Epidemiological trends of HIV-1 shown through phylogenetic trees
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CHAPTER 6

General discussion
GENERAL DISCUSSION

6.1 The importance of global monitoring of HIV-1 variation

HIV-1 is characterized by extensive genetic variation within individual patients, within infected communities, and on a global scale. Based on phylogenetic analysis, HIV-1 strains are divided into three groups M, N and O, of which group M is subdivided into nine genetic subtypes: A-D, F-H, J and K\(^1\), each with its own geographic distribution. Due to virus exchange between infected populations, the global distribution of HIV-1 subtypes is changing over time. Recombinant forms may arise. Currently, six have officially been identified as circulating recombinant forms (CRF)\(^1\), some with evidence for epidemic spread.\(^2\) The recent identification of the first two recombinants in Thailand including CRF01_AE (AE/B and AE/C) \(^3,4\) demonstrates that CRFs are relevant in the worldwide epidemic, and that the genetic diversity of HIV-1 is increasingly complex. Biological differences between HIV-1 subtypes have been shown in vitro.\(^5-7\) Although in vivo studies focusing on the relationship between transmissibility of infection and clinical progression between HIV-1 subtypes have been inconclusive. The variability of HIV-1 undoubtedly affects diagnostics and vaccine development. For example, some commercially available viral load assays - used both for clinical purposes and epidemiological studies - are less efficient in detecting non-B HIV-1 subtypes.\(^5,9\) If RNA levels, used to measure the effect of anti-retroviral therapy are underestimated, this may adversely influence the clinical management for the patient. As for the development of a widely effective or 'global' vaccine, a vaccine based on one subtype could be ineffective to prevent infections with other subtypes of HIV-1. The influenza vaccine has to be periodically modified and updated because of the genetic variations of the influenza virus. Possibly, the same might need to be done with a future HIV vaccine.

In conclusion, as described in chapter one, the continuously discovered new - and more complex - recombinants (as well as new subtypes or groups)\(^1\) and the implications of HIV-1 variation for diagnostics, treatment, vaccines, and epidemiology, underscores the need for systematic attempts to characterize HIV-1 strains and follow their spread. The extreme variability of HIV-1 makes it possible to conduct transmission studies on the basis of phylogenetic analysis, which appears to be a useful tool in tracing global and local patterns of spread. Currently, UNAIDS is supporting a global network for HIV isolation and characterization to monitor the distribution and emergence of new subtypes. The information collected is being used to monitor the dynamics of subtype distributions globally to facilitate the development and evaluation of vaccines [www.unaids.org].

6.2 Monitoring of HIV-1 subtypes in the Netherlands: the past

In this thesis, we demonstrate that studying the variation of HIV-1 by DNA sequencing and phylogenetic analysis can be used to successfully address a variety of epidemiological issues. Initial molecular studies of HIV-1 in the Netherlands were concentrated on injecting drug users and homosexual men in Amsterdam, and demonstrated that these epidemics are caused by subtype B strains.\(^10,11\) A pilot study
among women from AIDS-endemic areas showed that many other subtypes are present in Amsterdam. However, no systematic data on circulating HIV-1 subtypes and transmission patterns among heterosexually infected individuals in the Netherlands were available nor had long-term studies been undertaken. In chapters two and three, we describe the results from a nationwide surveillance project among heterosexuals in which we studied the circulating HIV-1 subtypes, their heterogeneity, and the epidemiological factors influencing their distribution. Providing HIV-1 sequences and epidemiological data for approximately 300 heterosexuals in the Netherlands, the project demonstrated that multiple HIV-1 subtypes are present and epidemiologically significant in the Netherlands: 40% of the heterosexually infected individuals carry a non-B subtype. We also identified a unique F/D recombinant circulating in the Netherlands and Belgium.

