Markers of HIV-1 infection and its pathogenesis

Maas, J.J.

Citation for published version (APA):
Chapter 2

An historic overview of the Amsterdam Cohort study among homosexual men (1984-1999)

J.J. Maas
2.1 Introduction

After years of loyal participation in the Amsterdam Cohort study among homosexual men, based at the Municipal Health service, we were obliged to transfer the participants to an outpatient clinic in town, the Jan van Goyen clinic due to financial constraints on our part. Currently, there is just enough money to keep the study group of HIV-1 infected men together and to maintain data collection. For that reason we consider it important to give a brief overview of the history and logistics of the project and also to give a description of the various sub-groups and studies.

2.1.1 The Amsterdam cohort study

The Amsterdam Cohort Study of Human Immunodeficiency virus (HIV-1) infection and AIDS amongst homosexual men (ACS) was started in 1984 \(^1\)\(^2\)\(^3\)\(^4\), followed shortly by the Amsterdam cohort study amongst intravenous drugs users in 1985 \(^5\)\(^6\). A multidisciplinary approach encompassing epidemiology, social science, virology, immunology and clinical medicine has significantly contributed to the knowledge and understanding of the various aspects of HIV-1 infection and AIDS. Four major fields of interest were explored. The first was to study the prevalence and incidence of HIV-1 infection and AIDS. This was followed by studies designed to describe the natural course of HIV-1 infection and AIDS. The third point of interest was to examine the various risk factors, and to monitor changes in sexual behaviour over time. Finally, several intervention studies were performed to investigate the antiretroviral effects and the emergence of resistance of several types of treatment \(^1\).

The ACS is a collaboration of the Municipal Health Service, the Department of Human Retrovirology, the AIDS unit of the department of infectious diseases and the National AIDS Therapy Evaluation Center (NATEC) - these latter three departments all being located at the Academic Medical Center, University of Amsterdam - and of the Department of Clinical Viro-Immunology, CLB division of Sanquin Blood Supply Foundation and Laboratory for Experimental and Clinical Immunology.
An historic overview of the Amsterdam Cohort study

Initially, risk factors and behavioural issues relating to HIV-1 transmission were studied by the department of Gay and Lesbian studies at the University of Utrecht. Since 1993, however, these social-scientific studies have been conducted by the department of Social science, also at the University of Utrecht.

2.1.2 Three monthly routine

After the first study visit all participants have been seen every three months, with the exception of HIV-1 seronegative individuals who have been seen six-monthly since 1992. At every visit a medical history is taken, a physical examination is performed - only on the HIV-1 infected individuals - and blood is drawn for immunological and/or virological laboratory evaluations. All the data gathered in the scope of the ACS was centrally collected at the Municipal Health Service until February 1999. Since then, data on HIV-1 infected participants has been collected at the Jan van Goyen clinic, at which the same procedure is followed. Although all participants were mainly seen at the location of the Municipal Health service, issues regarding medical treatment were co-ordinated from the AIDS unit of the department of infectious diseases at the AMC. As a rule, participants who developed an AIDS event during follow-up were referred to the AMC. In addition, risk factors and psychosocial correlates of sexual behaviour, were studied by means of six monthly questionnaires.

2.1.3 Routine laboratory

For every new included participant, the first task was to determine his antibody status for HIV-1. If a participant was found to be HIV-1 seropositive, a western blot was performed for confirmation. Over the years, the different laboratory tests used in the daily ACS routine have varied considerably. This will be further discussed elsewhere. Currently, routine testing includes: CD3⁺,CD4⁺,CD8⁺ T cell count, T cell reactivity after stimulation with monoclonal antibodies against the CD3 receptor, HIV-1 phenotype once a year and the NucliSens HIV-1 RNA load assay. Since the beginning of the study, both frozen serum and peripheral blood mononuclear cells (PBMC) have been stored.
The sera have been sent to the department of Human Retrovirology and the cells to the CLB.

