ISSN 1730-1270

International Journal of HIV-Related Problems

hiv & aids review

volume 7 • number 3 • 2008

Polish AIDS Research Society (PTN AIDS)

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Print and binding:
MKJ Druk
Poland, Białystok, Zwyciestwa 3A Str.

Published for:

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ISSN: 1730-1270, Warszawa 2008

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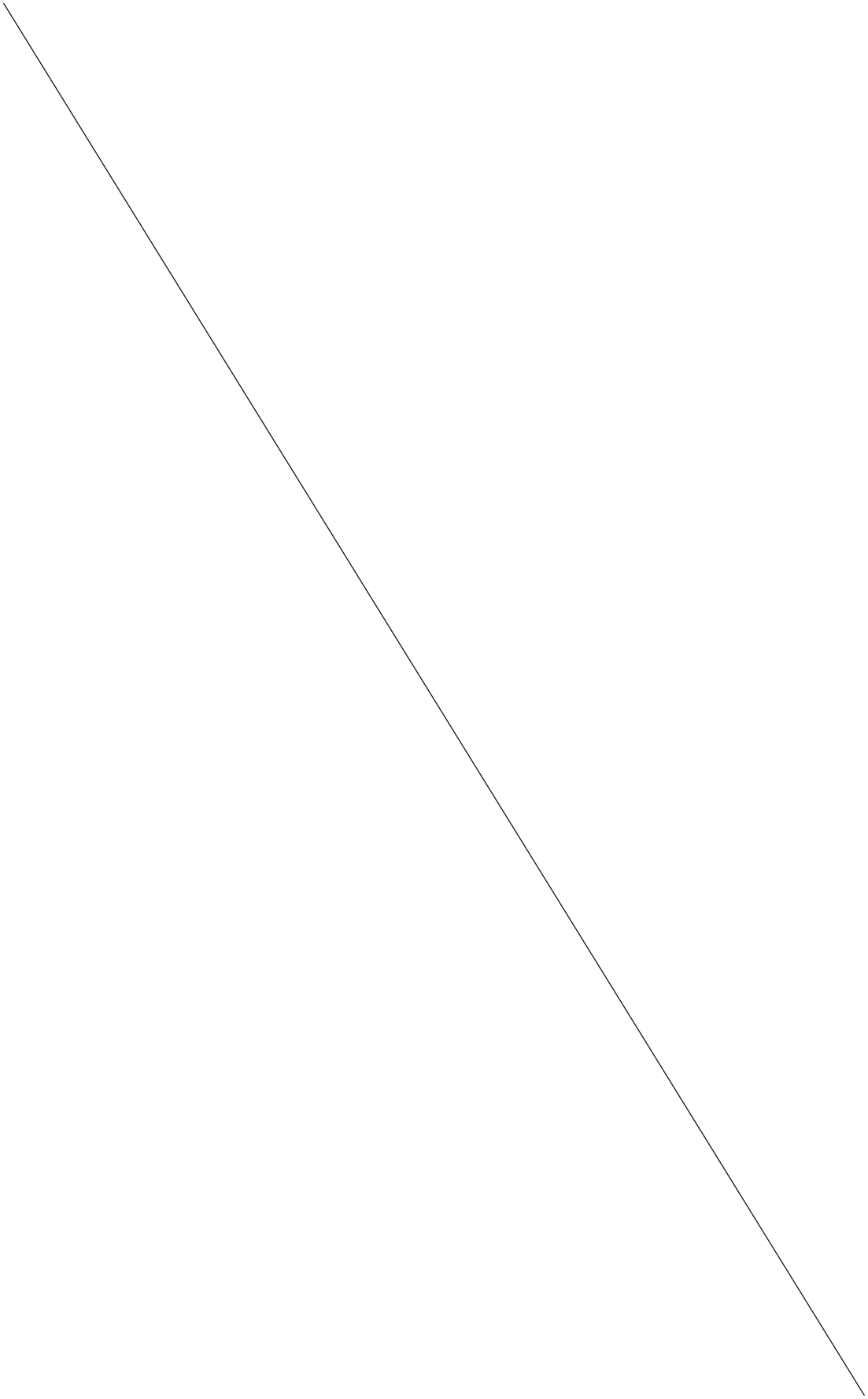
Full Text of Articles Avaiable on the Internet: **http://www.ptnaids.info**



title

Microbicides in HIV infection prophylaxis – not only ethical challenges

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summary

The increasing number of HIV infections transmitted through heterosexual contacts and the increasing incidence of infections in women make it urgently needed to find a chemical agent which could be applied locally and could prevent HIV and other sexually transmitted diseases. However, the previous experience with nonoxynol-9 and subsequent suspension of clinical studies on other preparations are not optimistic. In the absence of an effective HIV vaccine, even a partially active microbicide would still be a highly desirable intervention.

key words

microbicides, nonoxynol-9, clinical trials, ethics

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Almost from the beginning of the AIDS epidemics, attempts have been taken to find chemical agents to be applied locally (intravaginally or intrarectally) which could protect from sexually transmitted infections, mainly HIV. Microbicides, which could be applied by women with or without their partners' knowledge, are extremely important for inhibition of HIV infections in women. Recent statistics by UNAIDS suggest that 2.5 million (range: 1.8-4.1 million) people were newly infected with HIV in 2007. About 15.4 million (range: 13.9 – 16.9 million) women were living with HIV (1).

Nonoxynol-9

In mid-1980s the researchers' attention focused on nonoxynol 9 (N-9), used for more than two decades as a spermicidal agent. In the USA it was approved before introduction by the Food and Drugs Administration (FDA) of requirements of obtaining test results concerning security and efficacy of preparations introduced to market and that's why the knowledge about its efficacy as contraceptive agent – at different doses and forms – was very limited until recently (2). Subsequent studies revealed that N-9 dissolves and disrupts the sperm cells' plasmatic membrane (3), that it is classified as a detergent and that in experimental biology it is used for cytolysis (destruction of cells) (4).

Early experimental studies showed that in vitro it is active toward many microbes, e.g. against viruses of *Herpes simplex* (HSV) and cytomegalovirus and against bacteria – *Neisseria gonorrheae*, *Treponema pallidum*, *Bacteroides*, *Gardnerella vaginalis* and *Chlamydia trachomatis* (5,6). First reports suggesting that N-9 may have an effect of prevention of heterosexual HIV transmission were published in 1985. They indicated that a one-minute-long exposure to N-9 in 0.05% or higher concentration blocked infection of sensitive cells with HIV (7). Subsequent observations demonstrated that even shorter exposure to N-9 inactivates HIV virus (8).

In the late 1980s, N-9 was added to condoms and lubricants in many countries and campaigns were started to promote the preparation as an agent reducing the risk of HIV infection through sexual intercourses. However, even earlier there were reports showing that N-9 irritates mucous membrane. One of the first of them was published in 1964 (9).

Later studies, both in vitro and in vivo showed that the preparation is less active against pathogenic bacteria than against the *Lactobacillus* strains which produce hydrogen peroxide and protect women from sexually transmitted diseases, by keeping proper acidity of the vagina (pH 4,5) and otherwise (10). It was proved that although the preparation was active against HIV in the concentration of 0.01%, in the same concentration it was also cytotoxic for lymphocytes (11).

Clinical studies on efficacy and security of applying N-9 in HIV infections prophylaxis were undertaken after a broad marketing campaign of the preparation. Initially, results of the studies on small groups of patients were equivocal. They revealed that the preparation was often irritating and even caused ulceration of the vaginal mucous membrane and changed bacterial flora of the vagina, reducing the number of Lactobacillus-type bacteria, which may facilitate HIV infection (12, 13), but this fact was not confirmed by other researchers (14).

Broader reaction was caused only by results presented at the 13th International AIDS Conference in 2000, showing that HIV infection was about 50% more frequent in women who used N-9 than in the placebo group (15). Shortly after the Conference the American *Centers for Diseases Control and Prevention* – CDC published information about these studies, including a warning of inefficacy of N-9 in HIV infections prophylaxis (16). Full results of the research were published only in 2002. They revealed that women who used the preparation demonstrated 3 times a day almost twice as high a risk of HIV infection as in the case of women who used placebo and no influence on the preparation on *Neisseria gonorrheae* and *Chlamydia trachomatis* transmission was shown (17).

In July 2000 II/III-phase studies on N-9 initiated in August 1997 were terminated. They too confirmed that HIV infection affected significantly more frequently those women who used a preparation called COL-1492, a gel containing 52.5 mg of nonoxynol-9 (18).

Further studies did not prove N-9 to prevent other sexually transmitted diseases, such as viruses of *Herpes*, *Chlamydia,* gonorrhoea (19)*, Neisseria gonorrheae* (20), *Trichomonas vaginalis* (21), it did not prevent HPV infection either, and contrarily, it could increase this virus's ability to infect and survive in women's bodies (22), and applying the preparation many times in a day may facilitate transfer of HIV and other sexually-transmitted diseases (23). A little later it was proven that a single dose N-9 does protect from HSV-2 infection, but only for several minutes and after 12 hours it increases sensitivity to this virus about 20-30 times (24). In vitro studies confirmed that the preparation may disrupt the barrier generated by endometrium, and thus facilitate infection with HIV and other pathogenic microbes (25). Long-lasting exposure to N-9 disturbs the composition of the vaginal bacterial flora, increasing the risk of inflammation which may facilitate HIV infection (26).

In October 2001 at a meeting organised by the WHO and CONRAD programme (abbreviation of *Contraceptive Research and Development Programme*) drew the following conclusions:

1. N-9 is not efficient in preventing infection with HIV or other sexually transmitted diseases and if applied intravaginally several times a day it may cause injuries which will increase the risk of HIV infection in women,
2. even small doses of N-9 may cause extensive short-lasting injuries of the anus, increasing the risk of infection with HIV and other sexually transmitted diseases through anal intercourses,
3. although contraceptives containing N-9 are moderately efficient in prevention of unwanted pregnancies and are safe when used rarely (no more frequently than once a day), still condoms lubricated with small quantities of N-9 are not any more efficient in preventing pregnancy and sexually transmitted diseases than condoms lubricated with silicone or other substances (27, 28).

