in 75% people with CNS disorders and in 70.5% with T CD4 lymphocyte > 500 cell/ μL count. The patients who entered the study did not get yet cART and thus HIV viremia was connected with the lack of antiviral therapy.

During observation 16 (12% of the observed population) patients died including 5 women and 6 men. The average age of the patients who died was 39.87 years old.

JCV/BKV infections were found in 27 HIV-1 infected people, which constituted 25 % of this group: with 18 (16.7%) JCV cases and 9 (8.3%) BKV. The infections were confirmed on the basis of genetic material presence in urine. JCV and BKV was not found in any patients' blood or cerebrospinal fluid. The average age of the patients was 35.7 years old.

In the group of patients with no symptoms from CNS with T CD4 lymphocyte count < 200 cell/μL JCV DNA was detected in urine in 5 (11.4%), and BKV in 7 (15.9%) cases, whereas in the group with neurological conditions JCV in 3 (15%) and BKV also in 3 (15%) cases, (together in 6 (40%) people).

Among the tested people with T CD4 lymphocyte count > 500 cell/μL JCV DNA was found in urine in 9 (20.5%) cases and BKV DNA in 1 (2,3%) case . In the control group polyomaviruses presence in urine was not detected.

Higher proportion of BKV infections in comparison to JCV in patients with advanced impairment of immunological response: with T CD4 lymphocyte count < 200 cell/ μL and CNS disorders is noticeable.

However in the group of people with T CD4 lymphocyte count > 500 cell/μL JCV infections prevailed.

TCD4, TCD8 lymphocyte count, the ratio of T CD4/ T CD8 lymphocyte count were lower in women than men. T CD4 lymphocyte count result differences between men and women with JCV, BKV reactivation tested t=2,071 and

Table 1. Tested group characteristics

Group	< 200 TCD4/ µL	CNS infection	> 500 TCD4/ µL	Control group
number	44	20	44	23
Average age,range	36,59 (15-57)	35,15 (19-47)	33,16 (20-60)	25,74 (20-50)
Sex Male/female	29 (65,9%) / 15 (34,1%)	12 (60%) / 8 (40%)	31 (70%) / 13 (30%)	8 (35%) / 15 (65%)
IVDU/HTX/MSM	31 (70,5%) / 8 (18,2%)/ 2 (4,5%) / 4 (9,1)	12 (60%) / 8 (50%) / 0 / 1 (5%)	20 (47,62%) / 6 (14,29%) / 2 (4,76%) / 14 (33,33%)	
TCD4 cell count (cells/ul average,range	142,44 (1) 2-775	68,95 (1) 2-188		–
Lymphocyte proportion TCD4	13,02 (1-40%)	6,15 (0,6-23%)	35,34 (21-61%)	–
Infection stage HIV A1/A3/C3 (%)	0 / 25 (56,8) / 19 (43,2)	0 / 2 (10%) / 18 (90%)	44 (100%) / 0 / 0	–

Table 2. T CD4 and T CD8 lymphocytes in JCV/BKV infected

	< 200 TCD4/ µL Men / women	CNS Men / women	> 500_TCD4/ µL Men / women
CD4	210 / 81.6	134 / 8.5	663.17 / 602
CD8	723 / 567.2	567 / 216.5	1493 / 1904
CD4/CD8	0.18 / 0.15	0.265 / 0.04	0.48 / 0.42
%TCD4	24 / 5	13 / 1.3	29.6 / 26

Table 3. Detailed data on coexisting conditions.

Main diagnosis	Patients with CNS conditions n = 20	JC and BK viruses presence in urine testing		
		JC-viruria, n = 3	BK-viruria, n = 3	No viruria JC, BK
CNS syphilis	2	1	0	1
PML	5	2	1	2
CNS cryptococcosis	5	0	2	3
Herpes Simplex Encephalitis	1	0	0	1
CNS tumour	2	0	0	2
ADC	1	0	0	1
Neurotoxoplasmosis	2	0	0	2

were statistically significant=0,049, a very significant difference was observed for lymphocyte proportion in those groups t=2,664, and statistical significance 0,018. Table 2 contains detailed values.

