Male reproduction and HIV-1 infection

van Leeuwen, E.

Publication date
2009
Document Version
Final published version

Introduction and outline of the thesis

1

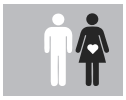
Introduction

In 1981, the Centers for Disease Control (CDC) reported about five men in Los Angeles with an idiopathic *Pneumocystis carinii* pneumonia, which in adults is a rare life-threatening opportunistic infection [1]. In the same year a report was published about an aggressive form of Kaposi's sarcoma amongst young gay men in New York [2]. In retrospect, these two reports mark the beginning of the acquired immunodeficiency syndrome (AIDS) epidemic. Initially, the disease was called 'gay-related immune deficiency (GRID) [3,4]. When it became clear that homosexuals were not exclusively affected, the name of the syndrome was changed into 'AIDS' in 1982 [5-7]. One year later, it became clear that AIDS was caused by an unknown sexually transmitted infectious agent [8,9].

A retrovirus that possibly caused AIDS was discovered in 1983 by Luc Montagnier of the Pasteur Institute in France, and was named 'the lymphadenopathy associated virus (LAV)' [10]. In 1984, 'the human T-cell lymphotropic virus type-3 (HTLV-III)' was isolated by Robert Gallo of the National Cancer Institute in the United States [11]. Both viruses were later shown to be identical and the virus was renamed 'human immunodeficiency virus type-1 (HIV-1) [12].

From its initial presentation in the early nineteen eighties until 1996, HIV-1 infection almost inevitably led to AIDS, which was a death sentence. Life expectancy after the diagnosis was on average only ten months in 1987, and 20 months in 1990 after the introduction of zidovudine [13]. Because of such a short life expectancy, patients were advised to delay pregnancy [14]. Couples with one HIV-1-infected partner, i.e. HIV-1-discordant couples, had a high risk of infecting the uninfected partner, i.e. horizontal transmission, and HIV-1-infected women had a high risk of infecting their child, i.e. vertical transmission [14,15]. As a consequence, these couples were advised to always use condoms, irrespective of other contraceptives. If women nonetheless did become pregnant, they were advised to undergo first-trimester abortion [16].

To reduce the risk of horizontal HIV-1 transmission, in the era before data on HIV-1 in semen or spermatozoa were available, the Italian gynaecologist Semprini started in 1990 with intra uterine inseminations (IUI) of HIV negative women with semen from their HIV-1-infected partners [17]. In the hope to select HIV-1 free motile spermatozoa, Semprini processed semen of HIV-1-infected men by combining density-gradient centrifugation with swim-up of spermatozoa. After HIV testing, the final sperm fraction was used for intra uterine insemination (IUI). Unfortunately, the sperm-yield after this type of semen processing was low, and therefore only men with good semen qualities qualified for treatment.



For more than 10 years, Semprini remained the only clinician providing fertility care for HIV couples and he received a lot of criticism from his colleagues [18]. Arguments against IUI with processed sperm during that era were: (1) the short life expectancy (2) the immunofluorescence test with monoclonal antibodies against HIV p17 that was used to detect any residual HIV in processed semen before insemination had very low sensitivity and therefore a high chance of a false-negative result, and (3) the CDC reported a case of HIV-1 transmission after IUI with processed semen in 1990, although in this specific case the semen was not tested for HIV before insemination [19].

In 1996, the introduction of highly active antiretroviral therapy (HAART) led to a spectacular increase in life expectancy, AIDS-free survival, and quality of life of HIV-1-infected men and women with access to this therapy [20]. The changes in the AIDS epidemic caused by HAART led to the publication of numerous ethical debates in authoritative journals with a plea to reconsider the ban on reproduction for HIV-1-infected couples [21-23]. The first argument to offer HIV-1-infected couples artificial reproductive technologies was "harm minimisation". IUI with processed semen dramatically decreased the chance of horizontal HIV-1 transmission, as seroconversions of the treated women or their offspring after IUI with HIV negative sperm had never been described [24]. Withholding these techniques could lead patients to practice unprotected intercourse with an unknown but certainly higher risk of HIV-1 infection. Second, in 1998 the US Supreme Court declared that an asymptomatic HIV-1 infection should be considered a handicap falling under the protection of the Americans with Disabilities Act (ADA) [25]. As discrimination of people with any handicap under the ADA is unlawful, it was felt that the categorical exclusion of people with a HIV infection from assisted reproductive technology programs was also unlawful [26]. The third moral argument was that medical interventions should not be discriminatory. Couples with HIV infection were not essentially different from couples with other chronic diseases or couples with an increased chance of having offspring with anomalies. Assisted reproductive technologies were already offered to couples with for example diabetes, and to women in their forties who have an increased chance of having a child with Down's syndrome. The final argument was that a doctor had to respect a patient's autonomy when the risks seemed acceptable, even if the patient's values, preferences and decisions conflicted with those of his own.