In 2002 *Cochrane systematic review* listed results of the previous studies on application of N-9 and concluded that the preparation does not protect women from sexually transmitted infections (19) or from HIV infection (30) and there is evidence that it may be harmful, increasing frequency of ulceration of sex organs.

Despite these findings, nanoxynol-9 is still used as a lubricant in condoms manufactured by many companies worldwide. Only in 2007 FDA decided to publish on intravaginal spermicidal agents sold without prescriptions an information that N-9 does not protect from HIV or other sexually transmitted diseases and that it may irritate the

vagina and anus, which in turn may increase the risk of HIV infection (30).

N-9 is still subjected to research. In 2003 the results of the 1st phase of studies held in India were published. In this country, N-9 is available as spermicidal agent. The authors claim that N-9 vaginal pessary was found to be safe and accepted in one daily dose in low risk women after consecutive use for 14 days (31). Only recently it was proven that a single dose of N-9 protected mice against HSV-2 for a few minutes but then rapidly increased susceptibility which reached maximum at 12 hours. There was no concentration at which detergents provided protection without significant increase of susceptibility (32).

N-9 is sometimes used intrarectally during anal intercourses with condom or instead of it. Experimental studies showed that application of 2% N-9 causes destruction of rectal epithelium, increasing the risk of HIV infection (33), and lubricants which contain it may increasing the risk of getting infected with HIV or other sexually transmitted diseases through anal intercourses (34).

New principles of clinical studies on microbicides

The experience with N-9 contributed to drawing more attention to studies on microbicides. In 2001 detailed recommendations were published concerning preparation and implementation of subsequent phases of clinical studies on new microbicides, developed by the *International Working Group on Microbicide* (IWGM), (35). The recommendations focused on ethical aspects of studies on microbicides, performed often in developing countries. They stressed the meaning of informed consent by women participating in studies which should be based on complete information delivered in a way and language understandable for the women.

The amended recommendation stressed also the necessity to include organisations of communities concerned in planning and realisation of the studies, to recommend to women participating in them to use condoms at all sexual intercourses and that condoms should be available to all participants. Management of sexually acquired diseases during the studies was declared as obvious but providing antiretroviral therapy to women who got infected during the study was controversial.

A perfect agent to destroy microbes and prevent HIV infection should meet many requirements. It has to be resistant to physiological changes which occur during a sexual intercourse and it should remain active in the presence of semen, despite significant changes of pH reaction and it should act for several hours at best. It should not affect the normal bacterial flora of the vagina, cause local toxic reactions and storage at high or low temperature should not reduce its activity. It would be recommendable for it to prevent transmission of other infectious diseases as well. A microbicide should be absorbed to a small extent, have a long using term, be compatible for use with latex condoms and it should be easy and simple to apply. Such an agent should not be expensive, it should have a long using term, it must not generate dirt, too intensive smell or taste or otherwise affect the feeling of pleasure (36), should not attract inflammatory cells to the vaginal canal (37). All these features make studies on an ideal microbicide very complex, labour and time absorbing as well as costly.

Ethical challenges in efficacy trials of microbicides for HIV prevention

Such trials should include sufficient participants to have adequate statistical power to measure the benefits and any adverse effects of the experimental product. Sample size depends on the expected incidence of HIV infection in the trial participants, on the predicted reduction in incidence related to microbicide use, and on other factors such as the retention and compliance of trial participants. Trials of this kind are often conducted in developing countries, among women who are at particularly high risk of heterosexually transmitted HIV infection (38).

Among issues of research ethics, informed consent has historically received the greatest attention. Nonetheless, there still remains a significant gap between the spirit of informed consent and what it actually means in practice. Often, informed consent is a one-way, one-time communication, a hurdle so that researchers can move on to the next stage of their research protocol. In 2005 in an international consultation, participants have accepted several point of a general agreement:

1. informed consent is a process, not a single action or moment in time,
2. emphasis should be put on comprehension and choice, not merely disclosure,
3. the amount of information should not be overwhelming or work against comprehension, and it must be conveyed in understandable language,
4. persons who choose to consent need to take some explicit action to indicate their decision,
5. reimbursements should be appropriate to the setting and circumstances (39).

During workshops conducted in Africa, Europe, and the United States it turned our that within 8 elements of informed consent according to the US Code of Federal Regulations 95 items ("points") of information were identified, but only 27 (28%) were identified as useful by all groups. This indicates the need for involvement of a variety of individuals and stakeholders, with different research and cultural perspectives, in the development of informed consent, particularly for research undertaken in international settings (40).

A lot of studies have shown that ensuring truly informed consent is challenging particularly when working with vulnerable populations. Participants often have difficulty understanding complex research concepts (such as randomization and placebo), and "wishful thinking" is common. Participants often believe that they are receiving an active microbicide, even though the efficacy of the microbicide under study is not known, and they could be randomized to the placebo group. Pistorius et al. observed that women from the RPA who participated in studies on microbicides felt they had received high quality medical care, and some participants appeared to have become dependent on services provided during the trial (41). In hospital-based research in developed countries many patients perceive that the hospital staff expect them to participate in the studies: this perception seems to have added a subtle element of coercion to ostensibly voluntary consent (42).

Mantell et al. in South Africa found that although the participants clearly indicated, that they had understood the experimental nature of the study microbicide, and they recognized that they had been informed after the trial that the product was ineffective, most continued to believe that

the study microbicide helped prevent HIV and other STI, alleviated reproductive tract pain and STI symptoms, and helped to clean the vagina. This underscores the importance of understanding women's perceptions of the efficacy of study microbicides and the rationale for these beliefs. These results also demonstrated how desperate many women at high risk of HIV infection may be for new prevention technologies (43). Women in South Africa understand that participation in clinical research may not affect their own situation but they hope that it may bring positive results for their younger sisters and daughters. They want to play a role and just participating empowers them (44).

Researchers are ethically obliged to provide all study participants with means that are known to reduce HIV risk, such as condom promotion, safer sex counselling, apart from sexual abstinence, and voluntary HIV counselling and testing. It is generally recommended that, at very least, all participants of a microbicide trial be given free supplies of condoms and urged to use both the vaginal product and a condom during each act of intercourse (38). But women in developing countries often have no chance to force their partners to use condoms (45, 46). But even when condoms are used consistently they may be used incorrectly (47). On the other hand, female sex workers suggest that financial and clients preferences resulted in poor condom use. Clients perceive condoms as barriers to sexual pleasure and therefore paid less for vaginal and anal sex when condoms were used. In South Africa unprotected vaginal sex costs Rand 120 (US$20) whereas with condom is worth only Rand 60 (US$10) (48).

HIV seroconversion is the studies' primary endpoint, therefore only HIV-negative women are included. At baseline, all potential participants are tested for HIV infection with pre- and post-test counselling. Like informed consent, preparing the women to receive a positive result should be a process. Test results should be confidential. But in practice it is difficult to keep the confidentiality.

After entry into the trial some women will seroconvert. All trial participants with HIV infection should be referred to services for essential medical care as well as social and psychological support. This raises a problem when appropriate services of this kind are not available and the social environment discriminates against persons living with HIV/AIDS (38).

Population Council stresses that HIV/AIDS prevention trials involve highly sensitive issues: stigma, sexuality, and gender-based power dynamics (49). Preferences and practices regarding lubrication during sex also influence microbicide acceptability and use. Vaginal practice should be taken into account during clinical testing of microbicides (50).

There are controversies about whether microbicides should prevent HIV only or pregnancy as well. In a study in California (USA), women expressed an opinion that an ideal microbicide should offer protection from pregnancy, HIV and sexually transmitted infections (51). In South Africa apparently for non-contraceptive properties were preferred by potential users (52). In Zimbabwe however, men were concerned that vaginal microbicides might prevent pregnancy and cause infertility (53). At the same time, high rates of pregnancy during clinical trials raise important methodological and ethical issues (54).

The organisation of microbicide trials is constantly discussed. Food and Drug Administration (USA) raised concerns that use of microbicides could decrease condom use, resulting in a net increase in HIV infection rates and require a three-arm study to compare HIV incidence rates not only with active versus placebo control gels, but also in a no-gel ("condoms only") controls group. Evidence of microbicide efficacy would be evaluated against both control groups. The recommendation for a third arm was also intended to address the concern that placebo gels may reduce or enhance HIV infection. Although such effects have not been detected in human or animal studies, they have not been formally excluded (55). To address these issues, a hydroxyethylcellulose (HEC) placebo formulation has been developed and has been adopted for use in clinical evaluations of investigational microbicides as "universal" placebo, which has adequate physical properties, is sufficiently stable as a vaginal gel formulation, and is safe and sufficiently inactive in the clinical study (56). Until 2003 nine studies have examined how the availability of microbicidal-like products such as spermicides (N-9) affected male-condom use. Six found that the availability of additional protection options along with counselling resulted in increased condom use (57). Stein and Susser stress that it is not ethical to authorize a study that recruits women as volunteers to a study arm that cannot be expected to yield scientifically useful information (58). Observations in the RSA have shown that many respondents thought that including a condoms-only arm would result in increased product-sharing and male partner resistance to trial participation (59).

One of the most difficult issues is the problem of informing male partners. In particular, whether they should be required to consent to their partners' participation in the research trial or not. In many relationships, men hold power over sexual decision-making, including condom use. Research has documented imbalanced gender roles for making sexual and reproductive health decisions, especially in developing world (60). In Zimbabwe (Africa) most men said that they could be supportive of their wives' participation in microbicide trials, if they were asked for permission first and if proper medical care and insurance coverage were provided (53). Studies have reported men's acceptance of hypothetical microbicides, although it is not clear if this is partially based on belief that such products will protect them from an infected partner. If microbicides offer protection only to the female partner, acceptability and use may be more problematic (61). Covert use of microbicides may have a negative impact on relationship leading to psychological harm of varying significance (54). In Malawi, Zimbabwe, India and Thailand men and women reported that use, which was kept in secret from an intimate partner, might be difficult and might „break the trust" in relationship (44).