JCV/BKV viruria was found in 6 (40%) cases in the group with neurological conditions. Presence of polyomaviruses in the cerebrospinal fluid was not seen in any patient.

Discussion

For over ten years there have been available viruses' genetic material tests. Owing to that 4 genotypes and 18 subtypes of JC virus which exist in Africa, Europe, America, Asia and Oceania have been isolated (3).

However, in our study there was a significant difference between the HIV positive groups and control group concerning occurrence of JCV and BKV infections, indicating increased prevalence in HIV positive individuals regardless the immunological status.

JCV presence can be found in HIV-1 infected patients who do not suffer from PML. Andreoletti showed JCV in 26 % people with T CD4 lymphocyte count < 200/µL, 18% of patients with TCD4 lymphocyte count >200/µL (12), Calderelli in 40% people in brain tissue and in kidneys (13), Ferrante in 49% patients in urine and 10 % of peripheral blood monocular cells (14), Lednicky in 30 % patients in urine (15), and Tornatore in 38 % patients in of peripheral blood monocular cells (16). In our test group the proportion of JCV/BKV infections in people who were not diagnosed with PML was 40.7% in patients with T CD4 lymphocyte count < 200/µL and similarly 40.7% in people HIV infected with T CD4 cells count > 500/µL. The achieved information is consistent with the data from Ferrante and co.'s report with regards to a low lymphocyte count and diverge from the data concerning people with a high T CD4 lymphocyte count (the tests concerned various materials) (14). Due to a different biological material being tested and little literary data the results achieved by us and other researchers are difficult to compare and it may be an approximation only.

Among HIV infected patients with PML symptoms Tornatore (16) found in 89 % tested people JCV DNA in peripheral blood mononuclear cells, Andreoletti (12) in 60% tested, and Ferrante (14) in all patients in cerebral preparations. Calderelli (13) observed JCV DNA in 100% diseased brain tissue preparations and in 60% of disease unchanged brain tissue, whereas Ferrante in 92% cerebrospinal fluid samples and 75% PBMC (peripheral blood mononuclear cell) confirmed virus presence (14). In our test group it is noticeable that both JCV and BKV were found in urine only. Presence of those viruses was found neither in cerebrospinal fluid nor in peripheral blood in PML patients as well. According to other researchers' reports and our tests results the data varies significantly as to the location of virus detection. It seems that the differences in diagnostic methods (e.g. tests sensitivity, quantitative test (17) versus

qualitative) may influence that fact. It needs to be emphasised that in a proportion of PML patients detection of JCV and/or BKV in cerebrospinal fluid is unsuccessful, despite unambiguous brain MR examination. Such negative results constitute a diagnostic problem of a severe condition such as PML and suggest a possible PML diagnosis despite a lack of confirmation of virological examination of cerebrospinal fluid. Additionally virological examination of brain biopsy specimen, despite nearly 100% sensitivity, is connected with complications risk and is hardly ever suggested and performed, which is an additional difficulty in diagnosis.

Reactivation of BKV infection in HIV positive patients is described rarely (9,10,18). In our research we paid attention to a higher proportion of BKV infections in comparison to JCV in patients with an advanced immunological response: with T CD4 lymphocyte count < 200 cell/μL and with CNS conditions (as listed in table 3). Whereas in the group with T CD4 lymphocyte count > 500 cell/μL JCV infections prevailed.

Nebuloni and co. have described nephropathy connected with BKV infection without kidney functions disorder in HIV infected patient at B3 stage with T CD4 lymphocyte count below 50/μL**.** Disseminated BKV infection was described in four patients infected with HIV with abnormally low T CD4 lymphocyte count – i.e. from 0 – 85 cells/μL. The infection concerned meninges in each case, tunica vasculosa bulbi and also lungs and kidney (19, 20).

