Impact of antiretroviral therapy on HIV-1 transmission dynamics

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Citation for published version (APA):

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I

Introduction

Twenty-eight years ago the human immunodeficiency virus (HIV) pandemic took the world by surprise [1]. After the eradication of smallpox and the introduction of childhood vaccines, developed countries thought to have won from infectious diseases. But new infectious diseases emerged besides HIV, Hepatitis C virus, Severe Acute Respiratory Syndrome (SARS) virus, Ebola virus, and new variants of Influenza virus [2]. In protection measures against infectious diseases it is as the Red Queen explains to Alice in Wonderland: “it takes all the running you can do, to keep in the same place” [3]. Virulence management is needed in order to understand the conditions for emergence and spread of infectious diseases [4, 5]. Molecular studies taught us that viruses are as old as life itself, shaped part of our DNA and have been an important drive of evolution [6, 7]. New infectious diseases can emerge from cross-species transmission, but might also hide in isolated persons and spread more easily when their contact networks change. Infectiousness is related to viral load [8], which often is related to virulence [9]. Evolution at the population level might select for more or less virulent strains through the process of inheritable diversity and selection thereon, by selecting the strains that are most successful to spread under certain conditions. Dependent on the contact network of the host the pathogen needs a certain amount of time to transmit in order to survive, resulting in a trade-off between virulence and transmission [10].

Global HIV pandemic

In 1981 the first healthy young gay men in the United States were diagnosed with Immuno Deficiency Syndrome, shortly after it was found to be Acquired (AIDS), the Netherlands followed in 1982 [11-14]. In 1983 infection with a new retrovirus was found to be responsible [15-17], later named Human Immunodeficiency Virus (HIV). Soon it became clear that HIV is transmitted not only via unprotected sexual contact among men having sex with men (MSM) but also via heterosexual intercourse, contaminated blood, and from mother to child. UNAIDS estimated that in 2008 globally 33 million people are infected with HIV, and yet another overall 25 million people have died of HIV-related causes [18].

Based on molecular phylogenetic clustering HIV is classified in two variants HIV-1 and HIV-2, both distinctly related to simian immunodeficiency viruses (SIV) that infect other primates [19, 20]. HIV-1 is subsequently classified in three groups M (main), O (out-group), and N (new), presumed to represent separate cross-species introductions [20]. The main group M is again classified in several subtypes and recombinants thereof [21]. This diversification is likely to have originated early in the epidemic as several subtypes and recombinants are present in the Repub-
lic of Congo, regarded as the epicenter of HIV-1 group M [22]. By retrospective searching the earliest man found to be infected with HIV-1 is from a sample taken in 1959 in Kinshasa [22]. In North America subtype B is believed to have been introduced via Haiti in the seventies, and is now the dominant circulating strain among MSM also in Western Europe [22-25].

**Viral host dynamics and evolution**

The unique strategy of HIV is that it infects CD4+ receptor T helper cells which themselves are involved in antigen recognition and triggering of immune response in defense against infectious agents [26]. Upon infection, HIV uses and thereby destroys CD4 cells for replication of new HIV, leading to CD4 cell depletion causing immune deficiency. By error-prone replication and high replication rates HIV forms a viral quasispecies that continuously changes upon selection as it escapes the host immune system. A dynamic process takes place between HIV and the immune system cells where, without treatment, some people die within months while others can live many years without disease [27]. This is influenced by both genetic variations in co-receptors, cytotoxic T cell (CTL) and human leukocyte antigen (HLA) polymorphisms and age amongst humans, and by inheritable and evolving properties of the transmitted virus [28-32]. These factors are also involved in determining if initial infection takes place. People homozygous for co-receptor CCR5 delta32 are resistant for HIV-1 infection, unless infected directly into the blood with HIV that attaches to co-receptor CXCR4 [33]. Humans also have innate immune defense mechanisms, one of which is the protein APOBEC3. It would cause HIV to hypermutate beyond its error-threshold [34] and thereby unable to maintain sufficient genetic information, if not that HIV evolved a protection by the gene *vif* [35].