Although it has proven to be a huge endeavour to maintain such an enormous tissue bank, both in financial and physical efforts, it has been shown to be worthwhile. The unique database of data on both disease and patients' characteristics, in combination with the stored samples, has enabled us, both prospectively and retrospectively, to investigate the relevance of and the predictive properties of newly developed markers such as HIV RNA load\textsuperscript{7} and Syncytium-inducing HIV-1 virus phenotype. Similarly, we have been able to investigate the significance of genetic determinants like HLA, CCR5 and CCR2\textsubscript{b}\textsuperscript{8} polymorphism.

2.2 Recruitment over time

2.2.1 In the beginning...\textsuperscript{21}

A year before the outbreak of AIDS in the USA in 1980, 680 homosexual men took part in a trial to evaluate the efficacy of immunisation with a hepatitis B vaccine. These were individuals who were at the high-risk of getting a hepatitis B infection because of their lifestyle\textsuperscript{9}. This Amsterdam based study was terminated in 1982. In 1984, shortly after the first cases of AIDS were diagnosed in The Netherlands\textsuperscript{10}, plans were made to start a new cohort study which allowed monitoring of the recently identified HIV-1 epidemic in Amsterdam. The first wave of enrolment in this study took place between October 1984 and April 1985 (n=748). The inclusion criteria were: asymptomatic homosexual men aged 18-65, with at least two sexual partners in the six months prior to intake. Many of the participants of the former hepatitis B vaccination study consented to follow-up testing for HIV-1 or to participate in the newly initiated study. Even so all participants were tested at entry. Recruitment was also made through announcements in the gay press, through advertisements and by word of mouth. When enrolment was completed in 1985, nearly one-third of the men appeared to be HIV-1 infected at the first enrolment visit. Obviously, HIV-1 had spread considerably among the population of gay men living in Amsterdam. Furthermore, using the frozen sera from the hepatitis B vaccination trial, it was shown that the virus was
An historic overview of the Amsterdam Cohort study

introduced to Amsterdam somewhere at the end of the seventies.  

2.2.2 Second enrolment
By 1985, the prevalence of HIV-1 among homosexual men living in Amsterdam was well established. Knowledge of the incidence and the natural history of the HIV-1 infection, however, was still very limited and could only be studied in persons with a known date of seroconversion. Therefore, the recruitment of HIV-1 infected individuals was terminated while recruitment of HIV seronegative individuals, with at least two sexual partners in the six months prior to the study intake, was continued (n=265). This second wave recruitment was started in April 1985.

2.2.3 Third enrolment
This policy, however, changed with the introduction of zidovudine. In 1986, zidovudine became available as the first drug found to be effective against HIV-1. The ACS offered an unique opportunity to study the immunological and virological effects of zidovudine in the early phases of the disease. Because of this, there was a new interest in intervention studies. To ensure that there were enough HIV-1 infected persons able to participate in future intervention trials, enrolment was re-opened to HIV-1 infected individuals from February 1988 until December 1998 (n=175).

2.2.4 Fourth enrolment
Fifty-one participants could not be classified in either of the earlier mentioned studies. Twenty-seven of these 51 participants entered the ACS because they were found to be HIV-1 positive while participating in another municipal health service study. The remaining 24 persons entered the ACS to start with antiretroviral treatment. At the end of 1996, protease inhibitors became available in the Netherlands causing an increased demand for antiretroviral treatment which could not be immediately met because of the lack of treatment facilities in Dutch Hospitals. To relieve this temporary congestion in treatment capacity, HIV-1 infected persons, both male and female, and various other risk groups were
Markers of HIV-1 infection and its pathogenesis

allowed to start their treatment within the ACS from February 1997 onwards.

2.2.5 Fifth enrolment
Since the beginning of the epidemic, several safe sex campaigns have been launched, targeting homosexual men. Anal receptive sexual practices emerged to be the most important risk factor in HIV-1 transmission among homosexual men. Those campaigns appeared to be effective, resulting in a reduction in the number of sexual partners, a higher proportion using condoms and a decline in the incidence of HIV-1. However, in the beginning of the nineties, the first signs of a reverse trend became apparent. In the STD clinics of Amsterdam an increase in other STDs such as anal gonorrhoea was seen in young homosexual men, indicating that these men were more commonly engaged in unprotected sex. Therefore, to address the question of whether there was a rise in HIV-1 prevalence and incidence among young homosexual men, a campaign was launched in February 1995 to recruit young homosexual men younger than 30 years old (n=438) 22-24. This study is still ongoing.