BKV infection in our test group was not connected with condition symptoms described by other researchers. In one observed PML patient viruria BKV was found.

In singular cases in PML sufferers in brain tissue coinfection of JCV and BKV was found. In none of our patients, including those with extreme immunological deficit, similar coinfection was found.

Conclusions

1. JCV and BKV infection may be asymptomatic in HIV-1 infected people and concerns patients at various HIV-1 infection stages.
2. In patients with extreme immunological deficit BKV infection prevailed.
3. BKV infection may be connected with development of PML in people HIV-1 positive.
4. Urine test for JCV and BKV presence constitutes an important diagnostic test facilitating PML diagnosis.

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title

Study comparing lipid metabolism disorders and effectiveness of treatment in HIV infected patients on long-term treatment with various thymidine analogues

authors

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summary

Disorders in lipid metabolism exist in most patients treated for the long time. In our study were included 186 patients on antiretroviral treatment lasting 96 weeks treated either with AZT/3TC or with D4T/3TC. Performed studies have shown that both d4T/3TC and AZT/3TC regimens are effective as backbone of CART. Similar immunological values are achieved using above mentioned regimens. Elevation of TG and TC level during antiretroviral therapy is much more frequent in persons treated with d4T/3TC and is not dependent on type of the third drug used.

key words

lipid metabolism, antiretroviral treatment, adverse effects

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Background

Prolonging use of antiretroviral therapy reveals numerous late adverse drug reactions. Disorders in lipid metabolism exist in most patients treated for the long time. To limit these complications multidirectional efforts are undertaken in everyday clinical practice. Recommendations related to the lifestyle, nutritional customs and cessation of habits are frequently unsatisfactory. Sometimes the change of antiretroviral treatment to agents having potentially less influence on lipid metabolism is necessary as well as implementation of lipid-lowering drugs. Although most antiretroviral drugs have direct influence on lipid metabolism, search for regimens least affecting the lipid concentration in blood continues.

Lipid and other metabolic disorders are more and more common problem in HIV infected patients, especially on antiretroviral treatment. Dyslipidaemia increases the risk of numerous diseases, and especially coronary atheromatosis which is the most frequent cause of myocardial infarction. Also other risk factors like age, smoking or inherited susceptibility in form of hereditary hypercholesterolaemia increase the risk of cardiovascular diseases in HIV infected patients [1-4]. All these risk factors cause that this population requires special monitoring of lipid parameters. Dyslipidemias in form of elevation of total cholesterol concentration (TC), high density lipoproteins (HDL-C) low density lipoproteins (LDL-C) as well as triglycerides (TG) were reported shortly after introduction of CART. In studies completed so far it was shown that frequency of adverse effects of antiretroviral treatment in form of lipid metabolism disorders correlated with duration of CART [2]. HIV infection itself is high risk factor of dyslipidaemia. Former studies in patients in advanced stage of HIV infection, antiretroviral naive, have shown lowering of total cholesterol and LDL-C and somewhat elevated concentration of TG [5]. Similar phenomenon was observed also in persons in early stage of infection [6]. Recently published data concerning lipid concentration in 50 persons just before and just after HIV seroconversion have shown decrease in TC, HDL-C and LDL-C before antiretroviral treatment [7]. Therefore, increase of serum lipid concentration after implementation of antiretroviral treatment should be systematically tested having baseline values in mind.