Another problem may be the wetness related to using microbicides. Orner et al. conducted conversations with men in Cape Town region (South Africa) and they learned that it would be difficult to distinguish what caused the wetness – the microbicide or sex with another man. Some respondents thought that changes in vaginal lubrication could led to accusations of infidelity or promiscuity, others suggested that added lubrication could be attractive for women experiencing vaginal dryness, and increased lubrication may be advantageous for sex workers (52). In such countries as Zimbabwe, where many women insert liquids, paper, cloth and traditional herbs in their vaginas to dry and tighten the vagina to increase the man's sexual pleasure ("dry sex"), microbicides may cause women to wash and wipe out their vaginas, thus compromising the effectiveness of this method (53).

Another important issue is enrolling adolescents and the issue of parental consent (39). Discussions with representatives of the black working class urban area close to Cape Town (South Africa), where rapes and sexual coercion are a serious problem, there appeared opinions that

there should be no age restrictions on using microbicides. This desire stemmed from a fear that children could be raped and infected with HIV (53).

Provision of antiretroviral treatment has moved to the forefront of current debates on the ethics of HIV prevention trials. In 2000, UNAIDS declared that "care and treatment for HIV/AIDS and its associated complications should be provided to participants in HIV preventive trials, with the ideal being to provide the best proven therapy and the minimum to provide the highest level of care attainable in the host country" (62). A similar discussion concerned research into maternal-infant transmission of HIV. After February 1994 zidovudine became the standard of care in industrialized nations (63). In developing countries the costs of the ACTG 076 regimen made it unavailable. It was, therefore, a matter of some urgency that trials begin to determine whether radically cheaper alternatives could reduce maternal-fetal HIV transmission. The Centers for Disease Control and Prevention and National Institutes of Health launched nine placebo-controlled trials in developing countries. Some said then that the justifications are reminiscent of those for the Tuskegee study. Women in the Third World would not receive antiretroviral treatment anyway, so the investigators are simply observing what would happen to the subjects' infants if there were no study (64). Two years later, in "Consensus statement of participants of Perinatal HIV Intervention Research in Developing Countries Workshop in 1999" it was accepted that there is no obligation to provide study participants with the highest standard of care attainable elsewhere in the world, and "ethical standards in designing research trials should always be applied so as to reflect the economic, public-health, medical, and social realities of the host country", and placebo-control studies were accepted as well (65). This stand raised many controversies, including such arguments as "… if the research is planned and financed by a sponsor from a developed country who cannot do it in their own country for ethical reasons, and especially when a developed sponsoring country is likely to benefit (albeit less than the host country) from successful results, then only the highest possible standards of care of the sponsoring country should be offered to consenting research volunteers" (66). However, studies among HIV prevention research participants, community stakeholders and health-care service providers in ten sites in seven countries (South Africa, Malawi, Tanzania, Zimbabwe, Zambia, India, US) revealed that many respondents viewed the provision of antiretroviral treatment by researches to HIV-infected trial participants as unfair if the treatment was not sustained beyond the end of the trial (67). Therefore more consideration has to be given to issues related to responsibility for participants of prophylactic studies and to rights of the participants.

Failed search for other microbicides

Nonoxynol-9 turned out to be the first unsuccessful microbicide, actually increasing HIV infection risk, but not the last one, unfortunately. Last years witnessed suspension of several clinical studies on microbicides, as no difference was shown between frequency of HIV infections in women who used the preparation and in the placebo group (as with the preparation SAVVY in 2006), or in women who received the active preparation the number of HIV infections was higher than in women who used placebo as in the case of cellulose sulphate, research on which was stopped in January 2007 (68). During the discussion concerning the preparation it was stresses that the anti-HIV activity of anionic polymers (including cellulose sulphate) may be compromised by seminal fluid (69), and clinically relevant concentrations of this compound reproducibly increase the in vitro infection rate of sexually transmissible R-5 tropic strains of HIV (70). Van Damme et al. rejected these charges, indicating that the potential increased risk of infection observed in our per-protocol analysis was driven by results from two sites (Benin and Uganda) where gel was reportedly used 20 times per week on average (9 infections with cellulose sulphate and 1 with placebo). This frequency of use was dramatically higher than the four-times-per-week use reported in South Africa, where there was essentially no evidence of an effect (12 infections with cellulose sulphate and 10 with placebo). Although not conclusive, these findings suggest that a mechanism related to very frequent exposure to cellulose sulphate is a more likely explanation for results (71).

In the early 2008 the organisation *Population Council* published the results of the 3rd phase of studies on a preparation called Carraguard, obtained from seaweed (*Rhodopyceae* or Irish moss). They proved that it is safe indeed but it can't prevent HIV infection – in the group of women who used the preparation, 134 new HIV infections were found and in the placebo group – 151 (72).

Studies held by the organisation *Methods for Improving Reproductive Health in Africa* (MIRA) where latex discs and lubricant gel were used together with condoms did not show lesser frequency of HIV infections in the study group (4.1%) as compared to the control group (3.9%), where only condoms were used. However, despite the researchers' efforts to promote safer sex, only 85% of women in the control group and 54% of women in the study group declared they had used condom at their latest sexual intercourse, which have had an effect on the results. Only 73% of the participants declared that they had observed the recommendation on application of discs and gel, which probably wasn't meaningless for the results either (73).

Ramjee et al. presented consequences of cancellation of research on cellulose sulphate for inhabitants of South Africa (74). Despite proactive steps to inform the wider community, some reporters wrote inaccurate and sensational stories, which instilled fear amongst all trial participants. Many people believed that gel contained HIV or that simply inserting the gel increased the risk of HIV infection. Participants from all other microbicide trials were affected by closure of the cellulose sulphate trial. Male partners who knew about women's participation in other trials raised concerns that using "gel" increased HIV risk. One of the major challenges is HIV prevention research is that there are no surrogate markers for efficacy. The only way to assess effectiveness of products is to measure new HIV infection as an outcome. It is thus extremely difficult to make the lay public understand that in all prevention trials participants are likely to become infected irrespective of the intervention, and it is not the researcher's aim to increase infection or risk of infection.

So many failed microbicide studies cause a danger that further bad news will see funders lose their interests for research on female-initiated prevention methods, so there is a tremendous pressure to avoid more failures. This field has always been difficult sell for policy-makers in any case. Lori Heise of the Global Campaign for Microbicides, quoted by the editorial in "Nature" says that it's about "women, vaginas and sexuality" – not a topic that government officials especially want to air public (75).

Search for new microbicides

New hopes are attached with tenofovir in gel, anti-retroviral drug of the nucleotide reverse transcriptase inhibitors group. Currently researchers of the *Centre for the AIDS Programme of Research in South Africa* (CAPRISA) conduct in the Republic of South Africa studies, co-funded by *US Agency for International Development* (USAID). This study has been controversial from the very beginning. The most problematic issue is that in the CAPRISA study women should apply the gel 12 hours before the sexual intercourse and 12 hours after the intercourse. This application mode is plausible for women whose partners are at home for a short time (76). The researchers from the RSA explain that in the studied community majority of new HIV infections concern women whose partners go far away from home to work and return only for short holidays (77). The studies are held in the city-centre of Durban where frequency of HIV infections in general population of women is 59.3%, and among women who sell sexual services – 59.4% and in countryside where 55% of women aged 20 – 24 were HIV-positive in 2004 (in 2001 – 44%) (78).

An earlier study on 48 women showed that application of a gel containing 1% of tenofovir twice a day for 14 days was well tolerated (79). The gel used in clinical studies by women in the USA was accepted by men as well (80).

Microbicides which contain antiretroviral drugs (ARV) could be highly effective. However, there is a concern that, if used by HIV positive women, ARV resistance may evolve. Wilson et al. also found that paradoxically, although microbicides would be used by women to protect themselves against infection, they could provide greater benefit to men. This suggests that use ARV-based microbicides could have surprising consequences (81).

At a conference on microbicides held in February 2008 in New Delhi, India, information was presented about new microbicides of the reverse transcriptase inhibitors group (tenofovir, dapivirine – TMC-120 – non-nucleoside inhibitor of HIV-1 reverse transcriptase [NNRTI], UC-781 – NNRTI, MIV-150), entry inhibitors (Maraviroc, RANTES analogs), gp-120 blockers, fusion inhibitors (as T-1249) and CD4 downmodulator – a novel class of cytotriazadisulfonamide – CADA) (82).

Different categories of microbicide drugs and lead compounds, their mechanism of action, current status of development, and progress in phase III trials were discussed by Balzarii and Van Damme (83) and Culter and Justman (84).

Microbicides for intrarectal application

It is also necessary to make research on microbicides which can be applied intrarectally. It is obvious that the vagina and anus are different anatomically, histologically, they have different bacterial flora and physiology. It has been shown than semen-stimulating substance applied intrarectally may dislocate to the splenic flexure, located about 60 cm from the anus, which suggests that a microbicide applied intrarectally should offer protection on such an extensive area (85). Such microbicides would also reduce the risk of infection in women, because in some cultures even up to 30% of heterosexual couples has anal intercourses (86). Research performed in New York (USA) in an *ethnically diverse sample of women* revealed that women overwhelmingly expressed their interest in intrarectal microbicides (87). Men in turn who have anal sex, use lubricants very often and majority of them would like to have access to lubricants with an anti-HIV microbicidal agent (88). However, currently there are few studies on microbicides to be applied intrarectally.

Conclusions

Although so far there is only one efficient method of preventing HIV infection through sexual intercourses – condoms, in many countries in the world women cannot discuss using condoms with their partners. In African countries south of Sahara, e.g. in Uganda, marriage poses the worst risk of HIV infection for women. Modelling suggests that even a microbicide shown to be only partially effective, if used by women in concert with promotion of condoms for men, could have a beneficial effect on the reduction of HIV transmission at a population level. In a sub-Saharan setting where HIV prevalence is currently 10.8%, the introduction of a microbicide of 50% efficacy covering 50% of sex acts in high-risk women could achieve a reduction in HIV prevalence to 8,1% after 20 years. Concurrent promotion of condoms additionally covering 50% of sex acts in high-risk men could potentially achieve a prevalence as low as 1,4% (89). In the absence of an effective HIV vaccine, a partially active microbicide would still be a highly desirable intervention.