Phylogenetic analysis and epidemiological data revealed that new introductions of HIV non-B strains have occurred regularly in the Netherlands, but have not caused a significant change in the frequency of HIV-1 subtypes over time, probably due to simultaneous introductions of subtype B strains from the Caribbean countries and Surinam. Furthermore, we demonstrated that virus transmissions occur across distinct exposure groups in the Netherlands, as well as in other European countries. For example, injecting drug users in Amsterdam appear to be a source of HIV-1 subtype B infections to the heterosexual population in Amsterdam. A molecular epidemiological study of acute hepatitis B infections among various exposure groups in Amsterdam also shows virus transmission from IDUs to heterosexuals. Our study among nosocomially HIV-1 infected children in Romania suggests that the subtype F viruses circulating among these children originated from the heterosexually infected adult population. Our large-scale European study among HIV-1 positive IDUs demonstrates virus exchange between drug user populations in different countries.

Taken together, these studies have shown that to a large extent, HIV diversity in the Netherlands and in other European countries, is the result of introductions of new HIV-1 variants through population migration and the travels of individuals. The mixture of HIV-1 subtypes may result in the emergence and spread of new intersubtype recombinant strains.

6.3 Monitoring of HIV-1 subtypes in the Netherlands: the future

Our surveillance for HIV-1 subtypes among heterosexuals in the Netherlands was set up as a three-year study to examine the geographic distribution of HIV-1 subtypes and its dynamics. It has recently ended, raising the question: should we continue the Dutch surveillance for HIV-1 subtypes in the Netherlands to detect potential subtype displacement in the future?

The need to continue such a monitoring system among heterosexuals is debatable, especially since we found no significant change in the HIV-1 subtype distribution in the Netherlands in the last few years. However, the spread of non-B subtypes was perhaps not revealed by our surveillance effort for two reasons. First, we showed that more than 30% of the heterosexuals diagnosed HIV-positive between 1997 and 1999 were subsequently diagnosed with AIDS. This indicates that we captured a significant proportion of individuals who were infected for a relatively long period, and although the
HIV-1 subtype distribution described in chapter two was based on recent diagnoses, it probably reflects HIV infections in the past. Second, African immigrants are relatively ‘new’ in the Netherlands compared to Antilleans and Surinamese,\textsuperscript{21,22} and since African individuals carry predominantly non-B subtypes, the spread of non-B subtypes may visibly increase in the Netherlands in the next years.

In this thesis, we demonstrate that human migration changes the HIV-1 epidemic. Various HIV-1 strains have entered the heterosexual population in the Netherlands and will no doubt continue to enter this country; as the inevitable result of immigration and travelling. However, it is difficult to predict the trend in the future. The Dutch institutes for public health should therefore continue its monitoring for new and re-emerging HIV-1 subtypes and recombinants, preferably as part of a national surveillance system that includes all exposure groups. Cross-sectional testing every few years, as a component of such a surveillance system is strongly recommended. Monitoring over time can detect changes in the distribution of various strains, and may guide selection of sentinel study sites as well as sentinel populations for DNA sequence studies in the future.

6.4 HIV surveillance in the Netherlands: the current situation and recommendations for the future

In this section, we discuss to what extent the Dutch HIV surveillance system can serve as a basis for monitoring of HIV-1 variation and what kind of improvements the system needs to adequately follow trends in the course of the HIV-1 epidemic. The future surveillance system should be capable of identifying individuals with primary infections (HIV incident cases), and may include monitoring for HIV-1 subtypes, recombinants, and resistance to antiretroviral drugs.