**Antiretroviral Therapy**

Monotherapy with AZT, was first available in 1987 [36, 37]. AZT inhibits the reverse transcription of the wildtype HIV RNA genome into viral DNA prior to integration into the host genome. But HIV resistance to AZT emerges within a few weeks by selection of strains with only 1- or 2 specific point mutations already present in the cloud of mutants formed upon infection [38-40]. These resistant strains were found to be capable of being transmitted [37]. A special viral mutant at amino acid position 215 of reverse transcriptase (RT) selected for during AZT mono therapy is two RNA mutations away from wildtype virus and is less fit than wildtype in absence of AZT. After transmission to a person not on antiretroviral therapy this resistant virus reverts to an AZT sensitive variant which is only a single RNA mutation removed from drug-resistant HIV, and which was found to be just as fit as wildtype in absence of therapy [41]. Here antiretroviral therapy helped HIV to pass low fitness gaps after which it can explore new routes in its fitness landscape [42, 43].

From 1996 in most developed countries combination antiretroviral therapy (cART) became available [40], consisting of three types of drugs attacking two processes of viral replication
simultaneously: nucleoside RT inhibitors (like AZT) that are chain terminators incorporated into the growing DNA; non-nucleoside RT inhibitors that bind to RT and stop it from functioning; and protease inhibitors that prevent cleavage of the gag precursor protein, which results in the production of non-infectious particles. cART proved effective, as now several mutations are needed for HIV to become resistant, and these combinations are not a priori there to be selected upon. cART can reduce virus load below the limit of ordinary detection methods of about 50 copies per ml blood. Death rates decreased drastically and cART turned HIV infection from a terminal into a chronic disease [44]. cART also drastically reduced infectiousness as this is shown to be related to the viral load [8].

Once integrated HIV can remain in latent stable viral reservoirs. It is estimated that it might take a life-time of successful treatment to completely eradicate this reservoir that is built up during the first weeks of infection [45-48]. Under full suppression the virus is not able to evolve, but as the therapies do not have similar half-life times and do not all get to every body part in sufficient concentration, low-level replication might continue, and resistant mutations to the combination of therapies might accumulate and recombine. Especially when a patient has poor adherence to the therapy, and when a patient was yet infected with a partial resistant strain or was on mono-therapy previously. The worst case scenario is that a multi-drug-resistant virus evolves that in addition is more virulent and infectious then wildtype both on and of antiretroviral therapy. At present multidrug resistant strains still result in a decreased viral load both with and without therapy and those strains are less efficiently transmitted compared to wild-type in a drug-free environment [49, 50].

Most antiretroviral therapies cause modest to severe toxic side-effects [51], in addition the human genome itself encodes for a related aspartic protease as HIV [6]. An effective vaccine that protects against HIV infection has not been realized and possibly by activating the immune response a recent trial rather did more harm then good [52]. Two new classes of antiretroviral medications for HIV treatment, fusion and integrase inhibitors, have recently been approved for use in patients in whom previous HIV treatment regimens have failed [53]. To the fusion inhibitor T20 emergence of drug-resistant HIV-1 variants have already emerged, including a curious case reported on a drug dependent strain [54]. Novel targets could make use of the intrinsic antiviral defense mechanisms against viral infection [55]. A few cases have been reported of people who were in need for bone marrow transplantation, because of leukemia, and by using stem cells with co-receptors resistant against HIV acquisition (homozygous for co-receptor CCR5 delta32) therewith also lost their HIV infection [56]. But this operation did not work for all patients, it is very risky and very expensive.
Behaviour – The Netherlands
In the Netherlands AIDS was initially predominantly found among gay men and injecting drug users. At present, of the 107,491 patients actively followed in one of the 24 national HIV treatment centers only 3% was infected by injecting drug use, 57% by homosexual, and 32% by heterosexual contact. Of those infected heterosexually 55% is from Sub-Saharan African origin [51]. A wide variety of HIV-1 subtypes (only 2% subtype B) is found among these immigrants reflecting the epidemic in Sub-Saharan Africa. HIV-1 subtype B is found amongst 96% of seropositive men having sex with men (MSM). This separation in subtypes indicates that there are different networks without substantial intermingling between risk-groups. Comprehensive programs including clean needle provision, and a shift in the nineties to non-injecting drug use, limited HIV transmission by injecting drug use [57]. Early in the epidemic MSM decreased their risk behaviour obviously in reaction to the increasing number of acquaintances dying of AIDS [58]. In response to beneficial effects of cART, increased risk behaviour among both positive and negative MSM were observed in several countries, including the Netherlands [59-64]. This was confirmed in an increase of sexually transmitted infections and indications for increasing HIV incidence [59, 64, 65]. The increase in HIV diagnoses was initially interpreted as the effect of improved HIV testing stimulated by the availability of effective treatment. Diagnostic antibody tests were available since 1984, but have a window period of 3 months for HIV infection to be identified. During these first months however HIV viral load and thus infectiousness is highest [8, 66-68]. In response to the knowledge of once HIV positive status MSM were found to reduce their risk-behaviour [69, 70], but when on successful cART a tendency of increased risk-behaviour was noticed, even though the last undetectable viral load test does not always reflect the actual viral load [60].