From commonly used three classes of antiretroviral drugs the protease inhibitors are regarded as the most hyperlipaemic drugs. Substantial lipid changes in form of elevation in concentration of TC, LDL-C, TG has been shown in patients treated with protease inhibitors, and especially ritonavir [8,9]. On the other hand, exactly this group of drugs was regarded as the most effective in treatment of advanced HIV stages because of the strongest inhibition of HIV replication. Recently more and more studies confirm that also the nucleoside analogues may influence lipid metabolism [10,11]. This group in turn is always used in CART. Till now the most frequently used drugs from this group were lamivudine, and than zidovudine and stavudine. The third group of drugs used in CART, non-nucleoside reverse transcriptase inhibitors show smaller influence on changes in lipid profile. There also exist reports of lipid concentration elevations in group of patients treated with zidovudine, lamivudine end efavirenz [12]. Less pronounced dyslipidaemia was observed after use of other NNRTI drug – nevirapine [13]. As result from previous data all classes of antiretroviral drugs adversely affect lipid profile, however to varying extent. Therefore it s advisable to determine to what extent particular drug combinations influence lipid metabolism.

Objective of the study was assessment of influence of chosen drugs from the NRTI group on lipid metabolism and comparison of effectiveness of treatment with these drugs.

Material and methods

In the study were included 186 patients on antiretroviral treatment lasting 96 weeks. Subjects were divided into two groups:

- Group I – 88 subjects treated with AZT/3TC
- Group II – 98 subjects treated with D4T/3TC

Moreover all patients were receiving protease inhibitor (PI) or non-nucleoside reverse transcriptase inhibitor (NNRTI).

In the first group 60,2 % of subjects were receiving PI, and in the second group 50% of subjects. This difference was not statistically significant in chi-sqare test.

Following values were determined in all patients: CD_4 cells count concentration of total cholesterol (TC) and triglycerides (TG) before implementation of antiretroviral treatment and during therapy every 3 – 6 months. For each group mean value and proportion of patients with elevated levels was calculated.

Effectiveness of treatment was assessed comparing the difference between CD_4 count before implementation of treatment, and value obtained at the end of observation. For each group mean value was calculated.

Concentration of cholesterol and TG was determined in blood serum in fasting condition. As normal values it was assumed: for total cholesterol < 5,2 mmol/L and for TG - <2 mmol/L – equal for both sexes.

Due to possible biases associated with outpatient settings no attempt was made to test statistical significance of observed changes.

Results

Table 1 presents the CD_4 count in both studied groups before implementation of treatment and at the end of observation.

After 2 years of treatment the mean increase in CD4 count was similar in both groups and amounted to 310 cells/μL and 361 cells/μL respectively. Even though these values were similar, the lowest increase in CD4 count was achieved in group treated with AZT/3TC and PI, whereas the highest in group treated with d4T/3TC and PI.

Table 2 presents concentration of triglycerides and total cholesterol before inclusion to the study and in week 96 of observation.

Table 3 presents the percentage of subjects with increased concentration of triglycerides and total cholesterol before inclusion to the study and in week 96 of observation.

In the first group elevated concentration of triglycerides was revealed in 38.5% of persons, and total cholesterol - in 13,5% of subjects. After 2 years of treatment these values increased to 59.3% and 45.6% of subjects respectively. In the second group elevated values of TG concentration were revealed in 40% of subjects, total cholesterol in 23.5%, and after 96 weeks of treatment 47.7% of subjects had elevated TG concentration, and 37.7% total cholesterol concentration. The highest increase of both TG as well as TC was observed in the group treated with d4T/3TC regimen.

Table 1. CD_4 count before implementation of treatment and in week 96 of treatment in relation to used CART regimen

Time of test	Mean (min-max) CD_4 count in treatment group:			
	AZT/3TC +		d4T/3TC+	
	PI	NNRTI	PI	NNRTI
Before start of treatment	128 (0-344)	226 (2-525)	143 (1-364)	190 (6-592)
At the end of observation	413 (121-775)	581 (220-1201)	521 (44-1153)	534 (99-1356)
Increase in CD_4 count	286 (0 – 976)	355 (20-917)	378 (316-1033)	344 (479 –1244)

Table 2. Concentration of triglycerides (TG), total cholesterol (TC) before implementation of treatment and after 96 weeks of treatment in relation to used CART regimen