6.4.1 HIV case notification

Currently, HIV surveillance in the Netherlands is based on an AIDS reporting system, three long-term HIV serosurveys (STD clinic visitors, pregnant women and IDUs) and various smaller studies.\textsuperscript{23-26} This system has severe limitations. The surveys provide data on HIV prevalence and related behavior, but only for a few selected populations and geographic regions. AIDS case reporting has played an essential role in the epidemiological surveillance of HIV infection in industrialized countries. In the Netherlands, the AIDS case data were used, together with information about the natural history of infection, to extrapolate the progression of the epidemic.\textsuperscript{27} However, since 1996, the Dutch national AIDS reporting system has been unable to follow trends in recent HIV infections, mainly due to the availability of early combination therapy that postpones the onset of AIDS.\textsuperscript{28-30} Attention to the spread of HIV through surveillance nevertheless remains important, because new generations become sexually active and lifestyles change. Also demographic developments (such as migration) continually influence the composition of the population at risk for HIV.

A nationwide (coded) HIV reporting system of newly identified cases as a basis for HIV surveillance is needed in the Netherlands for several reasons.\textsuperscript{31} Compared to AIDS case reporting, reporting of HIV infections provides a more up-to-date picture of the HIV
epidemic; it can detect recent HIV trends in all exposure groups. In addition, it can provide information on the clinical stage at which diagnosis is made. Ultimately, this information will be useful in the planning and evaluation of prevention programs, as well as in the cost-effective application of resources for controlling the HIV epidemic. A Europe-wide program of combined HIV and AIDS reporting and additional surveillance activities directed at established exposure groups is recommended by UNAIDS, for the reasons mentioned above. Ideally, in each country HIV surveillance that includes all exposure groups should be coupled with programs offering prevention strategies. Currently, the National Health Council in the Netherlands is reconsidering the public health value of HIV case reporting.

6.4.2 Heterosexual population

In the Netherlands, most of the epidemiological, clinical, and biological studies of HIV-1 infection are based on the Amsterdam cohort studies among homosexual men and IDUs. The few studies that include the heterosexual population are mainly restricted to HIV surveillance, and information on disease progression among heterosexuals is limited. The AIDS Therapy Evaluation (ATHENA) project, to monitor the HIV infection and its treatment, includes only 21% heterosexuals, while 65% of the treated individuals are homosexual men.

Clearly, we can conclude that heterosexual men and women should receive more attention in additional HIV surveillance activities and in clinical studies in the Netherlands. Although the AIDS notification data do not reflect the most recent HIV infections, the relative contribution of heterosexual AIDS cases is increasing over time. In 1999, the number of AIDS cases among heterosexuals passed the number of IDU AIDS cases in the Netherlands. Data from other sources indicate that HIV infections occur, more often than in the past, among women, among individuals with a non-Dutch origin, and in areas beyond the urban agglomeration in the west of the Netherlands.

Also in Belgium a recent increase in newly diagnosed HIV infections based on surveillance system data was observed; the proportion of women and individuals with non-Belgium nationalities increased significantly the last few years.

The relatively large percentage of heterosexuals with an AIDS diagnosis in the Netherlands (chapter 2) indicates that heterosexuals may not perceive themselves at risk for HIV. Compared to homosexual men, heterosexuals know their HIV status less often and thus receive early antiretroviral treatment less often. In chapter two we demonstrated that almost all individuals who did not know how they became infected - but denied drug use and homosexual contacts - were infected with subtype B. This shows that a particular subgroup of heterosexuals, those who occasionally travel within Europe or other areas where subtype B predominates, do not consider themselves at risk for HIV. Moreover, it may indicate secondary spread of the subtype B strains that have been introduced in the Netherlands. The role of immigrants in the HIV epidemic in the Netherlands, in particular those from areas with high or increasing numbers of HIV infections, requires specific attention in surveillance activities. Currently, a surveillance program is set up at the national institute for public health and environment directed at heterosexuals from HIV endemic countries living in the Netherlands, and will include HIV-prevalence as well as behavioural data. Physicians should be aware that persons originating from African countries and those with sexual partners from these areas may
be infected with a non-B subtype; they should take into account that RNA plasma levels, essential for the evaluation of treatment, may be underestimated by some commercial assays.\textsuperscript{8,9}

