Perinatal HIV-1 infection

Aspects on clinical presentation, viral dynamics and epidemiology

Lars Navér

Stockholm 2004
All previously published papers were reproduced with the kind permission of the publishers.

Published and printed by Karolinska University Press
Box 200, SE-171 77 Stockholm, Sweden
© Lars Navér, 2004
ISBN 91-7349-983-8
To my nearest and dearest
How many deaths will it take till he knows
that too many people have died?
Bob Dylan
Abstract

During the last 20 years HIV-1 infection has changed from being an unknown disease to being a widespread pandemic, in some areas affecting a large proportion of the human population. Mother-to-child transmission (MTCT) is the predominant route of transmission in children, who thus become infected during a period when their immune system is immature and developing. This thesis is based on a prospective follow-up of a population-based cohort of children to HIV-1-infected women in Sweden since the start of the HIV-1 epidemic.

We followed a total of 419 mother-child pairs between 1982 and 2003, 355 of them prospectively. The MTCT rate decreased during the period from 24.7% 1984-93 to 5.7% 1994-98 and 0.7% 1999-2003. The proportion of children born to women who received antiretroviral treatment or prophylaxis increased from 2.3% to 91.5%, and the elective caesarean section rate from 8.0% to 80.1%. Seventy-two children, 31 of whom were born in Sweden, were vertically infected. Eleven infected children died. Ten out of 51 children living in Sweden in 2003 had had an AIDS diagnosis and 29 were on antiretroviral treatment. Eleven lived with a non-parental caregiver.

In 24 prospectively followed HIV-1-infected children born during 1985-98, the disease progressed faster to severe immunodeficiency, AIDS or death in children with early symptoms related to HIV-1 than in those without early symptoms. Detectable virus during the first four days of life was not shown to affect disease progression. Two children developed symptoms suggestive of primary HIV-1 infection.

HIV-1 RNA load was determined in 32 infected children. The median HIV-1 RNA level was highest at 1.5-3 months of age, decreased between 1 and 8 years and then increased slightly. This pattern was not seen in all individuals. Both symptomatic and asymptomatic children displayed a varying pattern of viral load.

Stored samples from 24 infected children were analysed for genetic subtype and coreceptor use of the virus. The virus belonged to subtypes A, B, C, D, G and CRF01_AE. All isolates from the first year of life used chemokine receptor CCR5 as coreceptor. In virus from four patients, the coreceptor use changed from CCR5 to CXCR4. The change was associated with a decreased CD4+ cell count and disease progression, but appeared after immunological deterioration.

Infectious viral loads by limiting dilution culture and days-to-culture positivity (infectious index) and HIV-1 RNA were determined in 16 children. Limiting dilution correlated to the infectious index. The median HIV-1 RNA and infectious indices of plasma and PBMC rose rapidly to 6-8 weeks of age to a plateau level. The median index in plasma declined to zero by 2 years of age in contrast to that in PBMC and to HIV-1 RNA. A high-peak plasma index correlated with clinical progression. Children who progressed to AIDS had higher median plasma indices than those who did not. In children displaying a coreceptor change in plasma, there was a simultaneous reappearance of infectious virus, which was not related to changes in RNA.

In summary, the virological studies contribute to a better knowledge of the natural course and interaction between virus and host in perinatally HIV-1 infected children. The epidemiological study shows that good results can be achieved if prophylactic measures against MTCT of HIV-1 are used consistently, which highlights the importance of antenatal screening programmes.

Keywords: Perinatal, HIV-1 infection, disease progression, viral load, genetic subtype, coreceptor, mother-to-child transmission, epidemiology

ISBN: 91-7349-983-8
Original papers

This thesis is based on the following papers, which will be referred to by their Roman numerals:


Table of contents

Abbreviations ..............................................................................................................10
Introduction ..................................................................................................................11
  A pandemic ..............................................................................................................11
  The virus ...................................................................................................................12
    Evolution, origin and genetic diversity .................................................................12
    Structure ...............................................................................................................14
    Replication cycle ..................................................................................................14
  HIV-1 coreceptor use ............................................................................................15
  HIV-1 transmission ................................................................................................17
  Mother-to-child transmission ................................................................................17
    Risk factors ........................................................................................................18
    Prevention ............................................................................................................18
Negative effects of prophylaxis against mother-to-child transmission ..................19
  Pregnancy screening ..............................................................................................19
  Diagnosis ...............................................................................................................20
  Clinical course of HIV-1 infection ........................................................................20
  Viral load ................................................................................................................23
  Viral dynamics in acute infection ..........................................................................23
  Viral load and clinical course ................................................................................24
  Immune status and clinical course .......................................................................24
  Social situation and stigma ..................................................................................25
Aims of the study ........................................................................................................26
Patients and methods ..............................................................................................27
  Patients ...................................................................................................................27
    Paper I ................................................................................................................27
    Paper II ..............................................................................................................27
    Paper III ............................................................................................................27
    Paper IV ............................................................................................................27
    Paper V ..............................................................................................................28
  Clinical examination and blood sampling (papers I-V) ........................................28
  Virus isolation (papers I, III, IV) ..........................................................................28
  Detection of HIV-1 p24 antigen (papers I, III, IV) ...............................................29
  Quantification of infectious virus (paper IV) .....................................................29
    Limiting dilution ................................................................................................29
    Days-to-culture positivity ....................................................................................29
  Determination of coreceptor use by HIV-1 (paper III, IV) ....................................29
  Quantitative HIV-1 RNA (paper I-IV) ..................................................................30
  HIV detection by DNA PCR (paper I) ..................................................................30
  Determination of genetic subtypes (papers II-V) ................................................30
  Clinical and immunological classification .........................................................30
  Diagnostic criteria .................................................................................................31
  Statistical analysis ................................................................................................31
  Ethical consideration .............................................................................................31
Results and discussion ............................................................................................32
  HIV-1 detection in perinatally HIV-1-exposed children (paper I) .......................32
  Primary HIV-related symptoms in perinatally HIV-1-infected children (paper I) 33
  Disease progression in perinatally HIV-1-infected children (paper I) ...............33
Natural history of HIV-1 RNA load in perinatally infected children (paper II) .... 34
Importance of blood fraction, specimen collection, processing and storage on viral load detection (papers I-IV) ........................................................................................................... 35
Coreceptor use in perinatally infected children (paper III) .................................. 36
Infectious viral load in children (paper IV) ............................................................. 37
Quantification of HIV-1 RNA in children infected with HIV-1 of different subtypes (paper II-V) .................................................................................................................. 39
Zidovudine monotherapy and viral load (paper I-IV) ............................................. 39
HIV-1 genetic subtype and coreceptor use (paper III) ......................................... 40
HIV-1-infected women in Sweden (paper V) ......................................................... 40
Mother-to-child HIV-1 transmission in Sweden during 1985-2003 (paper V) ...... 40
HIV-1-infected children in Sweden (paper V) ......................................................... 42
  AIDS-defining diagnosis ....................................................................................... 42
  Antiretroviral treatment ....................................................................................... 42
  Caregiver ............................................................................................................. 43
  Psychosocial support and disclosure .................................................................... 43
Conclusions and future perspectives .................................................................... 44
Acknowledgements ............................................................................................. 46
References ............................................................................................................ 48
Papers I-V
## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIDS</td>
<td>acquired immune deficiency syndrome</td>
</tr>
<tr>
<td>CCR5</td>
<td>CC chemokine receptor 5</td>
</tr>
<tr>
<td>CD</td>
<td>cluster of differentiation</td>
</tr>
<tr>
<td>CD4+ cells</td>
<td>helper/inducer T-lymphocytes</td>
</tr>
<tr>
<td>CD8+ cells</td>
<td>suppressor/cytotoxic T-lymphocytes</td>
</tr>
<tr>
<td>CDC</td>
<td>Centers for Disease Control and Prevention</td>
</tr>
<tr>
<td>CXCR4</td>
<td>CXC chemokine receptor 4</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>ECS</td>
<td>European Collaborative Study</td>
</tr>
<tr>
<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>env</td>
<td>envelope gene</td>
</tr>
<tr>
<td>gag</td>
<td>group-specific antigen gene</td>
</tr>
<tr>
<td>gp</td>
<td>glycoprotein</td>
</tr>
<tr>
<td>HIV-1 and -2</td>
<td>human immunodeficiency virus type 1 and 2</td>
</tr>
<tr>
<td>IgG</td>
<td>immunoglobulin G</td>
</tr>
<tr>
<td>NASBA</td>
<td>nucleic acid sequence-based assay</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>PBMC</td>
<td>peripheral blood mononuclear cells</td>
</tr>
<tr>
<td>pol</td>
<td>polymerase gene</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>R5 virus</td>
<td>virus which use CCR5 as a coreceptor</td>
</tr>
<tr>
<td>RT</td>
<td>reverse transcriptase</td>
</tr>
<tr>
<td>SIV</td>
<td>simian immunodeficiency virus</td>
</tr>
<tr>
<td>X4 virus</td>
<td>virus which use CXCR4 as a coreceptor</td>
</tr>
</tbody>
</table>
Introduction

A pandemic

The first reports of a new disease causing immunodeficiency and affecting homosexual men [1,2] and intravenous drug users [3] in the USA were published in 1981. The patients displayed reduced CD4+ cell counts and opportunistic infections and the disease was named acquired immune deficiency syndrome (AIDS). Soon thereafter, AIDS was reported also to affect other groups of people, such as, haemophiliacs, transfusion recipients and female sex partners of intravenous drug users. The first report of children with AIDS was published in 1982 [4]. The causative agent, later named the Human Immunodeficiency Virus (HIV) was identified and characterised in 1983-84 by two different research groups [5,6]. The first known child with HIV-1 and AIDS in Sweden died in 1982 after a clinical course compatible with AIDS and was retrospectively diagnosed as being HIV-1-infected by stored serum [7].