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title

Association between serum bilirubin levels and *ABCB1* 3435 variant in HIV-1 infected, atazanavir treated individuals

authors

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summary

Background: It has been scientifically confirmed that gene variants influencing glycoprotein P (gp-P) activity are associated with better drug uptake and increase in serum atazanavir (ATV) levels.
Aim of the study: The aim of the study is the analysis of association between serum bilirubin levels before and after initiation of treatment with ATV and ABCB1 (MDR-1) *3435* genotypes.
Material and methods: Forty-nine HIV-1 infected patients treated with ATV as a part of the cART regimen were included into the study. Total bilirubin levels were measured before and after four months from treatment initiation. Frequency of the ABCB1 *3435* variants was assessed, by the *Taq-Man SNP genotyping assay* with real-time fluorescence detection.
Results: Bilirubin levels prior to the treatment have ranged from 0,3 to 3,77 mg/dL (mean = 0,86, MD = 0,76 ±0,54) and were significantly lower ($p < 0,01$) than levels after four months of the treatment (values ranging from 0,5 to 8,26 mg/dL, mean = 2,66, MD = 2,28 ±1,77). Genotype frequencies for ABCB1 3435 were: 30,61% (n = 15) TT homozygotes, 51% (n = 25) TC heterozygotes and 18,37% (n = 9) CC genotypes. No significant differences in changes of the bilirubin levels after ATV treatment initiation in association to the investigated ABCB1 was noted ($p > 0,25$).
Conclusions: Serum total bilirubin significantly increase after initiation of the ARV treatment. In the investigated group no difference of bilirubin levels after treatment initiation in association with ABCB1 3435 genotypes was found.

key words

atazanavir, pharmacogenetics, ABCB1 polimorphisms

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Introduction

Contemporary antiretroviral therapy is aiming at the highest clinical, virologic and immunological benefit for the patient, including reduction of the treatment adverse effect risk. Implementation of the pharmacogenomic discoveries into the practice opens the window of opportunity allowing for the selection of the antiretroviral regimen basing not only on individual's medical history and concomitant diseases but also genetic inheritance. A range of genetic variants is associated with modified response and adverse reactions to antiretrovirals, from widely investigated HLA-B5701, HLA-DR7 and HLA-DQ3 haplotype related hypersensitivity reactions, promoter and untranslated-region related variants of *APOC3, APOE* and ritonavir-associated hyperlipidemia, *-238 G/A* tumor necrosis factor polymorphism and lipoatrophy, *CFTR* mutations and pancreatitis, UDP glycosyltransferase 1 (UGT1) TATA box repeat and atazanavir-linked hiperbilirubinemia, to name just a few confirmed ones (1-7).

From the perspective of pharmacogenetic research, *ABCB1* variants, also known as multidrug receptor 1 (*MDR-1*) gene polymorphisms remain ones of the most widely studied and associated with altered plasma levels of digoxin, immunosuppressants such as cyclosporine and tacrolimus, as well as hypolipemic agents (7). ATP binding cassette transporter gene, *ABCB1* coding for the P-glycoprotein involved in transport of a range of substances, including antiretroviral drugs through the cellular membranes and body compartments (8). In the previous studies it was confirmed that intestinal fraction of this transporter, located at the apical membrane of the enterocyte, is restricting the drug uptake to the body by the retrograde flow of the drug back into the intestinal lumen (9). Additionally, presence of this glycoprotein in the barriers of other endothelial compartments, such as blood-brain, blood-ovary and testis, as well as on the maternal-fetal interface, might influence the distribution of the transported drug and its concentration (10, 11).

Of the reported to date over fifty *ABCB1* single nucleotide polymorphisms (SNP) C3435T variant of the exon 26 was described to be associated with the altered expression of the p-glycoprotein. In individuals with homozygous TT genotype for this SNP intestinal content of the transporter protein was significantly reduced resulting in higher plasma drug concentrations (12). Moreover, blood-brain transport is might also be influenced, as in *ABCB-1* knockout mice brain levels of protease inhibitors were found to be higher when compared to animals with the normal gene expression (13).

The influence of the *ABCB1* variants on protease inhibitors plasma concentrations was studied previously, with the results indicating that T allele might be an independent predicting factor for the lower atazanavir (ATV) plasma concentrations (both boosted and unboosted) (14). Hyperbilirubinemia remains to be most commonly reported adverse effect of atazanavir and is directly correlated with its plasma concentrations as well as with presence of the *ABCB1* 3435 T allele (15).

In our study we aimed to investigate the association between serum bilirubin levels before and after initiation of treatment with ATV and *ABCB1* (*MDR-1*) 3435 polymorphisms.

Material and methods

Forty-nine HIV-1 infected patients treated with ATV as a part of the cART regimen were included into the study. In 13 individuals prescribed atazanavir was boosted with ritonavir (RTV), while in 37 cases unboosted drug in the standard doses was administered. Total bilirubin levels were measured before and after four months from treatment initiation.

Genotyping of the *ABCB1* T3435C single nucleotide polymorphisms was analysed by implementation of real-time PCR based allele discrimination assay. For genomic DNA extraction from full blood samples previously collected to tubes containing EDTA anticoagulant QIAamp DNA Blood mini kit and (QIAgen, Hilden, Germany) was used. The extraction was performed according to the manufacturer's protocol, with DNA re-suspended in the 200 µL of AE buffer (QIAgen, Hilden, Germany) and stored in 4°C for further analyses.

The PCR were performed in the total volume of 10 µL of reaction mixture with ~20 ng of genomic DNA being used. The PCR mixture contained 2x TaqMan® Genotyping Master Mix, nuclease-free water and pre-designed set of primers and probes designed on-demand by Appliedbiosystems corporation *Taq-Man SNP genotyping assay*. PCR reactions and real-time fluorescence detection were performed on the Eppendorf Realplex S thermal cycler (Eppendorf, Germany) with the following temperature profiles: initial denaturation at 95°C for 10 min followed by 40 cycles of 15 s at 95°C and 1 minute annealing/extension at 60°C. Fluorescence detection was performed at the end of the one-minute annealing/extension step.

For statistics t-student test was used for dependent variables while univariate ANOVA test was implemented for *ABCB1* polymorphisms.

Results

Bilirubin levels prior to the treatment have ranged from 0,3 to 3,77 mg/dL (mean = 0,86, MD = 0,76 ±0,54) and were significantly lower ($p < 0,01$) than levels after four months of the treatment (values ranging from 0,5 to 8,26 mg/dL, mean = 2,66, MD = 2,28 ±1,77). On the figures 1a and 1b the distribution of the bilirubin levels prior to the treatment and after four month of treatment initiation are presented. Figure 2 depicts the changes of the bilirubin levels.

All analysed genotypes were checked for conformity with the Hardy Weinberg equilibrium with none presenting significant deviation. The following genotype frequencies for ABCB1 3435 were noted: 30,61% (n = 15) TT homozygotes, 51% (n = 25) CT heterozygotes and 18,37% (n = 9) CC genotypes. ABCB1 3435 T allele frequency was 56,12% (55/98), and for C allele 43,88%.

No significant differences in changes of the bilirubin levels after ATV treatment initiation in association to the investigated ABCB1 genotypes was noted ($p > 0,25$). Mean bilirubin concentration differences for the analyzed ABCB1 genotypes were 2,4 mg/Dl for TT genotype, 1,6 mg/dl for TC and 1,3 mg/Dl for CC homozygotes (SD 2,04; 1,64 and 1,36 respectively). Association between ABCB1 genotypes and change in bilirubin levels are presented on the figure 3.

Figure 1a. Histogram of the total serum bilirubin concentrations prior to the antiretroviral treatment with atazanavir. On the x axis the bilirubin levels (mg/dl) are shown, while the y axis depicts the number of patients

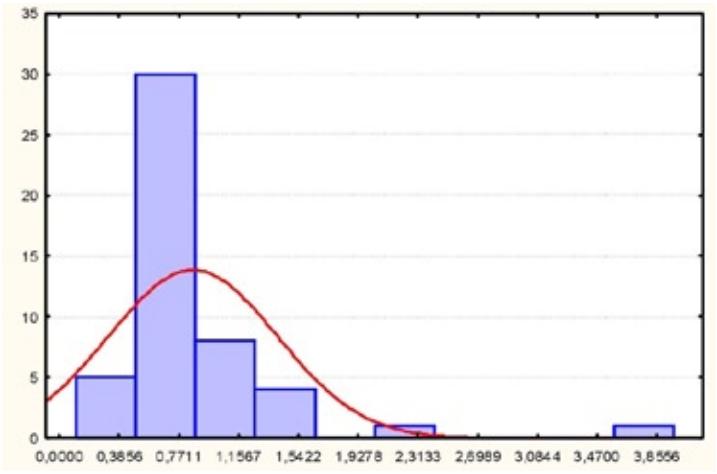


Figure 1b. Histogram of the total serum bilirubin concentrations after the 4 months of antiretroviral treatment with atazanavir. On the x axis the bilirubin levels (mg/dl) are shown, while the y axis depicts the number of patients