2.2.6 The remaining HIV seronegative participants
In February 1996, the follow-up of all remaining HIV seronegative participants was terminated for three reasons. Firstly, due to ageing, this group was no longer considered representative of the Amsterdam gay population. Illustrative of this was the observation that the HIV-1 infection rate among these persons was lower than the infection rate found in a cross-sectional study of visitors to the Amsterdam gay bars 25. Secondly, a bias had been introduced by extensive counselling at the cohort visits. Finally, due to a lack of motivation, questionnaires were not being adequately completed.
An historic overview of the Amsterdam Cohort study

2.2.7 The Jan van Goyen clinic

In February 1999, follow-up of all HIV-1 infected participants of the study was transferred to the Jan van Goyen clinic (n=227). This transfer of participants was necessary because of a decrease in the funding of the ACS by the Dutch government. In addition, in an area where many patients are being treated with Highly Active AntiRetroviral Therapy (HAART) in the early stages of infection, a clinical setting such as the Jan van Goyen clinic is more appropriate than the Municipal health service. One hundred and eighty-four HIV-1 infected participants agreed to continue their follow-up and medical treatment at the Jan van Goyen clinic (table 1). The remaining participants were scattered over several hospitals in Amsterdam and in other hospitals near their homes outside Amsterdam. Although in the table 1. Overview of the diaspora of the 227 participants after termination of the ACS at location Municipal health service, February 1999.

<table>
<thead>
<tr>
<th>Migration of the ACS participants</th>
<th>Number of participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moved to the Jan van Goyen clinic (JvG)</td>
<td>184</td>
</tr>
<tr>
<td>Continued their follow-up in another hospital</td>
<td>23</td>
</tr>
<tr>
<td>Withdraw from the study due to lack of motivation</td>
<td>10</td>
</tr>
<tr>
<td>Refused to continue their follow-up at location JvG</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>227</td>
</tr>
</tbody>
</table>

Although in the scope of the National Athena monitoring project all participants are still being followed in the context of the ACS, the main study interest is narrowed to the monitoring of blood values, event registration, and treatment.
2.3 The seroconverters cohort

The participants who were initially HIV-1 seronegative and then seroconverted during follow-up provided the most complete and the least biased information on the incubation time of disease and death. This was because the time between the last HIV negative and the first HIV-1 seropositive sample was less than one year.

The moment of seroconversion can be easily estimated as the midpoint of the interval between the last seronegative visit and the first seropositive visit. In total 139 seronegative participants seroconverted during follow-up (January 1999). Ninety-two from the first recruitment, 38 from the second wave of recruitment and 9 from the most recent recruitment of young homosexual men. From these seroconverters, the moment of HIV-1 seroconversion is known within a median range of three months (table 2). Additionally, in 24 seroprevalent men who participated also in the hepatitis B vaccine trial, seroconversion intervals could be determined retrospectively using stored material. However, because in most cases the

Table 2. Overview of cases with known interval of seroconversion.

<table>
<thead>
<tr>
<th>Prospectively seroconverted participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>First enrolment</td>
</tr>
<tr>
<td>Second enrolment</td>
</tr>
<tr>
<td>Fifth enrolment</td>
</tr>
<tr>
<td>Retrospectively identified seroconverters</td>
</tr>
<tr>
<td>Seroconverted during the Hepatitis B trial (between 1980 and 1982)</td>
</tr>
<tr>
<td>Participants, being HIV- during the Hep-B trial, but HIV+ at the first visit first enrolment.</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

Seroconverters intervals were too wide (in some cases more than 7 years), none of those were used as seroconverters and included in the 139 cases mentioned above. This seroconverters cohort mentioned above has played a very prominent role in AIDS research and in our understanding of the natural history of HIV-1.

2.3.1 Seroprevalent participants

Among seroprevalent individuals, some of the infection time had already elapsed by the time of
entry into the study. The results of a survival analysis can therefore become severely confounded if certain risk and co-factors of interest are associated with the unknown duration of the infection before enrolment. Using retrospectively identified HIV-1 seroconverters, it is nowadays possible to estimate the time since the exposure to HIV-1 of a given seroprevalent individual, thus enabling greater sized, unbiased studies, with a more efficient use of all available cohort data.