In general, more research needs to be undertaken among heterosexuals to study potential differences in pathogenicity and responses to antiviral therapy among individuals infected with various subtypes or recombinants. Preferably, prospective studies should examine seroconverters who have not yet received antiretroviral therapy. A few cross-sectional studies have shown small differences in disease progression by HIV-1 subtype,\textsuperscript{37,38} but results were inconclusive.\textsuperscript{37-41} Studies of this kind are usually difficult to interpret, because the observed differences in disease progression between subtypes could be biased by many factors, for instance, the length of time a subtype has been circulating. Differences in disease progression, HIV transmissibility or vaccine efficiency are most likely influenced by a multitude of host, viral and behavioural factors, which may be difficult to unravel. A long-term prospective cohort study among drug-naive individuals, in a country with good treatment opportunities, which includes a significant number of non-B infected patients, would probably be the best design to control for other factors of influence. If no individual country has an adequate number of recent cases, one could consider conducting a study at the European level.

\section*{6.4.3 Primary infections: incidence of HIV-1 subtypes, drug resistant strains, and recombinants}

An HIV reporting system should be developed in the Netherlands. It is a key element for HIV surveillance and already exists in most European countries.\textsuperscript{29} HIV infection reports should include information allowing the characterization of patients (age, gender, region, and risk group) and identification of those recently infected (the incident cases).\textsuperscript{29-31} Adding this last variable to the HIV case report is recommended for various reasons. First, it provides HIV-1 incidence data for the various exposure groups. HIV incidence data are available in the Netherlands only for homosexual men and IDUs participating in the Amsterdam cohort studies, not for other regions in the country, nor for the heterosexually infected population. Second, a register of individuals with a primary HIV infection provides the opportunity to select individuals for monitoring of HIV-1 variation. From these incident cases, samples can be collected with informed consent (through the patient's physician) for DNA sequence analysis and subtyping to study the HIV-1 strains that are currently being transmitted.

Finally, the increase of gonorrhoea and syphilis among clients of the Amsterdam sexually transmitted diseases clinic visitors, underlines the need for a continuous surveillance system for HIV-1 infections. The increase of these STDs indicates an increase in unsafe sexual behaviour, possibly because of a change in attitudes about AIDS, now that effective antiretroviral treatment is available.\textsuperscript{42}

The incidence of different subtypes can be carried out in relation to genotypic drug-resistance patterns. Monitoring of viral drug resistance should be a permanent component of HIV surveillance. In Western Europe and the United States, subtype B predominates, and most studies for drug resistance include mainly patients infected with subtype B. However, non-B subtypes are increasingly documented in the West, and we know little about the drug resistance patterns among non-B infected patients. Drug-
resistant HIV-1 mutants have been observed in response to most, if not all, available reverse transcriptase (RT)\textsuperscript{43,44} and protease inhibitors,\textsuperscript{45-47} even among drug-naive patients\textsuperscript{48,49}, but the incidence of transmission of drug-resistant strains within the different risk groups in the Netherlands is unknown. Transmission of multi-drug-resistant viruses has been documented in other countries, resulting in drug resistance in newly infected persons.\textsuperscript{50}

At the Department of Human Retrovirology of the Academic Medical Centre in Amsterdam, HIV-1 seroconverters who participate in the Amsterdam cohort studies are routinely tested for drug-resistant mutations in the HIV-1 pol gene. Since the availability of highly active retroviral therapy (HAART) no new cases of drug resistance have been found. However, in the ATHENA project it was demonstrated that 50\% of those without previous treatment must change the first HAART combination within the first 24 weeks of starting treatment.\textsuperscript{51} The percentage is 80\% for previously treated patients.\textsuperscript{33} In 32-40\% of all treated patients, the therapy-switch was made because of serious side effects. Furthermore, half of the patients did not follow the instructions for the prescribed anti-retroviral therapy,\textsuperscript{33} which suggests that transmission of drug-resistant HIV-1 strains in the Netherlands may increase in the future.