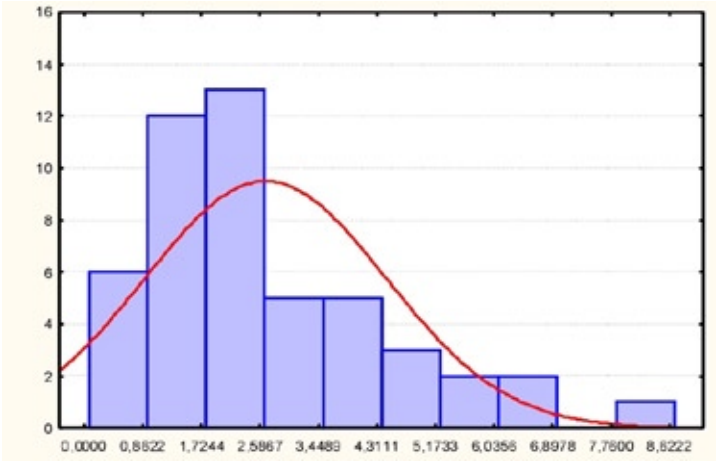
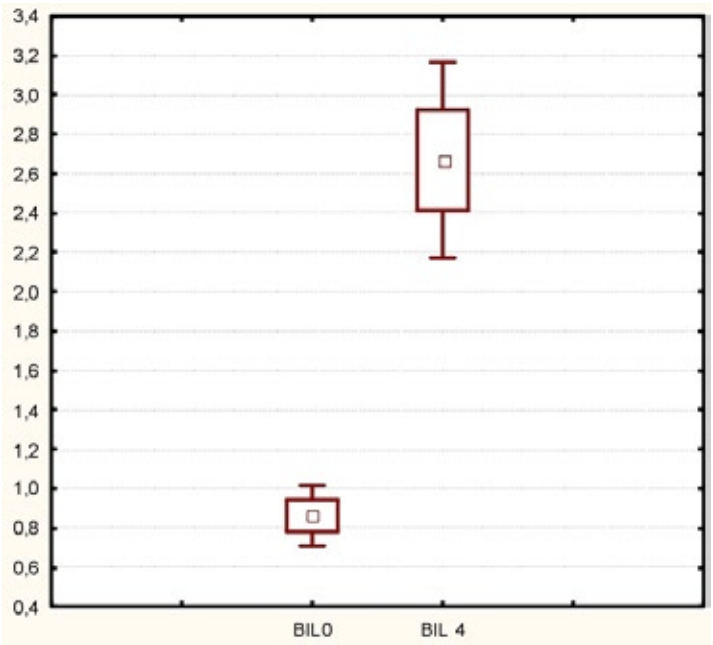


Figure 2. Mean changes in the serum bilirubin concentrations prior to and after initiation of antiretroviral regimen with atazanavir. Axis y depicts the serum bilirubin concentrations (mg/dL). Bil0 – bilirubin levels prior to the ARV treatment (baseline) , Bil4 – bilirubin levels after four months of treatment



Discussion

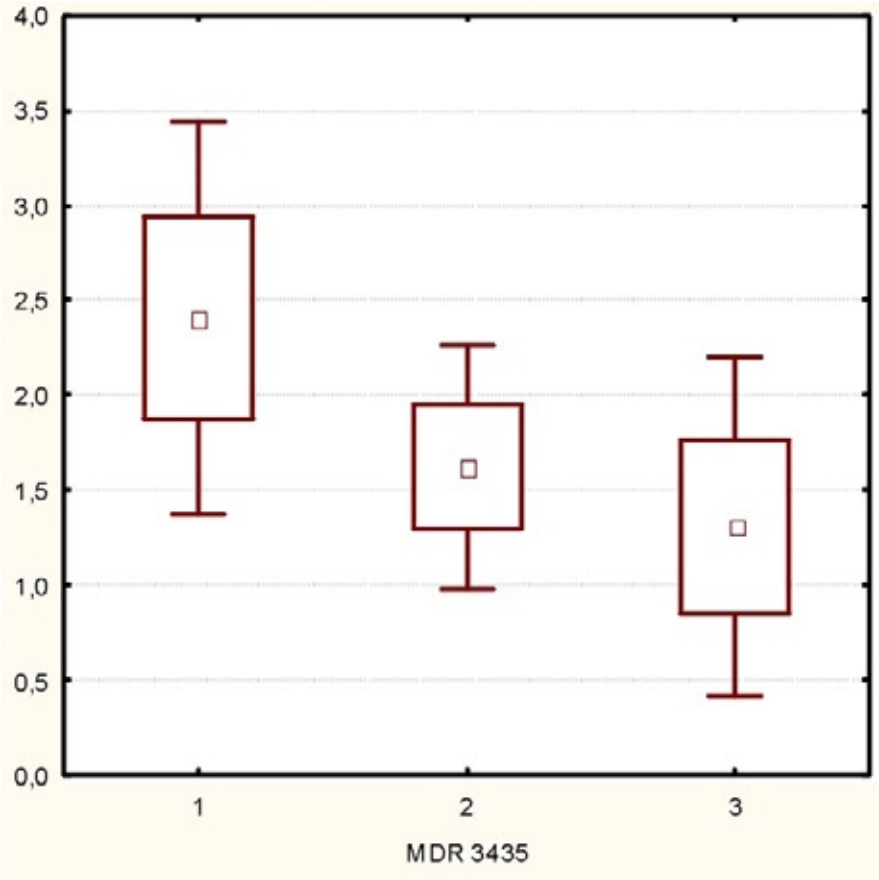
In the light of the necessity for the prolonged, often lifetime antiretroviral therapy best possible selection of the antiretroviral drugs seems the key to the successful patient management. P-glycoproten, being the major drug transporter in the gut is influencing plasma atazanavir levels, by the active efflux activity (16, 17). The ABCB1 gene polymorphisms influence the P-gp function influencing plasma drug levels (18). In this study association between *ABCB1* 3435 genotypes and changes of the bilirubin levels after four month of the treatment. It was previously observed that the *ABCB1* 3435 genotypes are associated with the success of the antiretroviral treatment with the greater rise of CD4-cells observed in individuals bearing the homozygous TT genotype after 6 months of the treatment initiation, and earlier virological failure among patients with CC genotype (19,20). Of the range of associations between this polymorphisms and protease serum protease inhibitors levels, the only influences on pharmacokinetics were noted for atazanavir and indinavir. Higher plasma atazanavir concentrations were observed among patients with *ABCB1* 3435 C allele (15). In our study the association between the *ABCB1* 3435 genotypes and change in bilirubin levels after the treatment initiation was unconfirmed.

Additionally, it was previously shown that the CT 3435 genotype positively influences the serum nelfinavir concentrations in children (21), however study by Hass et al., have not confirmed these results (22).

One of the key atazanvir adverse effects, negatively influencing the patient quality of life is unconjugated hiperbilirubinemia. While being usually clinically insignificant and transient, it might be the factor limiting patient adherence to the treatment and individual treatment acceptability (14, 23, 24). In our study the association between increased bilirubin levels and ATV treatment initiation was presented, with significant statistical difference ($p < 0.01$).

Additionally it must be noted, that to date the reports analysing frequency of 3435 C > T polymorphisms among Poles are infrequent, with mean frequency reported by Kurzawski et al., being 0,525 for the T and 0,474 for C allele (25). These allelic frequencies are similar to the ones reported in our study.

Figure 3. Association between changes in bilirubin levels after four month of atazanavir treatment and *ABCB1* genotypes. Axis y depicts the serum bilirubin concentrations (mg/dL). Axis x: 1-TT genotype, 2-TC genotype, 3-CC genotype



Conclusions

Serum total bilirubin significantly increase after initiation of the ARV treatment. In the investigated group no difference of bilirubin levels after treatment initiation in association with ABCB1 3435 genotypes was found.

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title

Who do Polish patients inform about their HIV infection?

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summary

Background: The study's objective was to obtain information about whom – apart from their sexual partner – Polish patients inform about their HIV infections and what are the consequences.
Material and methods: The questionnaire survey included people living with HIV/AIDS (PLWHA), who were asked about the changes in their lives which resulted from HIV infection and about whom they informed about the infection.
Results: The most often PLWAs informed their parents about the infection, either one of them (mother more frequently than father) or both (75.3%). The fewest patients informed their workmates about the infection (4.7%), and 6.8% told nobody. 75 persons (23.4%) replied that their family and friends were informed by "someone else" – doctors the most often (43 cases – 57.3%), employees of drug addicts rehabilitation centres (14 cases – 18.7%), in 13 cases (17.3%) members of family and in 5 (6.7%) – court or police.
Consequences of disclosing the result of a HIV test: refusal to admit to a secondary school (in 4 cases – 1.2%), forced resignation of school (in 1 case – 0.3%), deterioration of relationships with parents (in 12 cases – 3.7%), with siblings (in 25 cases – 7.8%), with friends (in 30 cases – 9.3%). Five respondents (1.6%) replied that the result of a HIV test performed in a medical care institution in a small town was known to all the inhabitants.
Conclusion: In Poland still information about HIV infection is delivered by the medical care staff not only to the persons concerned but also to their family members, workmates and other persons and the fact has sometimes negative effect on the PLWA's further life.

key words

HIV, serostatus, disclosure

address

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Background

In Poland, the HIV/AIDS epidemic began later than in Western Europe and the USA. The first cases of HIV infection were reported in 1985 and referred to 6 haemophilic patients, 4 homosexuals and one female prostitute (1). The first case of AIDS identified one year later was a man who caught the infection during his long stay in the USA; he died shortly after coming back to Poland (2). The first cases of HIV infection among injection drug users (IDU) were diagnosed in Poland in 1988. Epidemic among the IDUs was spreading fast until the mid-1990s when they constituted 70% of infections and then there was a marked increase of the number of persons infected through sexual contacts. Still, the State Department of Hygiene at its website reports the epidemiological data as follows: since implementation of research studies in 1985 till March 2008, HIV infection was diagnosed in 11,431 Polish citizens, including at least 5,444 (48.1%) infected through injection drug use. This contributes, probably inadvertently, to supporting the common opinion that HIV/AIDS is a problem for persons whose lifestyle is not acceptable for the society, i.e. IDUs and men who have sex with men (MSM).

Due to the later onset of the HIV/AIDS epidemic, Poland and Polish medical staff missed the discussion about treating HIV/AIDS differently than other infectious diseases, a trend called "HIV/AIDS exceptionalism". In the developed countries exceptional status of HIV/AIDS has enhanced communication between doctors and patients and has made medicine less formal, autonomy has been strengthened and patients have become more involved in decisions about their own care, individuality has been more readily acknowledged, respect for informed consent and confidentiality has increased, and patent advocacy has emerged as a force for change (3). In Poland, people living with HIV/AIDS (PLWHA) often encounter unfriendliness, sometimes other doctors than anti-retroviral therapy specialists refuse to help them (4, 5).

PLWHA's possibility to reveal their serological status is important for several reasons. At the individual level, it can improve self-esteem and boost morale, decrease isolation and depression, and improve health through access to better information about care and prevention. Within organisations, the participation of PLWHA can change perceptions, as well as provide valuable experiences and knowledge. At the community and social levels, public involvement of PLWHA can break down fears and prejudices by showing the faces of PLWHA and demonstrating that they are productive members of, and contributors to, society (6).