2.4 Lost to follow-up management

Even when participants were lost to follow-up, special efforts were made to obtain information on the most crucial study parameters: the first AIDS diagnosis, death and cause of death. Once a year, information on survival status was obtained through active follow-up and matching with local population registries. The cause of death was obtained from the Amsterdam AIDS surveillance registry, hospital records and from next of kin. Of the 1239 participants included (but not considering participants of the young homosexual cohort), 553 were found to be HIV-1 seropositive at entry and 139 seroconverted during follow-up (total 692). Of those 692 persons, 273 have since died (Table 3). 101 participants prematurely withdrew from the study due to a lack of motivation. In all of them, however, follow-up data on survival were recovered from

<table>
<thead>
<tr>
<th>Death classification</th>
<th>Numbers of deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death by AIDS</td>
<td>243</td>
</tr>
<tr>
<td>Death by a non-HIV/AIDS related course</td>
<td>13</td>
</tr>
<tr>
<td>Died of AIDS, but date of death unknown</td>
<td>1</td>
</tr>
<tr>
<td>Cause of death not formally confirmed*</td>
<td>12</td>
</tr>
<tr>
<td>Cause of death unknown</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>273</td>
</tr>
</tbody>
</table>

* In 12 cases, the AIDS diagnoses could not formally be confirmed by medical documentation.
death announcements in the newspapers and from next of kin. Additionally 53 participants, explicitly withdrew their informed consent and 14 participants moved abroad. Of this group of 67 persons, no follow-up data could be obtained from 39 persons.

2.4.1 Endpoints
As well as the earlier mentioned fixed endpoints, the first AIDS event and death, the dichotomised number of CD4\(^+\) T cell count (\(< 200 \times 10^6/\)l)\(^{27}\) is also frequently used as an endpoint. However, other surrogate markers such as non-syncytium-inducing (NSI) to syncytium-inducing (SI) HIV-1 virus phenotype switch and the HIV RNA concentration above the threshold of quantification can be used as endpoints as well. Furthermore, in etiological studies that investigate whether there is an association between the presence of herpes 8 antibodies and the development of KS, KS can also be used as an endpoint.

2.4.2 Clinical follow-up.
If participants developed AIDS associated complaints during follow-up, those persons were automatically referred to the AMC for medical treatment. In a check to compare the event registration at the AMC with the registration at the ACS, it became apparent that the follow-up data of approximately 275 persons was either missing or incomplete. Since 1996 therefore, much effort has been put into updating and aligning both registries. However, due to missing medical records etc., this has turned out to be a difficult task that has still not been completed.

2.5 Intervention studies
In 1987 the results of a clinical trial of zidovudine was published\(^{13}\). In this placebo-controlled trial, using individuals with either advanced AIDS-related complex (ARC) or AIDS, there was a significant reduction of HIV-1-associated morbidity, a significant rise in CD4\(^+\) T cell count and an extended survival. In another study, the first indications were found that zidovudine was protective against AIDS dementia complex\(^{28-30}\).
An historic overview of the Amsterdam Cohort study

At the beginning of 1987, within ACS, 24 asymptomatic HIV-1 infected subjects with a long-term HIV-1 antigenaemia took part in a preliminary study of zidovudine. This study was one of the first, worldwide, to address the question of the efficacy of zidovudine among asymptomatic HIV-1 infected individuals. The ACS was an appropriate setting for this study as the participants were already well characterised in respect of follow-up and of the established markers of progression at that time.

Enrolment for a second (multi-centered) zidovudine study started one year later in April 1988. In this double-blind placebo-controlled study, asymptomatic participants were randomised to receive either zidovudine or a placebo. In this study, with a follow-up of two years, 56 persons from the ACS were included. From these studies much has been learned about therapeutic intervention and the mechanisms of antiviral resistance.