Time of test	D4T/3TC +			ZDV/3TC+		
	Total	PI	NNRTI	Total	PI	NNRTI
Concentration of TG [mmol/L]						
Before start of treatment	2,15	2,44	1.83	1,91	1,89	1,95
In wk. 96 of observation	2,52	2,91	2,02	2,27	2,41	2,05
Concentration of TC [mmol/L]						
Before start of treatment	4.2	4.4	3.7	4.4	4.3	4.5
In wk. 96 of observation	5.1	5.0	5.3	4.9	5.1	4,7

Table 3. Percentages of subjects with increased concentration of triglycerides >2 mmol/L and total cholesterol > 5,2 mmol/L before treatment and in week 96 of study in relation to used CART regimen

Therapeutic regimens	% of subjects with increased concentration:			
	TG	Total cholesterol	TG	Total cholesterol
	before treatment		in wk 96 of study	
d4T/3TC +				
NNRTI	32.0	4.5	47.8	42.6
PI	51.8	20	66.5	49.4
Total	38.5	13.5	59.3	45.6
AZT/3TC+				
NNRTI	38.9	30	33.5	26.0
PI	40.6	19.3	51.1	43.8
Total	40.0	23.5	47.7	37.4

Discussion

Performed studies have shown that both d4T/3TC and AZT/3TC regimens are effective as backbone of CART. Similar immunological values are achieved using above mentioned regimens. Elevation of TG and TC level during antiretroviral therapy is much more frequent in persons treated with d4T/3TC and is not dependent on type of the third drug used. This regimen in persons with hyperlipidaemia should be used only in exceptional situations, and systematic monitoring of concentration of both cholesterol and triglycerides is necessary during therapy.

Antiretroviral treatment with use of three drugs in most drug combinations leads to changes in blood lipid concentrations, mainly in form of elevation of cholesterol and triglycerides concentration. Changes in blood lipid concentrations in HIV infected patients were revealed even before antiretroviral treatment for many years [14-16]. This problem has definitely increased from the time of introduction of CART. However there is no perfect drug combination, careful choice of drugs, based on former analysis of patient's predispositions is key issue for effective long-term therapeutic effect.

It was shown in performed studies that elevated triglycerides concentration occurs in 40% of subjects, whereas elevated total cholesterol in 13% of subjects even before starting CART. In observations of other authors elevated cholesterol and triglycerides concentration was shown in 60% of subjects [17-19].

In own studies it was shown that biggest changes in lipid metabolism occurred in patients receiving stavudine. Similar results were obtained by other authors. In group of per-

sons receiving stavudine increase of triglycerides and total cholesterol concentration occurred much earlier, as compared to group treated with other reverse transcriptase inhibitors [20-22] .

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title

Lack of the proper diagnosis during primary HIV infection – case study

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summary

Diagnosis of HIV infection at the stage of the primary infection is a challenge for physicians. Its clinical symptoms are unspecific, sometimes they are few and they recede within 2-3 weeks regardless of attempts to treat them. In such circumstances it may be helpful to gather detailed history, including especially the patient's sexuality and risky behaviours he/she has undertaken. The article describes a case in which the patient himself brought about early diagnosis of the HIV infection.

key words

primary HIV infection, diagnosis

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Background

Regardless of the infection route, during the first 2-3 weeks, the virus becomes well established in a lymphatic tissue reservoir. This reservoir is the principal site of virus production, storage, persistence and pathology (CD4+ T-cell depletion and destruction of follicles and the lymphatic tissue architecture). By the time the infection becomes clinically symptomatic, it is associated with seroconversion (1). The symptoms are usually unspecific, the most often they include fever, fatigue, enlargement of lymph nodes, myalgia, nausea and diarrhoea (2). Both number and severity of primary HIV infection symptoms are variable, most of patients suffer more than one symptom. It is difficult to diagnose HIV at this stage. In a study including 103 seroconverters in vaccine preparedness cohorts between 1995 -1998, acute HIV infection was diagnosed in only in 8 out of 50 persons who sought medical care (3).