In Poland, so far no research has been made on HIV-positive persons' disclosing their serological status to other persons than their sexual partner, e.g. family members, acquaintances, workmates or on potential consequences of such disclosure.

Material and methods

In order to compare the life of HIV positive patients before and after they learnt they were infected, we designed a questionnaire in consultation with sociologists, psychologists, doctors concerned with anti-HIV therapy and HIV-infected patients themselves. Among other questions in this questionnaire, patients were asked about who – apart from their sexual partner – knows about their HIV infection and about consequences of the disclosure. The questionnaire forms were distributed among HIV-infected patients during their meetings (at the Polish National Meeting for People Living with HIV and at the "Salon of Acceptance" in Warsaw, a monthly meeting of those who have recently learnt that they are HIV positive), or were sent to patients of the HIV/AIDS reference centres in (alphabetic order) Białystok, Bydgoszcz, Chorzów, Kraków, Łódź, Poznań, Szczecin, Warszawa (Medical University Department of Infectious Diseases, Provincial Hospital of Infectious Diseases) and in Wrocław. The questionnaires were not discussed with patients. The respondents were asked to complete them at home and send them back in enclosed envelopes to the person conducting the research. The questionnaires were distributed in the period of June 2004 - May 2005.

Statistical analysis was performed using SPSS 11.5 PL program.

The study protocol was approved by the Ethical Committee at the Medical University of Białystok.

Results

The response was obtained from 321 subjects out of 500 questionnaires distributed (64.2%). Demographic characteristics of the patients have been presented in Table 1.

Responses to the question who knows about the patient's HIV infection have been presented in Table 2. The most often the information was given to the parents: either one of them (mother more frequently than father) or both. This option was selected by 75.3% of the patients. The fewest patients informed their workmates about the infection (4.7%), and 6.8% told nobody.

The researchers asked the patients, whether they delivered the information to their family and acquaintances themselves. The answer "someone else" was selected by 75 persons (23.4%). In most cases the information about the patient's HIV infection was given to family members, workmates or other medical staff by doctors (43 cases – 57.3%), in 14 cases (18.7%) by employees of drug addicts rehabilitation centres, in 13 cases (17.3%) by members of family and in 5 (6.7%) – by court or police.

Among the consequences of disclosing HIV infection there were refusal to admit to a secondary school (in 4 cases – 1.2%), forced resignation of a school (in 1 case – 0.3%, lack of a separate toilet served as grounds for the decision), deterioration of relationships with parents (in 12 cases – 3.7%), with siblings (in 25 cases – 7.8%), with friends (in 30 cases – 9.3%). Five respondents (1.6%) replied that the result of a HIV test performed in a medical care institution in a small town was known to all the inhabitants.

Discussion

Disclosure of their serological status to other people, including their sexual partners is not easy for PLWHA. In a study held in Los Angeles in the era of the combined antiretroviral therapy (cART) it was revealed that within the last 12 months only 5.5% of sexual partners were informed about the infection risk by their HIV-positive partners (7).

Table 1. Demographic characteristics of patients (n = 321)

Gender	women: 117 (36.4%)
	men: 201 (62.6%)
	no data: 2 (0.9%)
Age at the time of questionnaire completion:	mean 35.29 ±8.0 years (min – 21 years, max – 65 years)
Age at the time of HIV diagnosis:	mean 27.8 ±7.9 years (min – 15 years, max – 64 years)
Possible way of infection*:	
injecting narcotic drugs:	190 (68.9%)
women:	80 (68.4% out of 117 examined women)
men:	110 (54.7% out of 201 examined men)
heterosexual contacts:	88 (37.8%)
– women:	47 (40.2% out of 117 examined women)
– men:	41 (20.4% out of 201 examined men)
homosexual contacts:	61 (23.5%), (29.9% out of 201 examined men)
blood transfusion:	2 (0.6%)
others:	5 (1.6%)**
unknown:	3 (0.9%)

* sometimes patients admitted more than one way of contracting infection, with injection drugs and heterosexual contacts being most common.
** tattoos made using unsterile equipment (2 men), social contacts with HIV-infected flatmates (2 men), sharing a shaver with an infected person (1 woman).

Table 2. Whom except for their sexual partner, do Polish patients tell about their HIV infection?

Who, except for the sexual partner, knows about your HIV infection?*	n (%)
Parents – both or one of them	177 (75.3)
Friends	142 (60.4)
Siblings	129 (54.9)
Other doctors than those who treat the HIV infection	64 (27.2)
Support group	42 (17.9)
Other HIV-positive people	42 (17.9)
Workmates	11 (4.7)
Nobody	16 (6.8)

* patients were allowed select more than one answer

At the beginning of the HIV/AIDS epidemic it was believed that the serological status gets disclosed as the infection progresses to AIDS, when the disease can hardly be kept as a secret. Now when in developed countries cART is available, competing consequence theory (8) has been developed. According to this theory, persons with HIV are likely to reveal the fact of infection to significant others and sexual partners once the rewards for disclosing outweigh the associated costs (9). However, none of these theories explains all issues related to revealing one's serological status to one's family and/or friends.

As the HIV/AIDS epidemic lasts, as the society knows more and more about the infection route and the prognoses for HIV-positive patients improves, the attitude to PLWA changes. Serovich et al. (10) found that out of 76 HIV-positive MSMs only 4.2% of the respondents declared that they regretted disclosing their serological status to their family, friends or sexual partners. No differences were found between the situations when the HIV infection was revealed by the person concerned or by someone else. The authors suggest that this may result from the fact that PLWA reveal their serological status only to those persons, who they expect to react positive or at least neutrally and that positive impact of the disclosure may grow in time and eradicate any initial consequences experienced. Studies on HIV-positive women showed that fear of stigma from family and friends significantly impacts decision about disclosure (11). In a different study on women, Serowich et al. (12) found that women were the most likely to disclose their HIV status within the first seven years after diagnosis, and mothers and sisters were the most likely to be told. Rates of disclosure were not significantly impacted by indicators of disease progression, frequency of contact, physical proximity or relationship satisfaction.

A possibility to be frank with their families and acquaintances is very important for the perfect adherence related to taking anti-retroviral drugs. Being seen with pills risks disclosure of a stigmatized status – being HIV-positive. Ware & al. (13) suggest that rather than take that risk, people choose to compromise their adherence. When health and social interests are experienced as being in conflict, social interests may well take precedence.

Majority of our respondents informed their friends and family about their serological status by themselves. Only 23.4% of them indicated someone else as the person who delivered this information. In most cases these were doctors (43 cases – 57.3%), employees of injection drug users rehabilitation centres in 14 cases (18.7%), members of family in 13 cases (17.3%) and in 5 (6.7%) – court or police. Analogically to the other researchers' studies, PLWHA were rarely affected by any negative consequences of disclosure of their serological status.

Our study was somewhat limited. The respondents were patients who participate in PLWHA meetings and are under regular care of HIV-therapy specialists, therefore persons who have managed to accept their infection. This is why it is impossible to generalise the obtained results on the entire population of PLWHA in Poland.

Conclusions

Against the fears that disclosing their serological status to other people may have negative consequences for PLWHA, only 6.8% of the respondents decided not to reveal the infection to anyone among the family or friends. However, in Poland still information about HIV infection is delivered by the medical care staff not only to the persons concerned but also to their family members, workmates and other persons and the fact has sometimes negative effect on the PLWA's further life.

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title

Risk factors of liver dysfunction among HIV-infected intravenous drug user's

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summary

The definition of hepatotoxicity of antiretroviral therapy (ART) in the current literature is heterogenous and its pathogenesis multifactorial. All the etiologic factors have still not been recognized, therefore a study trial has been made to evaluate hepatic toxicity in a group of drug - addicts while taking antiretroviral therapy. A group of 388 HIV-infected patients has been analyzed. For the past two years (2007, 2008) these patients were hospitalized in a drug-addict ward in Infectious Diseases Hospital in Warsaw. In 110 subjects (32.5%) liver damage was confirmed, among them: 96 patients (87.2%) had positive anti HCV antibodies, 1 patient had positive markers of HBV infection, 10 patients (9.09%) presented HBV and HCV co-infection, and 3 individuals (2.7%) had elevated transaminases without viral markers. Half of the 110 patients mentioned above, with liver disease were taking ART. Only in one case increase in aminotransferase was caused probably by direct toxic effect of antiretroviral medications.

key words

HIV infection, drug addicts, side effects, antiretroviral drugs, liver failure

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Introduction

In drug-addicts using intravenous psychoactive substances, we can observe multifactoral etiology of liver damage. Defining particular factor responsible for decompensation of liver efficiency is very challenging. Nevertheless, patients with HIV infection and co-infection of HCV and/or HBV, as well as those using heroin, amphetamine, benzodiazepines, cocaine, and illegal substances have much higher risk of hepatic toxicity (1, 2, 10). Cytotoxic liver damage may also be caused by alcohol abuse togheter or alternatingly with stimulant substances. Necrosis of hepatocytes and chronic liver infection leads to fibrosis and finally to cirrhosis of alcoholic liver. Main function in pathogenesis of hepatic toxicity is attributed to medicines used in prophylaxis and treatment of opportunistic infections (isoniazid, rifampcin, fluconazole, ketoconazole, amoxicillin with clavulonic acid, sulfonamides).

Multifactorial character of liver damage in patients hospitalized in 3 Ward in Hospital for Infectious Diseases, inclined us to analyze disorders of liver function.

Aim

The aim of our presentation was to evaluate causes of liver dysfunction among intravenous drug user's.

The second aim was case report, concerning hepatotoxicity in HIV-infected patient with antiretroviral treatment and with alcohol abuse.

Methods and Materials

In a retrospective research, we analyzed a group of 388 patients, hospitalized in a drug abusers ward in a Hospital for Infectious Diseases in Warsaw in past two years (2007, 2008). Strong addiction to psychoactive substances, opiates and heroin, amphetamine and benzodiazepines – was a common trait in these patients. Analyses included earlier detected HBV and HCV infection as well as alcohol and other hepatotoxic substances. Presentation is descriptive in nature and aims at examining the phenomenon of hepatotoxicity in drug addicts while taking antiretroviral therapy. There are no statistics included due to lack of sufficient numbers in trial groups.