2.5.1 The last ACS comparative intervention study

Although the ACS has participated as a study-site in several different multi-centered trials since then (Delta, Triple, Atlantic, Prometheus, and Native study), one comparative intervention study solely held in the ACS was the “D4T/3TC” study (1996-1997). In this study (n=48) asymptomatic participants were randomised to two treatment arms: zidovudine and lamivudine or stavudine and lamivudine. Depending on the HIV RNA load (if above 500 copies/ml) on week 8, or thereafter the protease inhibitor indinavir was added on the next visit. Furthermore, in a sub-study the drug penetration through the blood-brain barrier was assessed. Viral suppression in the Cerebrospinal fluid (CSF) was compared with the drug levels and viral suppression obtained in peripheral blood. This study demonstrated that antiretroviral drugs other than zidovudine are also capable of penetrating sufficiently into the CSF.

2.5.2 Vaccination studies

As well as these multi-centered interventions studies mentioned above, the ACS has also participated as a study-site in three vaccination studies, one of which is still ongoing. Using HIV-1 infected individuals, the main object of
the first two studies was to determine whether
immunotherapy with either a p24-HIV or rgp120
containing vaccine would result in sufficient
levels of antibodies and whether the vaccine was
effective in delaying progression to AIDS. The
aim of the third (multi-centered) study is to
examine the extent to which HIV-1
seroconversion can be prevented by vaccination
of high-risk HIV uninfected individuals.

Recruitment of the first two vaccination
studies started between March 1993 and March
1994. In the first multi-centered study, 21 HIV-1
infected participants from the ACS were
included. They were assigned in a double-blind
fashion in which they were either vaccinated
with a p24-VLP vaccine (HIV-1 p17/p24:Ty-like
particles) or with a placebo. The
immunological efficacy was assessed by
measurements of the concentration of p24, p17
and Ty-antibody. In the second study which was
a simultaneous held double-blind placebo-
controlled study, 18 participants with a known
date of seroconversion were vaccinated with
either a rgp 120 containing HIV-1 vaccine
(n=12) or with a placebo (n=6). Finally, in the
still ongoing (multi-centered) double-blind
placebo-controlled study with as primary
outcome the frequency of HIV-1 seroconversion,
the efficacy of the vaccine is assessed by the
vaccination of high-risk HIV-1 uninfected
homosexual individuals with either a rgp 120
HIV-1 vaccine or with a placebo. Of the 5000
subjects totally included, 120 will be recruited in
the context of the ACS.

2.6 Topics of ACS research

The Amsterdam seroconverters cohort consists
of 139 HIV-1 infected individuals with a known
date of HIV seroconversion. By alignment of the
participants at the time of HIV seroconversion,
we were able to provide adequate estimations of
the median incubation time. It has became clear
that the time between HIV-1 seroconversion and
the first signs of AIDS is highly variable (8-15
years). It was recently shown that this
variation is strongly associated with the
polymorphism of the genes encoding for the
CCR, CXCR chemokine receptors and
SDF-1. In addition, Keet et al. recently
An historic overview of the Amsterdam Cohort study

described an association between progression to AIDS and death and HLA polymorphism \textsuperscript{61-65}.

In the beginning of the epidemic especially, much of the effort was focused on causal determinants and predictors of rapid progression \textsuperscript{66}. Equally important, however, was to identify of the characteristics of persons who remained asymptomatic for a long period. It was found that those persons were characterised by a stable and relatively preserved T cell reactivity after stimulation with antibodies targeting the CD3 cell receptor \textsuperscript{67}.

Our well-characterised cohort is also of great value to the present body of knowledge about the kinetics, pathogenesis and predictive capacities of viro-immunological markers such as T cell subsets, p24 antigenemia \textsuperscript{68-74}, antigens/antibodies complexes \textsuperscript{68,72,73,75,76} and HIV RNA viral load \textsuperscript{7} as well as for the development of new and useful markers such as the syncytium HIV-1 virus phenotype \textsuperscript{77,81} and T cell reactivity \textsuperscript{82-86}.

In addition to applied research, the ACS is also involved in extensive fundamental research. Recently a hypothesis, proposed by Ho et al was questioned \textsuperscript{87-90}, and this sparked lively discussion on the international scientific forum \textsuperscript{91}.