It were these debates that gradually changed the initial unwillingness to accept HIV-1-infected couples into assisted reproductive technologies programs. In addition, far more sensitive polymerase chain reaction-based methods to detect the presence of HIV-1 not only in blood

but also in other body fluids including semen had become available since 1996 [27]. Following this mindshift, Semprini's method has been copied and refined by many others [28,29].

While IUI and other reproductive technologies like in vitro fertilization (IVF) and intra cytoplasmatic sperm injection (ICSI) for HIV-1-infected patients were carefully being introduced in clinical practice, there was an important change in HIV-1 treatment policy caused by concerns for long-term side effects of HAART. These concerns resulted in 2002 in new guidelines for the treatment of HIV, which advised to postpone antiretroviral therapy until CD4 levels around 200 cells/mm^3 were reached [30].

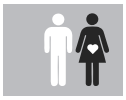
Background of the research described in this thesis

When we started the studies described in this thesis, possible implications of delaying HAART treatment in HIV-1-infected patients, thus lengthening exposure to untreated infection, on semen qualities were unknown. Longitudinal data concerning any potentially detrimental effects of HAART on semen quality were also very limited [31]. These data were urgently needed, because a negative effect of either HIV-1 infection or HAART on semen quality would exclude many men from this technique, as the sperm-yield after semen processing is low [17,32]. An important goal of our research therefore was to evaluate the effects of both the natural course of HIV-1 infection and of HAART on semen quality in HIV-1-infected men.

Some of the long-term side effects of HAART, including, but not limited to, lipid abnormalities, insulin resistance, premature atherosclerosis, neuropathy and lipodystrophy may at least partially be mediated by mitochondrial toxicity [33-37]. Mitochondrial toxicity could theoretically also affect spermatozoa, thereby leading to their functional impairment, i.e. reducing their motility and fertilization potential. We therefore also set out to try and explore possible mitochondrial toxicity of HAART against spermatozoa.

In order to exhibit toxic effects on spermatozoa, antiretroviral agents have to penetrate into the male genital tract [38]. Data on penetration in seminal plasma were not available for all antiretroviral drugs, particularly the more recently introduced ones. Therefore, we evaluated drug concentrations of didanosine alone or when combined with tenofovir in seminal plasma and concentrations of atazanavir in seminal plasma.

It has been suggested that the male genital tract is a sanctuary site for HIV-1, which is defined an anatomical site that is highly impermeable to (some) antiretroviral drugs, and in which



viral replication may continue during systemic treatment, thus allowing the local selection of drug-resistant strains [38]. Absence of adequate local drug concentrations and the presence of cells that are susceptible to HIV-1 are two prerequisites for defining a viral sanctuary site. As no data existed on HIV-1 susceptible cells in the male genital tract, we evaluated the presence of HIV-1 susceptible cells in semen.

Finally, we aimed to evaluate the effectiveness of our clinical practice of IUI with processed semen of HIV-1-infected men in the Netherlands, and tried to investigate which risks the couples undergoing IUI were willing to accept in order to conceive, and to explore anxiety for HIV-1 transmission amongst these couples.

Outline of the thesis

In CHAPTER 2 we give an overview of the presence of HIV-1 in the male and female genital tract and the effect of HIV-1 and HAART on male and female fertility. We report available data concerning artificial reproductive technologies for HIV-1 positive men and women and introduce an algorithm for the reproductive treatment of couples of which both partners are infected with HIV-1.

In CHAPTER 3 we report the unique case of a semen donor who became HIV-1 infected and describe this man's semen quality before acquiring HIV-1 infection, during the stage of primary HIV-1 infection, and finally during chronic stage of infection.

In CHAPTER 4 we describe the course of semen quality during 96 weeks of observation of untreated HIV-1 infection. A cohort of 55 men with variable prior duration of HIV-1 infection, but without previous or current antiretroviral therapy, underwent biannual blood and semen analyses. We examined the changes in semen parameters over time using a repeated measurements mixed-effects model.

In CHAPTER 5 we describe the course of semen quality during 48 weeks of highly active antiretroviral therapy (HAART). We hypothesized that HAART may result in lower semen quality, possibly because of mitochondrial toxic effects of HAART on spermatozoa. Thirty-four men with different estimated duration of HIV-1 infection, who were about to start various combinations of HAART, underwent blood and semen analyses before the start of HAART and 4, 12, 24, 36 and 48 weeks thereafter. The effect of HAART on semen parameters was examined by a repeated measurements procedure using a mixed-effects model.