Results

Among all the 388 hospitalized patients liver damage was diagnosed in 110 (32.5%). In that group 96 patients (87.2%) presented markers of HCV infection confirmed with anti HCV antibody tests. In 10 patients (9.09%) ascertained co-infection of HBV and HCV. Three patients (2.7%) demonstrated toxic liver damage without markers of HCV/HBV. Clinical and laboratory findings of cirrhotic liver were present in 11 patients (10%). One case revealed HBsAg and HBeAg positive. Half of the hospitalized 110 patients were taking antiretroviral therapy.

Exacerbation of hepatitis and / or symptoms of decompensated liver function, were causes for admission in 8 patients (14.2%) out of the 56 mentioned above. Alcohol abuse was the reason for liver failure in 5 patients (62.5%). Disseminated neoplastic process – B-cell lymphoma, after 4 courses of chemotherapy was the cause of liver failure in a patient with secondary cortex of adrenal gland failure and HBV / HCV co-infection. One case presented prominent decompensation of cirrhotic liver.

Only in one patient increase in transaminases activity was credibly related to possible together – toxic effect of antiretroviral therapy and of alcohol toxicity.

Case Report

40 years old male, with HIV infection confirmed 4 years earlier, with strong addiction to psychoactive substances, currently staying on substitutional methadone therapy (100 mg/24 h), was admitted to hospital due to increased activity of transaminases.

Patient has been addicted to psychoactive substances since he was 12 years old. At the beginning he used to sniff various glues and illegal substances. At the age of 17 years, he started using heroin, and unknown home-made opiates "kompot". Patient has been 7 times detoxified, but since three years, participates in a substitutional methadone therapy. Patient currently consumes few bottles of beer every day.

On the basis of positive anti-HCV and PCR HCV RNA methods, chronic hepatitis C was diagnosed. Patient never received Interferon with Ribavirin. Laboratory tests additionally confirmed past infection with HBV (HBs antigen negative, anti-HBc total antibody positive).

Patient has been receiving antiretroviral therapy for 68 weeks months: retrovir plus 3TC (Combivir) 2× 150/300 mg, nevirapine (Viramune) 2× 200mg. Before introduction of antiretroviral therapy, the level of HIV-1 viremia was 72 300 copies/ml, CD4 count was 295 and CD8 – 1301 cells/µl. After 40 weeks of therapy PCR HIV-1 viremia was undetectable, CD4 was 472 and CD8 was 1270 cells/µl. During 64 weeks of antiretroviral therapy the level of transaminases did not exceed its double norm values. Only in the 65th week, significant increase in liver enzymes erupted.

At the admission to the hospital, patient complained of pain in the right upper quadrant of the abdomen and gastric discomfort. On the physical examination, abdomen was soft with slightly painful upper right sub costal area, liver without signs of enlargement. In ultrasonographic examination of abdominal cavity we observed slightly enlarged liver, with irregular echogenicity, but without any focal changes. Laboratory tests revealed Alat – 481 U/L, Aspat – 725 U/L, and GGTP – 492 U/L. Levels of bilirubin and alkaline phophatase were normal.

On the second day of hospitalization antiretroviral therapy was discontinued, due to suspecting hepatotoxic effect of nevirapine. New treatment program was introduced: emtricitabine/tenofowir (Truvade) 1× 200/245 mg, saquinavir (Invirase) 2× 1000 mg, ritonavir (Norvir) 2× 100 mg. Within 4 days after nevirapine discontinuation we observed decline in the level of aminotransferases, especially of Aspat (Alat – 402 U/L, Aspat – 157 U/L). The level of GGTP remained high (717 U/L), probably due to toxic effect of alcohol.

Within next few months gradual decrease in the activity of transaminases and GGTP was observed. Five months after discharge from the hospital the results were as follows: Aspat – 117 U/L, Alat – 133 U/L and GGTP – 104 U/L.

Conclusions

From the group of 56 intravenous drug abusers with chronic liver pathology while on ART, only 8 (14.2%) showed clinical and laboratory sigs of liver damage. Alcohol abuse was the cause of changes in liver function in 5 patients (62.5%). Only in one patient increase in aminotransferases activity was a result of using antiretroviral therapy – particularly nevirapine. Important factor of Aspat and GGTP elevation was alcohol in this case.

According to current literature, from the group of nonnucleoside analogue reverse transcriptase inhibitors, nevirapine is most often mentioned drug causing hepatic toxicity (4). Studies reveal that 5% of patients taking nevirapine have improper activity of transaminases (5). Immunocompromised patients have higher risk for hepatic toxicity due to hypersensitivity reactions. In Pharmaceutical Product Characteristics nevirapine is not proposed to male patients with the level of CD4 > 400 cells/μl and in female with level of CD4 > 250 cells/μl. However study performed by Manfredi and Calze undermines the role of immunocompetence as a risk factor for hepatotoxicity (6). Low body mass index (BMI) and presence of HLA-DRB1*0101 are additional risk factor for nevirapine toxicity (3, 8, 9).

The clinical course of presented case report proofs, that discontinuation of potentially hepatotoxic nevirapine influence to rapid improvement in laboratory tests results. Alcohol abuse is the main causative agent (62.5%) of liver dysfunction in drug abusers infected with HIV/AIDS. Unfortunately, even stabilized drug abusers while on sustitutional methadone therapy overuse alcohol, which can be due to low efficacy of such programs.

Careful monitoring should be required in drug abusers with HIV infection who are receiving antiretroviral therapy and thoughtful consideration should be given to all potential causes of liver enzyme elevation.

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title

Disseminated cryptococcosis as primary manifestation of HIV infection – case report

authors

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summary

Skin lesions in generalised cryptococcosis can resemble wide-spread molluscum contagiosum skin infection. They are often the first symptom of disease dissemination. The patient presents at that time a relatively good status for days or even weeks before deteriorating. Generalised cryptococcosis can be the first manifestation of acquired immunodeficiency syndrome. It is important to consider HIV infection in the differential diagnosis and always perform HIV testing in these patients.

key words

Cryptococcosis, HIV infection, AIDS, Molluscum contagiosum

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Background

Cryptococcosis is caused by yeast *Cryptococcus neoformans*, which is found in soil contaminated by droppings of different animals, in particular pigeons. In humans it can present in various clinical manifestations and different severity. It usually results from inhalation of yeast spores. According to the host's immune alteration, the infection remains totally isolated in the respiratory system, mainly the lungs or spreads hematogenously invovling the internal organs, bones, skin and central nervous system. From then on it occurs as generalised cryptococcosis.

Due to weakened immune system in HIV infected patients disseminated cryptococcosis presents commonly, while in an immunocompetent humans it is rather rare (1). Disseminated disease may affect any organ, but meningoencephalitis and meningitis followed by pneumonia happen most frequently (2).

Case Report

A 37 year old Caucasian heterosexual male was diverted from Dermatology Department to Infectious Diseases Unit due to suspected HIV infection, fever and massive, hard, nodo-papular skin lesions of the whole body which were presumed by dermatologists to be spread molluscum contagiosum lesions (Figures 1, 2).

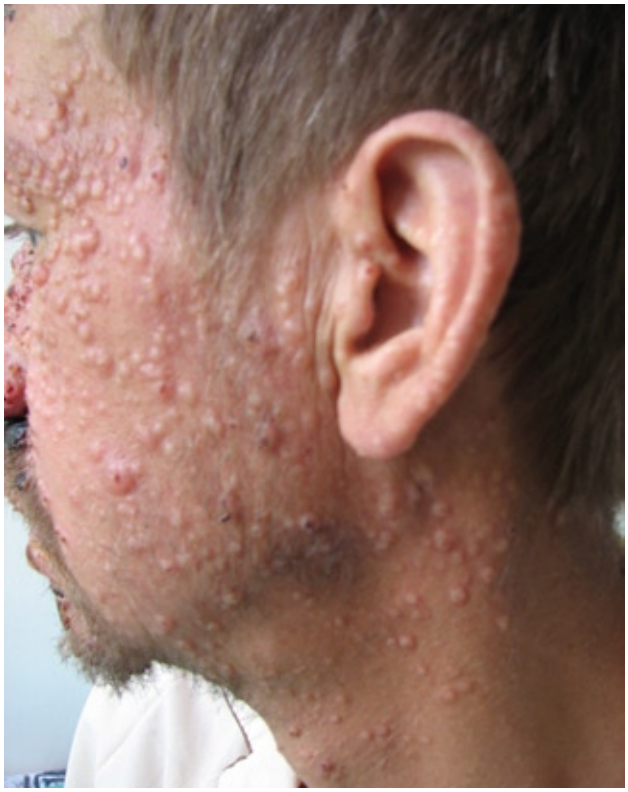

Figure 1. Nodo-papular, "molluscum-like" skin lesions on the face

The HIV infection was presumed upon positive results of the ELISA test performed at the Dermatology Department just before the patient's arrival at Infectious Diseases Unit.

On physical examination at the admission: patient was febrile and fatigued, with oral candidiasis, nodo-papular skin lesions of the whole body, most concentrated on the skin of face, thorax, abdomen and distant part of the extremities, in some places covered by crusts, peripheral lymphadenopathy (the size of lymph nodes up to 2,5 cm, hard, not tender to palpation), hepatosplenomegaly and a mild neck stiffness (without other meningeal signs). The remainder of the physical exam was unremarkable.