New approaches to control HIV spread are pre-exposure prophylaxis of high risk HIV-negative MSM [71], and testing for acute primary infection, but also ordinary health care interventions such as earlier treatment and more frequent testing [72].

Impact of cART on transmission dynamics?
Mathematical modelling showed that widespread treatment with cART decreasing viral load and infectiousness has the potential to decrease the annual number of HIV infections, but that an increase in risk behaviour has the potential to counterbalance this beneficial effect [37, 73-80]. However these models did not take into account that the majority of people treated with cART successfully suppress their viral load, and that only for a small group their virus becomes resistant to all available therapies.
The aim of this thesis is to study the impact of cART on the transmission dynamics of HIV in the national well monitored population of MSM infected with HIV-1 subtype B. We used data from the prospective Amsterdam Cohort Studies and the nationwide ATHENA observational cohort. These cohorts allowed for in-depth analysis of the HIV epidemic in the Netherlands in the cART era. For that, we used a combination of molecular epidemiology methods and developed a mathematical model on HIV transmission and cART use. The main objectives were to study not only the proportion, but also the magnitude and the source of new infections with (and without) a resistant strain, and to quantify the impact of cART in intervention on transmission at the population level.

**Amsterdam Cohort Studies**

The ‘prospective Amsterdam Cohort Studies (ACS) on HIV infection and AIDS’ initiated in 1984, followed HIV negative MSM and drug users over time. Participants are seen every 3-6 months to fill in questionnaires on risk behaviour and symptoms, and have blood samples taken for virological and immunological testing. For the participants who seroconverted during follow-up the distribution in time from HIV-1 infection to AIDS and death without the interference of cART was estimated. The ACS are also part of CASCADE, a collaboration between the investigators of 22 cohorts following persons with a well-estimated dates of HIV seroconversion.

**ATHENA**

To monitor HIV and the effect of cART the ATHENA observational cohort was initiated in 1996. The ATHENA cohort includes all patients treated and monitored longitudinally in one of the 24 treatment centers. Patients who died before 1996 are not included, neither are those who object (only 1.8%). Before ATHENA only the annual AIDS diagnoses were monitored by Statistics Netherlands. ATHENA collects anonymous information on patients’ first positive HIV test, and when available the last negative test, CD4 cell count, HIV viral load, and initiation, content, success, failure or toxicity of antiviral therapy [51]. Since resistant strains are transmittable, as a standard of care in most hospitals patients have their HIV polymerase gene sequenced, coding for both protease and RT, to check for the presence of resistance associated mutations to antiretroviral drugs before choosing the therapy combination, and this is often also done when a person fails therapy [51, 81-84].

**Molecular epidemiology (Chapters 2-4 and 7)**

Variation of the HIV polymerase gene is sufficient for reconstruction of HIV transmission networks [85]. As HIV polymerase gene sequences are obtained for many patients and collected in ATHENA, this gave us the unique opportunity to use these sequences to study the percentage
transmission of resistance over calendar time using only sequences from persons with an identified year of infection (Chapters 2 and 7), and to study the transmission networks these strains are in (Chapter 7). We studied the evolution of resistant strains after being transmitted to a person not on antiviral therapy (Chapter 3), and checked if transmission of resistant strains contributes to the failing of therapy in people who were initially successfully treated (Chapter 4). The sequences were also used to study the consistency of the MSM transmission network and in an attempt to estimate the time between infection and onward transmission (Chapter 7).

Mathematical model (Chapters 5 and 6)
A mathematical model was developed as a tool to estimate the number of annual new infections and the changes in risk behaviour and time from infection to diagnosis needed to explain and so to fit the timing and magnitude of the longitudinal data on HIV diagnosis and first AIDS diagnosis, incorporating information on survival distribution, cART initiation and failure. Here-with we could calculate the reproduction number, which summarizes the state of the epidemic in the number a newly infected MSM will on average infect over his life time: when larger then one the epidemic will increase, when smaller then one the epidemic will contract. It was also possible to estimate the number of undiagnosed infections and their impact on transmission, and to perform hypothetical scenarios on how many cases cART has actually prevented since it was used. The mathematical model was also applied to study the impact of: cART use and - efficacy, changes in risk-behaviour, and changes in time from infection to diagnosis.

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