In CHAPTER 6 the effect of HAART on mitochondrial DNA (mtDNA) content in spermatozoa as a possible marker for mitochondrial dysfunction is described in a subgroup of 10 patients of our HAART-treated cohort described in CHAPTER 5. A quantitative real-time duplex nucleic acid-based amplification assay was used to determine mtDNA content longitudinally before, and 4, 12, 24, 36 and 48 weeks after start of HAART. Using the same method mtDNA content was also measured in spermatozoa from a semen donor with documented seroconverion for HIV-1.

In CHAPTER 7 and CHAPTER 8 we measured concentrations of didanosine (ddI), tenofovir (TFV) and atazanavir (ATV) in seminal plasma and compared these to concentrations in blood plasma.

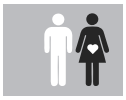
In CHAPTER 9 we quantified the presence of HIV-1 susceptible cells in semen of HIV-1-infected men using HAART, HIV-1-infected therapy-naïve men, and HIV seronegative men. Paired seminal and blood lymphocytes were stained with monoclonal antibodies against CD45, HLA-DR and CD38, and analyzed using flow cytometry. Lymphocytes with CD45, HLA-DR and CD38 expression were considered to be susceptible to HIV-1 infection.

In CHAPTER 10 we describe our first experiences in the Netherlands with IUI for HIV-1 discordant couples of whom the male is HIV-1 infected.

In CHAPTER 11 we evaluated anxiety for HIV-1 transmission and the willingness to undergo assisted reproductive technologies in 50 HIV-1 discordant couples of whom the male is HIV-1-infected. We used the State-Trait Anxiety Inventory to assess anxiety. A hypothetical transmission risk of HIV-1 to the woman was systematically increased from 0.5% to 3%, to assess the willingness to undergo assisted reproductive technologies. For each patient, we sought to obtain the transmission risk at which they would switch from "choose ART" to an "unacceptable HIV-1 transmission risk by ART".

In CHAPTER 12 we provide a general discussion of the results of the studies presented in this thesis, and suggest their implications for future work in HIV-1 and fertility research.

In CHAPTER 13 we give a summary of the data presented in this thesis.



References

(1) Pneumocystis pneumonia--Los Angeles. *MMWR Morb Mortal Wkly Rep.* 1981;30:250-252.

(2) Kaposi's sarcoma and Pneumocystis pneumonia among homosexual men--New York City and California. *MMWR Morb Mortal Wkly Rep.* 1981;30:305-308.

(3) Brennan RO, Durack DT. Gay compromise syndrome. *Lancet.* 1981;2:1338-1339.

(4) Kehr, U. A Name for the Plague. Time Magazine. 1982.

(5) Pneumocystis carinii pneumonia among persons with hemophilia A. *MMWR Morb Mortal Wkly Rep.* 1982;31:365-367.

(6) Opportunistic infections and Kaposi's sarcoma among Haitians in the United States. *MMWR Morb Mortal Wkly Rep.* 1982;31:353-1.

(7) Update on acquired immune deficiency syndrome (AIDS)--United States. *MMWR Morb Mortal Wkly Rep.* 1982;31:507-4.

(8) A cluster of Kaposi's sarcoma and Pneumocystis carinii pneumonia among homosexual male residents of Los Angeles and Orange Counties, California. *MMWR Morb Mortal Wkly Rep.* 1982;31:305-307.

(9) Anderson DJ, Yunis EJ. "Trojan Horse" leukocytes in AIDS. *N Engl J Med.* 1983;309:984-985.

(10) Barre-Sinoussi F, Chermann JC, Rey F, Nugeyre MT, Chamaret S, Gruest J et al. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science.* 1983;220:868-871.

(11) Markham PD, Sarngadharan MG, Salahuddin SZ, Popovic M, Gallo RC. Correlation between exposure to human T-cell leukemia-lymphoma virus-III and the development of AIDS. *Ann N Y Acad Sci.* 1984;437:106-109.

(12) Coffin J, Haase A, Levy JA, Montagnier L, Oroszlan S, Teich N et al. What to call the AIDS virus? *Nature.* 1986;321:10.

(13) Beck EJ. HIV infection and intervention: the first decade. *AIDS Care.* 1991;3:295-302.

(14) Recommendations for assisting in the prevention of perinatal transmission of human T-lymphotropic virus type III/lymphadenopathy-associated virus and acquired immunodeficiency syndrome. *MMWR Morb Mortal Wkly Rep.* 1985;34:721-722.

(15) Scott GB, Buck BE, Leterman JG, Bloom FL, Parks WP. Acquired immunodeficiency syndrome in infants. *N Engl J Med.* 1984;310:76-81.

(16) Henrion R. Pregnancy and AIDS. *Hum Reprod.* 1988;3:257-262.