During the primary HIV infection the peak level of HIV replication is established in blood, oropharyngeal tissues and genital tract (4). Primary stage infections were estimated to be 26 times more infectious than the asymptomatic infection (late stage 7 times more infectious). High infectiousness during primary infection was estimated to last for approximately 3 months after seroconversion (5). This is why early diagnosis of HIV infection is so important.

Case report

In 2006, a 32-year old single and professionally active man was referred by a general practitioner for diagnostics at an infectious diseases ward in a big city in Poland because of fever (> 39°C) which lasted more than a week and marked debilitation. During several days of hospitalisation no deviations were revealed in a substantial examination, and the only irregularity found in ancillary examination was low level of platelets. The fever receded and the patient's condition improved. Before being discharged, the patient himself asked for a anti-HIV antibodies test. The data chart received by the patient did not stipulate the method of the test. The result turned out negative and the managing physician assured the patient that his temperature was not related to HIV infection.

During the short hospitalisation the patient was never asked about potentially risky behaviours which might have led to HIV infection. The patient had difficulties in accepting his homosexuality, he had had only several anal intercourses in his life and these were always safe sex. However, 3 weeks before he fell ill, he had had the first unprotected anal intercourse in his life.

After being discharged from the hospital, the patient called one of the Counselling and Diagnostics Points in Poland, where anti-HIV antibodies tests are performed without a doctor's referral, for free and anonymously. He told his story and learned about the window period and about symptoms of the primary HIV infection. Therefore, he waited for 3 months and had another anti-HIV antibodies test at a Counselling and Diagnostics Point. The screening test proved positive and the result was confirmed with the Western blot test. When the patient was informed where to go to begin treatment in the city where he lived, he was surprised to learn that the right place was an outpatient clinic at the hospital where he was hospitalised.

Discussion

Diagnosis of HIV infection at the primary infection stage is of paramount importance both for the patient and for his sexual partners. If the patient is aware of the infection, he/she has a chance to modify his behaviour so as not to transfer HIV to sexual partners. It has been known for a long time that during the primary infection, the high plasma viral load (6) probably corresponds to semen viral load (7), it is strongly correlated with the risk of sexual transmission (8) and therefore epidemic growth, and transmission of drug resistance.

It is difficult to diagnose HIV infection at this stage, because the acute retroviral syndrome mimics many common febrile illnesses and because confirmatory HIV antibody tests remain negative during the diagnostic window (9). Fourth-generation HIV assays are not always helpful either in diagnosis of the infection at the early stage. Delforge et al. (10) presented a case where diagnosis of primary HIV infection was delayed due to one of these tests. Assays for HIV RNA are more sensitive than those for p24 antigen in diagnosing primary infection, but they may have false-positive results (11).

A similar case to the one discussed above was presented by Dobec et al. (12), who described a man who referred to a physician and reported high fever (39.8°C), tonsillopharyngitis, fatigue and myalgia, persistent for a week. No rash or lymphadenopathy were observed in this patient. At one moment lymphopenia (0,58 $\times 10^3$ cell/μL) and thrombocytopenia (65 x 10^3 cells/μL) were found. The patient reported that he had lately returned from a holiday in Cuba. Malaria, dengue, Streptococcus pyogenes group A, Enteroviruses, Shigella, Salmonella, Epstein-Barr virus, cytomegalovirus, Hepatitis C virus and Treponema pallidum were all excluded as potential causes of his clinical condition. Considering the progress of lymphopenia and thrombocytopenia, an anti-HIV antibodies test was recommended and the patient agreed. A third generation HIV test gave a negative result and two fourth-generation HIV EIA tests, which detect both antigen p24 and antibodies, were reactive. The authors performed a HIV nucleic acid amplification test (NAT), which revealed a positive HIV result of 4,150,000 copies/mL. Only then did the patient confirm that he had had unprotected heterosexual intercourses during his stay in Cuba.