Since there are limited epidemiological data on the occurrence of drug-resistant mutants among drug-naive patients, and their transmission has important implications for society, there should be broad implementation for sequencing the RT and protease regions of the pol gene isolated from seroconverters. When genotypic drug resistant viruses are identified, they should be analyzed by phenotypic assays. Measuring the frequency of drug-resistance in various groups and compiling drug-resistance profiles could help predict treatment failure and may assist in the design of treatment regimes.

As a pilot study to determine whether there are indications for the emergence of drug-resistant HIV strains among drug-naive individuals in the Netherlands, HIV-1 positive individuals from various populations (pregnant women, homosexual men, IDUs, and STD clinic visitors) can be examined for genotypic drug resistance in the pol gene. Such a study could also provide some indication of recombination in HIV-1 strains, since the V3 region of the env gene of those infected by heterosexual contact have already been sequenced (chapter 2). When individuals are found to have a discordant subtype in the env and pol genes of discordant subtypes, full-genome sequencing can be applied to reveal the complete recombinant structure of the genome.

To discriminate individuals with a recently acquired HIV-1 infection from those infected for longer periods samples can be screened with the detuned assay, as described in the next paragraph.\textsuperscript{52} The individuals with a primary infection are eligible for a drug resistance study. So far, the detuned assay has been validated only for subtype B viruses, but validated also for non-B subtypes, it can be used to identify individuals with primary infections for all studies on HIV-1 subtypes and intersubtype recombinants.

6.4.4 Identification of HIV incident cases

Until recently, HIV seroconverters were difficult to detect beyond the setting of a cohort study in which individuals are regularly tested for HIV and may become HIV-1 positive over time. The best data for understanding recent changes in transmission and HIV incidence (the number of new infections in a defined period), is provided by such longitudinal studies among persons at risk.
One could also select individuals with symptoms of a recent HIV-1 infection, but some will be overlooked since not everyone has symptoms recognized as an acute HIV-infection and not everyone with symptoms needs to be hospitalized. An alternative to selecting individuals with primary HIV-1 infections, is to screen HIV infected samples with the detuned assay, a new two-part HIV-1 enzyme immunoassay (EIA) strategy that takes advantage of the progressive development of HIV antibody response during the initial phase of the HIV infection.

When persons who are first tested with the standard EIA, then retested with a less sensitive EIA, those with recently acquired infections have lower antibody levels and will test negative on the less sensitive EIA. This sensitive/less sensitive assay can thus distinguish persons infected less than approximately 130 days previously from those infected more than 130 days previously. The assay can be used as a measure for HIV incidence, if combined with a questionnaire that addresses risk behaviour. It has accurately diagnosed 95% of persons with an early infection.

6.4.5 Outbreaks

Finally, it should be clear that in case of suspected local outbreaks of HIV infections, the use of molecular techniques is essential. Outbreaks of HIV-1 with rapid spread and extremely low interpatient diversity of strains have been reported among IDUs in Eastern Europe and recently also in Finland. The latter report concerned 100 new cases of HIV infection among Finnish IDUs, which were preceded by an outbreak of hepatitis C in the same group. When the HIV cases were noted by the HIV case reporting system, DNA sequencing revealed that several newly infected IDUs carried a homogeneous strain: the circulating recombinant form CR01_AE. Thus the outbreak was evident.

6.5 Concluding remarks

Monitoring of HIV-1 variation by using DNA sequencing and phylogenetic analysis - as part of the HIV surveillance system - is a very useful tool to follow epidemiological trends of HIV-1.

From a public health perspective, these studies can direct prevention efforts more accurately, may help in partner tracing, and provide insights in populations at greatest risk. Identifying recently infected persons is not only important in the study the HIV-1 subtypes, recombinants and drug-resistant strains currently being transmitted, but in stopping the transmission chain, which is the best strategy to reduce the worldwide spread of HIV, as long as a global vaccine is not available.
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