(17) Semprini AE, Levi-Setti P, Bozzo M, Ravizza M, Taglioretti A, Sulpizio P et al. Insemination of HIV-negative women with processed semen of HIV-positive partners. *Lancet.* 1992;340:1317-1319.

(18) Edlin BR, Holmberg SD. Insemination of HIV-negative women with processed semen of HIV-positive partners. *Lancet.* 1993;341:570-571.

(19) HIV-1 infection and artificial insemination with processed semen. *MMWR Morb Mortal Wkly Rep.* 1990;39:249, 255-256.

(20) Hogg RS, Heath KV, Yip B, Craib KJ, O'Shaughnessy MV, Schechter MT et al. Improved survival among HIV-infected individuals following initiation of antiretroviral therapy. *JAMA.* 1998;279:450-454.

(21) Drapkin LA, Anderson J. Human immunodeficiency virus and assisted reproduction:

reconsidering evidence, reframing ethics. *Fertil Steril.* 2001;75:843-858.

(22) Bendikson KA, Anderson D, Hornstein MD. Fertility options for HIV patients. *Curr Opin Obstet Gynecol.* 2002;14:453-457.

(23) Baker HW, Mijch A, Garland S, Crowe S, Dunne M, Edgar D et al. Use of assisted reproductive technology to reduce the risk of transmission of HIV in discordant couples wishing to have their own children where the male partner is seropositive with an undetectable viral load. *J Med Ethics.* 2003;29:315-320.

(24) Semprini AE, Fiore S, Pardi G. Reproductive counselling for HIV-discordant couples. *Lancet.* 1997;349:1401-1402.

(25) Bragdon v. Abbott. *Wests Supreme Court Report.* 1998;118:2196-2218.

(26) Minkoff H, Santoro N. Ethical considerations in the treatment of infertility in women with human immunodeficiency virus infection. *N Engl J Med.* 2000;342:1748-1750.

(27) FDA approves first viral-load test. Food and Drug Administration. *AIDS Alert.* 1996;11:94.

(28) Bujan L, Hollander L, Coudert M, Gilling-Smith C, Vucetich A, Guibert J et al. Safety and efficacy of sperm washing in HIV-1-serodiscordant couples where the male is infected: results from the European CREAThE network. *AIDS.* 2007;21:1909-1914.

(29) Savasi V, Ferrazzi E, Lanzani C, Oneta M, Parrilla B, Persico T. Safety of sperm washing and ART outcome in 741 HIV-1-serodiscordant couples. *Hum Reprod.* 2007;22:772-777.

(30) Yeni PG, Hammer SM, Carpenter CC, Cooper DA, Fischl MA, Gatell JM et al. Antiretroviral treatment for adult HIV infection in 2002: updated recommendations of the International AIDS Society-USA Panel. *JAMA.* 2002;288:222-235.

(31) Robbins WA, Witt KL, Haseman JK, Dunson DB, Troiani L, Cohen MS et al. Antiretroviral therapy effects on genetic and morphologic end points in lymphocytes and sperm of men with human immunodeficiency virus infection. *J Infect Dis.* 2001;184:127-135.

(32) Nicopoullos JD, Almeida PA, Ramsay JW, Gilling-Smith C. The effect of human immunodeficiency virus on sperm parameters and the outcome of intrauterine insemination following sperm washing. *Hum Reprod.* 2004;19:2289-2297.

(33) Carr A, Samaras K, Burton S, Law M, Freund J, Chisholm DJ et al. A syndrome of peripheral lipodystrophy, hyperlipidaemia and insulin resistance in patients receiving HIV protease inhibitors. *AIDS.* 1998;12:F51-F58.

(34) Brinkman K, Smeitink JA, Romijn JA, Reiss P. Mitochondrial toxicity induced by nucleoside-analogue reverse-transcriptase inhibitors is a key factor in the pathogenesis of antiretroviral-therapy-related lipodystrophy. *Lancet.* 1999;354:1112-1115.

(35) van der Valk M, Bisschop PH, Romijn JA, Ackermans MT, Lange JM, Endert E et al. Lipodystrophy in HIV-1-positive patients is associated with insulin resistance in multiple metabolic pathways. *AIDS.* 2001;15:2093-2100.

(36) Lee H, Hanes J, Johnson KA. Toxicity of nucleoside analogues used to treat AIDS and the selectivity of the mitochondrial DNA polymerase. *Biochemistry.* 2003;42:14711-14719.

(37) Deeks SG. Antiretroviral treatment of HIV infected adults. *BMJ.* 2006;332:1489.

(38) Lowe SH, Sankatsing SU, Repping S, van der Veen F, Reiss P, Lange JM et al. Is the male genital tract really a sanctuary site for HIV? Arguments that it is not. *AIDS.* 2004;18:1353-1362.