The discussed cases document the necessity to include HIV infection in the differential diagnosis in patients with leukopenia, thrombocytopenia and fever and the necessity to exclude HIV infection in the diagnostic process. They also demonstrate how important it is to gather detailed history of the patients' sexual habits.

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title

Generalized hair loss as an adverse effect of antiretroviral therapy in an HIV-1 positive man – a case report

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summary

Hair loss can be one of adverse effects of antiretroviral therapy among HIV infected patients. There are only few reports in literature concerning alopecia in patients treated with protease inhibitors. Most of them are related to indinavir use and only few to other drugs. A 40-year-old HIV-1 infected man developed complete hair loss after 3 months of effective antiretroviral therapy with lopinavir/ritonavir plus saquinavir. Hair loss was diffuse, progressive and complete affecting scalp, eyelids, eyebrows, beard, axillar and pubic areas, arms, legs and body hair. After 4 weeks of new treatment, the new regimen being nevirapine, emtricitabine and tenofovir, thin and sparse moustache hair began to appear. No further signs of hair re-growth have been seen. To our knowledge, there are 3 reports of lopinavir/ritonavir-related hair loss in the literature, our being only the fourth one – if the described alopecia was actually caused by lopinavir/ritonavir use. On the other hand, there have been no reports concerning alopecia after saquinavir use. We cannot establish whether generalized hair loss in our patient was due to lopinavir or saquinavir alone or both drugs used simultaneously. Although generalized hair loss after PIs use is rare, clinicians should be aware of this side effect, especially because of its possible negative influence on the quality of life.

key words

AIH (autoimmune hepatitis), HIV infection, antiretroviral therapy

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Abstract

Hair loss can be one of adverse effects of antiretroviral therapy among HIV infected patients. There are only few reports in literature concerning alopecia in patients treated with protease inhibitors. Most of them are related to indinavir use and only few to other drugs. A 40-year-old HIV-1 infected man developed complete hair loss after 3 months of effective antiretroviral therapy with lopinavir/ritonavir plus saquinavir. Hair loss was diffuse, progressive and complete affecting scalp, eyelids, eyebrows, beard, axillar and pubic areas, arms, legs and body hair. After 4 weeks of new treatment, the new regimen being nevirapine, emtricitabine and tenofovir, thin and sparse moustache hair began to appear. No further signs of hair re-growth have been seen. To our knowledge, there are 3 reports of lopinavir/ritonavir-related hair loss in the literature, our being only the fourth one – if the described alopecia was actually caused by lopinavir/ritonavir use. On the other hand, there have been no reports concerning alopecia after saquinavir use. We cannot establish whether generalized hair loss in our patient was due to lopinavir or saquinavir alone or both drugs used simultaneously. Although generalized hair loss after PIs use is rare, clinicians should be aware of this side effect, especially because of its possible negative influence on the quality of life.

Background

Combination antiretroviral therapy (cART) has been associated with improvement in the immune and viral status of patients infected with HIV-1 and has significantly decreased HIV related morbidity and mortality. On the other hand, a lot of adverse events related to cART have been observed. One of them could be hair loss. There are a few reports on hair loss during use of different antiretroviral drugs, i.e. indinavir, lopinavir/ritonavir, atazanavir, zidovudine, lamivudine (1,2,3,4,5,6,7). In most cases the described hair loss was more or less expressed alopecia areata and only in a couple of cases generalized alopecia (1,2,3,4,5,8). Here we report on the first case of generalized and complete hair loss in an HIV-1 positive man treated with saquinavir plus lopinavir/ritonavir.