Finally, the following research topics were also studied within the ACS: the epidemiological patterns and mechanisms of co-infections with varicella Zoster \textsuperscript{92,93}, herpes Simplex type I and II \textsuperscript{94} and the KS associated herpes virus 8 \textsuperscript{95,96}, primary infection \textsuperscript{97,98}, AIDS Surveillance \textsuperscript{99-107}, neutralising antibodies \textsuperscript{108}, T cell dynamics in HIV-1 infection \textsuperscript{109-111}, T cell dysfunction \textsuperscript{112} and HIV-1 variation and transmission \textsuperscript{113}. 

30
2.7 Immunological assays used in the daily ACS routine

In Table 4, an overview is given of the immunological tests routinely assessed in the scope of the three monthly ACS.

<table>
<thead>
<tr>
<th>Period</th>
<th>Proliferation assays</th>
<th>T cell subsets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PHA</td>
<td>ALS</td>
</tr>
<tr>
<td>1984</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1985</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1986</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1987</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1988</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1989</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1990</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1991</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1992</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1993</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1994</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1995</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1996</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1997</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1998</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1999</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Although the MT-2 assay is not really an immunological assay, it is presented here because it is developed and routinely determined in the Clinical Viro-Immunological laboratory. PHA: phytohemagglutinin, before 1994, human pooled serum (HPS) was added to the culture medium, after 1994, no HPS was added: PHA responses with and without HPS were not comparable, ALS: horse anti-lymphocyte stimulation test, ACD3: T cell function measured after stimulation with monoclonal antibodies (mAb) against the CD3 receptor, CD228: T cell function stimulation with CD2 and CD28 mAb, CD328: T cell function stimulation with CD3 and CD28 mAb. T cell immuno-phenotyping CD2, CD3, CD4 and CD8: before 1988, the single indirect staining, a single indirect immuno-fluorescence staining on Ficoll isolated peripheral blood mononuclear cells (PBMC) was used and replaced by a double direct staining thereafter. Since 1994, lymphocyte immunophenotyping was accomplished in whole blood.

2.7.1 T cell subsets

The hallmark of HIV-1 infection is a decline of CD4⁺ T cell count, which ultimately leads to AIDS defining illnesses such as opportunistic infections and or malignancies. Although our knowledge about the underlining mechanisms is still limited, this decline has been shown to be a strong and useful surrogate marker in the monitoring of both the natural course of HIV-1.
An historic overview of the Amsterdam Cohort study

infection and the effectiveness of antiretroviral treatment.

From 1984 onwards, immunophenotyping of T cell has been part of the three monthly ACS routine. Over time, however, several changes have been introduced. Before May 1988, a single indirect immuno-fluorescence staining on Ficol isolated peripheral blood mononuclear cells (PBMC) was used. This was replaced by a double direct staining thereafter. The EPICS flow cytometer was replaced by a FACS in 1991. In addition, since 1994, lymphocyte immunophenotyping was accomplished in whole blood. For the previously mentioned changes, no longitudinal retrospective corrections for possible inter-assay variations were necessary.

2.7.2 T cell proliferation markers

Low T cell reactivity to CD3 mAb and to the combination of CD3 plus CD28 mAb have been shown to be strong predictors for developing AIDS, independent from CD4+ T cell count and syncytium-inducing virus phenotype. Preserved T cell reactivity was also shown to be a strong determinant among long-term asymptomatic individuals with a CD4+ T cell count below 200x10^6/l and an useful marker for predicting survival after the determination of AIDS.

T cell function is measured in a whole-blood lymphocyte culture after stimulation with various mitogens. In table 4 an overview is given of the various protocols used. In summary, before 1994, the proliferation test on phytohemagglutinin (PHA) and the horse anti-lymphocyte serum test (ALS) was performed using a culture-medium supplemented with human pooled serum. Furthermore, stimulation with ALS was eliminated from the protocol in 1994 and replaced by stimulation protocols using combinations of CD2 plus CD28 mAb (CD228) and CD3 plus CD28 mAb (CD328).

To obtain comparability between the different generations of tests, adjusted longitudinal test outcomes were calculated, taking those inter-assay variations into account. In addition, to adjust for the intra and inter-individual variations and the assay variations, the proliferation reactivity was expressed as the...
percentage of the median responses detected in concurrently running cultures of 5 healthy controls.

2.7.3 HIV-1 phenotype

Although the HIV-1 phenotyping is not really an immunological assay, nevertheless it is presented here because it is since 1992 part of the daily routine of the department of Clinical Viro-immunology.