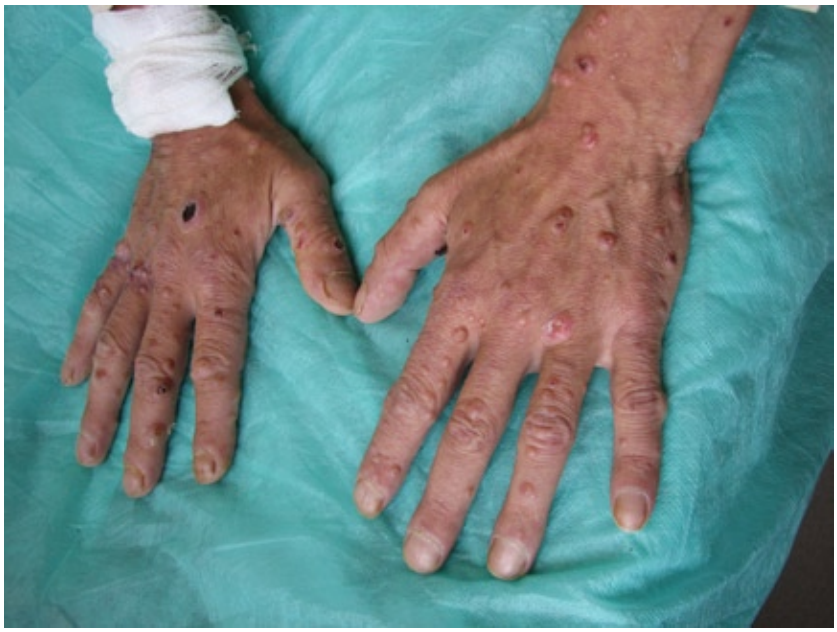

Figure 2. Nodo-papular, "molluscum-like" skin lesions on the hands

Lab tests revealed: normal white blood cell count, normal platelets count, moderate anaemia, highly elevated GGTP and ALP with normal AST, ALT and bilirubin levels; normal renal function and electrolyte levels; elevated LDH and lowered serum albumin levels; mildly prolonged prothrombin time. Blood cultures were drawn.

No alterations on fundoscopic exam were detected.
A lumbar puncture was performed, which revealed elevated cerebrospinal fluid (CSF) opening pressure. On examination: CSF clear and transparent, with 2 lymphocytes/mm^3, protein of 0,70 g/l and glucose of 1,66 mmol/l.

In the further investigation the HIV infection was confirmed with Westren-Blot test, the CD4 count was 463 cells/mm^3, CD4/CD8 ratio – 0,12, and serum HIV RNA – 300 000 copies/ml.

Cryptococcus antigen in CSF and patient's blood was detected. Blood cultures revealed no bacteria growth but *Cryptococcus neoformans*. Histopathological exam of skin lesion suggested a microbial (fungal) infection.

On a chest X-ray a bilateral, interstitial shadowing (pneumonia) was confirmed.

Ultrasound exam of the abdomen reveled a hepato- and splenomegaly; no focal hepatic lesions were found. At the upper external part of the spleen a hypoechogenic space was discovered with a few enlarged lymph nodes of the epigastrum (up to size of 1,7 cm). Gastroduodenscopy revealed no remarkable alterations. For the first 48 hours a treatment with fluconazole was initiated, followed by am-

photericin B. Primary prophylaxis of pneumocystis pneumonia (PCP) and toxoplasmosis were initiated (cotrimoxazole – TMX/SMX). The patient's condition began to improve: no fever, no headache and the slow regression of the skin lesions were observed. After 2 weeks of treatment a rough worsening of the condition occured, with fever over 39°C, dyspnoe and hypoxia. PCP was suspected and the treatment with intravenous TMP/SMX and steroids was introduced. Despite this treatment the patient deteriorated further and was transfered to Intensive Care Unit, where a use of mechanical ventilation was necessary. The treatment with TMP/SMX and antifungal therapy was continued and a wide-spectrum antimicrobial therapy was introduced. In the meanwhile the PCP was confirmed by the presence of *Pneumocystis jiroveci* DNA in sputum and the blood cultures showed metycyllin resistant *Staphylococcus aureus* (MRSA). According to the susceptibility of the MRSA vancomycin was introduced. Despite treatment the patient's condition deteriorated. The syndromes of multi-organ failure occured. On the 24th day of treatment the patient died after unsuccessful resuscitation.

An autopsy was performed which revealed as the primary cause of death the pulmonary artery embolism. Furthermore the multi-organ cryptococcosis was confirmed (skin, liver, spleen, lungs, CNS, pancreas, kidneys, suprarenal glands, lymph nodes). Figures 3-5 show different organ manifestation of cryptococcosis in a post mortem examination.

Discussion

Cryptococcus neoformans is an encapsulated yeast, its capsule is antiphagocytic and plays a significant role of an anti-defence mechanism. Cryptococcosis can develop in individuals with HIV compromised immune system as well as in those immune-suppressed after organ transplantation or anti-cancer chemotherapy or corticosteroid therapy (3). Its prevalence among AIDS patients in Poland is about 3-4% (4). It is a life-threatening infection.

The range and the spectrum of disease differs in HIV-positive patients and in those immunosupressed from other causes (5). Dissemination of this particular infection is very common. It can go unnoticed until it is whole body spread, although sometimes pneumonia-like symptoms may develop. Disseminating manifests with other symptoms, i.e. meningitis, skin lesions; it affects internal organs such as liver, spleen and bone marrow. Skin disorders in AIDS patients may be the initial manifestation of HIV-infection or indicate progression of the disease process (6). In case of cryptococcosis, cutaneous manifestations are rare and usually appear in patiens with advanced immunosuppression. They most commonly appear as papules or nodules that resemble *molluscum contagiosum* (7, 8). The differential diagnosis of papular lesions includes molluscum and fungal infection, such as cryptococcosis, histoplasmosis, coccidioidomycosis and warts, epitheliomas or

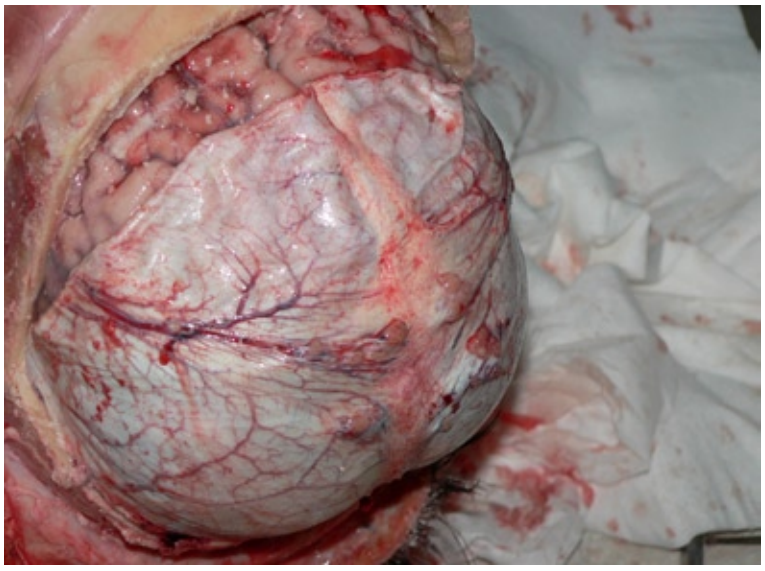

Figure 3. Meningeal cryptococcosis

Figure 4. Splenomegaly with cryptococcal foci

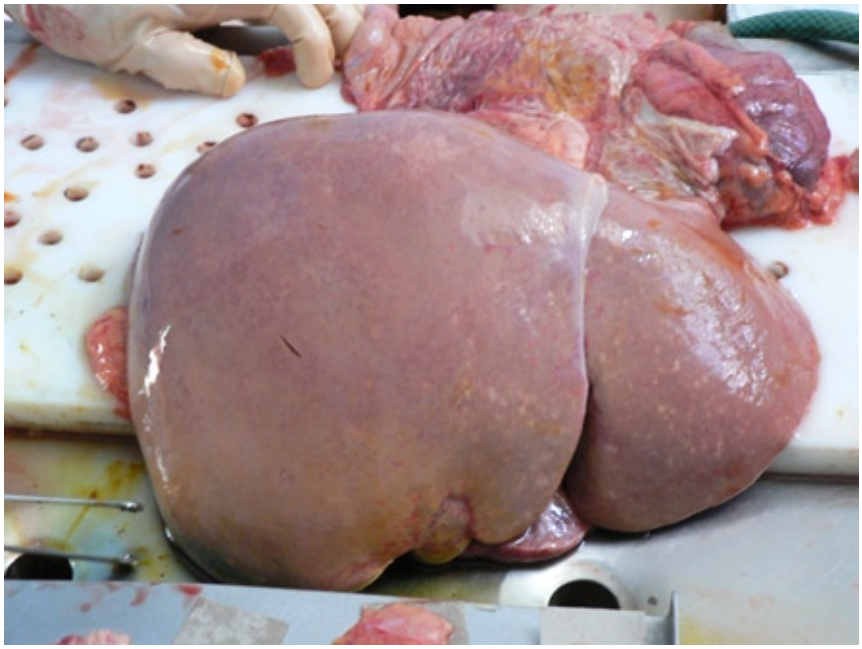

Figure 5.
Hepatosplenomegaly with cryptococcal foci

Kaposi's sarcoma (9). It is important to biopsy skin papular lesions in HIV-infected patients in order to confirm the diagnosis of cryptococcosis and then search for systemical involvement. In the case described above the CNS involvement followed the presence of skin lesions first.

Generalised cryptococcosis results in high mortality 15-20%, despite of introduction of the right treatment. The treatment of choice in case of HIV-infected individuals is amphotericin B or liposomal amphotericin B with flucytosine for two weeks and then consolidation therapy with fluconazole for ten weeks or until CSF culture is sterile, with fluconazol suppression therapy afterwards (10). Data show that chance of achieving a cure after the "induction" treatment course are low in HIV-infected patients with cryptococcal meningitis (30% or less). It differs a lot in comparison to other immunosuppressed patients. This is why long-term maintenance therapy is mandatory. The superiority of combination therapy (amphotericin B with flucytosine) to amphotericin monotherapy in immunocompromised patients has been proven (11, 12). In the case described above amphotericin B monotherapy was administered because flucytosine was temporarily unavailable.

On the basis of observations made in presented case, confirmed by others (13), cutaneous lesions of disseminated cryptococcosis can be the first clinical evidence of acquired immunodeficiency syndrome even with relatively high CD4 count.

Acknowledgement

To Dr Z. Kamiński, PhD, from the Department of Pathology at Hospital for Infectious Diseases in Warsaw for the results of autopsy.

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