Case presentation

A 40-year-old Caucasian man diagnosed with HIV-1 infection in 2000 (intravenous drug use) started cART for the first time in March 2001 with combination of stavudine, didanosine, and nevirapine. At the beginning of cART, CD4 T cell count was 388 cells/mm^3 and HIV RNA viral load 531.000 copies/mL. The patient was clinically asymptomatic. There was no history of any AIDS defining illnesses neither of any other disorders. Despite effective treatment (CD4 T cell count above 500/ µl, viral load (VL)< 50 copies/mL) the regimen was changed in September 2003 because of severe thrombocytopenia (1 x 10^3/ µl) and symptoms which could be related to mitochondrial toxicity (unexplained severe weakness and myalgia as well as chest and abdomen pain). The patient received a new nucleoside analogue – sparing regimen consisting of saquinavir and lopinavir/ritonavir (part of a clinical trial). The new regimen was well tolerated and all previously observed symptoms resolved. Platelet count raised to above 100 x 10^3/ µl , CD4 T-lymphocyte count reached 750 cells/ µl , and HIV RNA viral load was stable at <50 copies/mL. In January 2004 (after 3 months of treatment) the patient noticed diffuse progressive hair loss in the scalp, eyelids, eyebrows, beard, axillar and pubic areas, arms, legs, and body hair, leading to complete loss of hair from the whole body. No other dermatologic abnormalities were seen. As there was time coincidence between treatment initiation with protease inhibitors and hair loss, the patient was asked to change the regimen. However, because the therapy was effective and very well tolerated, he decided to continue it despite the generalized alopecia. He and his relatives accepted this side effect. Finally after four years of treatment with protease inhibitors, in April 2008 the patient asked for drug change to try and check if hair re-growth was possible. He received nevirapine, emtricitabine and tenofovir. After 4 weeks of new treatment he noticed thin and sparse moustache hair beginning to re-appear. Until now (6 months after discontinuation of PIs) no other signs of hair re-growth have been seen.

Conclusion

Generalized alopecia (alopecia universalis), a variant of alopecia areata, is a disease of unknown etiology, although is believed to be of autoimmune origins arising from a combination of genetic and environmental influences (2). Among HIV infected patients, alopecia could be a possible adverse event associated with the use of different drugs, especially protease inhibitors (8). The most commonly mentioned cause of alopecia induced by protease inhibitors is the alteration of retinoids metabolism (2,5). There have also been reports on alopecia in patients treated with indinavir, lopinavir/ritonavir, atazanavir (1,2,3,4). We report this case of hair loss in a patient treated with combination of saquinavir and boosted lopinavir. To our knowledge, there are 3 reports of lopinavir/ritonavir-related hair loss in the literature (2,3,4), our being the fourth one – if alopecia in the described patient was actually caused by lopinavir/ritonavir use. There have been no reports concerning alopecia after saquinavir use so far. We cannot establish whether generalized hair loss in our patient was due to lopinavir or saquinavir alone or both drugs used simultaneously. We also do not know what influence treatment with two boosted PIs had on the severity of this side effect. In our patient, other reasons of hair loss, such as immune reconstitution syndrome, secondary infections, nutritional disorders, endocrine dysregulation and exposure to other drugs were excluded (2). According to literature reports, alopecia was rapidly reversible in most patients and hair re-growth was usually seen 6 – 8 weeks after stopping the regimens in question (1,2,3,5). In our patient, 4 weeks after discontinuation of PIs use, slow re-growth of new hair, however moustache only, was noticed. In comparison to other reports, in this case the duration of treatment probably responsible for alopecia was very long, as well as the duration of hair loss (1,2,3,5). We do not know if there would be any relationship between the duration of treatment and the possibility of achieving hair re-growth and to what extent (complete or partial only). Although generalized hair loss due to PIs use is rare, clinicians should be aware of this side effect, especially because of its possible negative influence on patients' quality of life (2). A switch to a PI-sparing regimen may be required in some cases. Other antiretroviral drugs could also cause dermatologic disorders, alopecia among others.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Jacek Gasiorowski and Brygida Knysz took care the patient during treatment. Jacek Gasiorowski and Brygida Knysz prepared the manuscript, Bartosz Szetela participated in translation. All authors read and approved the final version of the manuscript.

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