Early in HIV-1 infection, but also among subjects with an asymptomatic infection, slow-replicating and mainly macrophage-tropic, non-syncytium-inducing (NSI) viruses are the predominant isolates in the peripheral blood. However, a shift of the virus population towards the more rapidly replicating T cell tropic, syncytium-inducing (SI) phenotype virus heralds in approximately 50% of the homosexual cases the onset of AIDS. The mutation-prone nature of HIV-1 replication is the driving force behind this variation in cellular tropism. In particular, mutations of certain amino acids located within the V3 loop, have been shown to be associated with the observed change of cellular tropism.

As mentioned before, since 1992 HIV-1 phenotyping is part of the daily routine. However, using cryopreserved PBMC, we were able to complete the individual follow-ups retrospectively (table 4).

2.8 Virological assays used in the daily ACS routine.

When a person agrees to participate in the ACS, his HIV-1 antibody status is determined. At that point the presence of those antibodies is tested with the commercially available enzyme-linked immunosorbent assays (IMX: Microparticle Enzyme Immuno assay, Abbott laboratories, North Chicago, Illinois), and confirmed with a Western blot (Diagnostic Biotechnology Ltd., Singapore). In table 5 an overview is given of the various assays and protocols used. Furthermore, to compare test results, some assays have been used simultaneously.

The presence of P24 antigens was routinely determined in the ACS from 1984, the beginning of the study, until 1998. HIV p24
An historic overview of the Amsterdam Cohort study

antigen was detected with a solid phase,
sandwich-type enzyme immunoassay (EIA, Abbott laboratories). Soon, however, a new p24 antigen assay will be implemented in the ACS (Vironostika, Organon). P24 antigen has been shown to be an important predictive marker in monitoring the natural history of the HIV-1 infection and it is successfully used as one of the first marker in monitoring the antiretroviral capacities of zidovudine.

In May 1996, the HIV RNA PCR was introduced into the daily ACS routine. The HIV-1 RNA concentration in peripheral blood has been shown to be strongly associated with progression to AIDS and death. The first test used in the ACS was the NASBA assay (Nucleic Acid Sequence-based amplification assay, Organon Teknika, Boxtel, The Netherlands), with a quantification threshold of 1000 RNA copies/ml. Since 1997, however the more sensitive NucliSens test has been used (quantification threshold 400 RNA copies/ml).
Table 5. Overview of the used virological assays.

<table>
<thead>
<tr>
<th>virological assays</th>
<th>Company</th>
<th>period</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HIV screenings assays:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HTLV-III screening test</td>
<td>home made</td>
<td>1984-1985</td>
</tr>
<tr>
<td>HTLV III EIA</td>
<td>Abbott</td>
<td>1985-1989</td>
</tr>
<tr>
<td>recombinant HIV-1/HIV-2 EIA</td>
<td>Abbott</td>
<td>1989-1993</td>
</tr>
<tr>
<td>Wellcozyme anti-HTLV III</td>
<td>Wellcome</td>
<td>± 1986</td>
</tr>
<tr>
<td>Vironostika anti-HTLV-III ELISA</td>
<td>Organon International</td>
<td>1985-1987</td>
</tr>
<tr>
<td>IMX (MEIA system)</td>
<td>Abbott</td>
<td>since 1998</td>
</tr>
<tr>
<td><strong>Other used HIV-antibody assays:</strong></td>
<td>Abbott</td>
<td>1986-1992</td>
</tr>
<tr>
<td>HIV-1 anti-CORE EIA</td>
<td>Abbott</td>
<td>1986-1992</td>
</tr>
<tr>
<td>ENVACOR HIV-1 EIA</td>
<td>Abbott</td>
<td>1989-1992</td>
</tr>
<tr>
<td>Detection of anti-CORE</td>
<td></td>
<td>1987-1990</td>
</tr>
<tr>
<td>Detection of anti-ENV</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>HIV-antigen assays:</strong></td>
<td>Abbott</td>
<td>1987-1990</td>
</tr>
<tr>
<td>HTLV-III Antigen EIA</td>
<td>Abbott</td>
<td>1990-1998</td>
</tr>
<tr>
<td>HIVAg-1 EIA polyclonal</td>
<td>Abbott</td>
<td>1990-1998</td>
</tr>
<tr>
<td>Vironostika HIV-Ag</td>
<td>Organon Teknika</td>
<td>Will be implemented soon</td>
</tr>
<tr>
<td><strong>Confirmation assays:</strong></td>
<td>Home made</td>
<td>1984-1985</td>
</tr>
<tr>
<td>HIV-1 Western Blot</td>
<td></td>
<td>± 1986</td>
</tr>
<tr>
<td>LiaTek HIV-1/HIV-2</td>
<td>Organon Teknika</td>
<td>Since 1986</td>
</tr>
<tr>
<td>HIV Blot 2.2 (HIV-1 en HIV-2)</td>
<td>Diagnostic Biotechnology</td>
<td>Since 1986</td>
</tr>
<tr>
<td>HIV-2 Blot version 1.2</td>
<td>Diagnostic Biotechnology</td>
<td>Since 1995</td>
</tr>
<tr>
<td><strong>HIV RNA assays:</strong></td>
<td>Organon Teknika</td>
<td>1996-1997</td>
</tr>
<tr>
<td>NASBA HIV-1 QT</td>
<td></td>
<td>Since 1997</td>
</tr>
<tr>
<td>NucliSens HIV-1 QT</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*EIA: Enzyme Immuno Assay, ELISA: Enzyme Linked immunoSorbent Assay, Liatek: Line Immuno Assay Technique, MEIA: Microparticle Enzyme Immuno Assay, Nasba: Nucleic Acid Sequence-based Amplification. Remark: To compare test results, some assays have been used simultaneously.*
An historic overview of the Amsterdam Cohort study

Reference List


Markers of HIV-1 infection and its pathogenesis


An historic overview of the Amsterdam Cohort study


26. Geskus RB: Methods for estimating the AIDS incubation time distribution when date of seroconversion is censored, 1999:(submitted)


Markers of HIV-1 infection and its pathogenesis

31. Centers for Disease Control: Revision of the CDC surveillance case definition for acquired immunodeficiency syndrome. *MMWR* 1987;36:1S-5S.


40. de Jong M, Veenstra J, Stilianakis NI, et al: Host-parasite dynamics and outgrowth of virus containing a single K70R amino acid change in reverse transcriptase are responsible for
An historic overview of the Amsterdam Cohort study


Marker of HIV-1 infection and its pathogenesis


60. van Rij RP, de Roda Husman AM, Brouwer M, Goudsmit J, Coutinho RA, and Schuitemaker H: Role of CCR2 genotype in the clinical course of syncytium-inducing (SI) or non-SI human immunodeficiency virus type 1 infection and in the time to conversion to SI virus variants. *Journal of Infectious Diseases*. 1998;178:1806-1811.


An historical overview of the Amsterdam Cohort study


70. Goudsmi J, Lange JMA, Paul DA, and Dawson GJ: Antigenemia and antibody titers to core and envelope antigens in AIDS, AIDS-related complex and subclinical human immunodeficiency virus infection. *Journal of Infectious Diseases* 1987;155:558-560.


73. Lange JMA, Goudsmi J: Decline of antibody reactivity to HIV core protein secondary to increased production of HIV antigen. *The lancet* 1987;i:448.

Markers of HIV-1 infection and its pathogenesis


84. Schellekens PTA, Roos MTL, de Wolf F, Lange JMA, and Miedema F: Low T-cell responsiveness to activation via CD3/TCR is a prognostic marker for acquired immunodeficiency syndrome.
An historic overview of the Amsterdam Cohort study


94. Dukers NHTM, Bruisten SM, van den Hoek JAR, de Wit JBF, van Doornum GJJ, and Coutinho RA: Strong decline in herpes simplex antibodies over time among young homosexual men is associated with changing sexual behavior. *AIDS* 1999:(submitted)

Markers of HIV-1 infection and its pathogenesis


An historic overview of the Amsterdam Cohort study

infection rate and risk behaviour with back-calculation for the Netherlands.


Markers of HIV-1 infection and its pathogenesis


An historic overview of the Amsterdam Cohort study


Markers of HIV-1 infection and its pathogenesis