Initially, most reports of HIV-1 infection originated in the USA, but it soon became evident that all continents were affected. Since then, HIV-1 infection has emerged as a widespread pandemic affecting a large proportion of the human population. The impact of the infection is the greatest in Sub-Saharan Africa, but it is rapidly increasing in many other parts of the world such as Asia, the former Soviet Union and North Africa.

The geographical distribution of worldwide HIV infection is shown in figure 1. According to UNAIDS, 40 (34-46) million people in the world were living with HIV by the end of 2003.

Figure 1. WHO/UNAIDS estimates of adults and children living with HIV/AIDS at the end of 2003.
The number of HIV-infected children under 15 years of age in the world is estimated to be 2.5 (2.1-2.9) million (Figure 2). During the year 2003, 5 (4.2-5.8) million adults and 700,000 (590,000-810,000) children were newly infected with HIV and about 2.5 (2.1-2.9) million adults and 500,000 (420,000-580,000) children died of AIDS [8].

Figure 2. WHO/UNAIDS estimates of children (< 15 years old) living with HIV/AIDS at the end of 2003.

Despite low rates of new HIV infections in the industrialized world, the total number of people living with HIV continues to rise due to longer survival as a result of widespread access to antiretroviral treatment. In Europe, fewer than 100 children were estimated to have died of AIDS during 2003, compared to 400,000-540,000 children in Sub-Saharan Africa [8]. Also in Western Europe many newly reported HIV-infected individuals were infected in countries with high HIV prevalence.

Since the start of the HIV epidemic, 6299 individuals in Sweden have been reported to carry HIV virus. By the end of 2003, approximately 3200 people were living with HIV in Sweden [9].

The virus

Evolution, origin and genetic diversity

Two types of HIV can be distinguished, HIV-1 and HIV-2, of which HIV-1 is by far the most important. HIV-1 is divided into three groups: M (main), O (outlier) and N (non-M-non-O). Group M represents the main group of HIV-1 strains and has been subdivided into different subtypes. The group M subtypes are phylogenetically associated groups of HIV-1, and are labelled A1, A2, B, C, D, F1, F2, G, H, J and K [10]. The sequences within a subtype or sub-subtype are more similar to each other than to sequences from other subtypes throughout their genomes. Recombination of
genes from different subtypes can occur if a cell is infected with two different viral genomes. If an inter-subtype recombinant virus is transmitted from one patient to others, and becomes one of the circulating strains in the HIV epidemic, it can be classified as a circulating recombinant form [10]. Sixteen circulating recombinant forms (CRFs) are identified [10]. The most important intersubtype recombinants are CRF01_AE, CRF02_AG, and CRF03_AB.

HIV and the closely related African monkey virus Simian Immunodeficiency Virus (SIV) belong to the lentivirus subfamily of retroviruses. Both HIV-1 and HIV-2 are considered to have been transmitted to man from monkeys. HIV-1 is thought to have emerged from SIVcpz from the common chimpanzee (Pan troglodytes troglodytes) [11] that lives in an area where all HIV-1 groups have been found. These non-human-primate lentviruses do not induce an AIDS-like disease in their hosts, suggesting that they have been associated and evolved with their hosts over an extended period of time. The three HIV-1 groups (M, N and O) are the result of at least three independent cross-species events [12]. The phylogenetic relationship of primate lentivirus is shown in figure 3. An HIV-1 sequence has been found in a plasma sample from an African man dating from 1959 [13] and the time of origin of HIV-1 in humans has been estimated to be the 1930s [14,15].

![Figure 3. Phylogenetic relationship of primate lentiviruses. Alignment of pol gene sequences of HIV-1, HIV-2 and SIV strain SIV\textsubscript{m~ng}: SIV of sooty mangabeys or macaques experimentally infected with SIV\textsubscript{m~ng}. Reproduced with the kind permission of Human Retroviruses and AIDS (Los Alamos, New Mexico; Theoretical Biology and Biophysics Group, Los Alamos National Laboratory) (Kuiken et al., 1999).](image)

The genetic diversity of HIV-1 is a distinguishing feature of the AIDS epidemic. The associated evolution of variants may influence the ability of the immune system to control HIV-1 infection. Much of our understanding of the biology and pathogenesis of HIV-1 comes from analyses of subtype B, which predominates in Europe and the
United States, but is infrequent in Africa, where the prevalence of HIV-1 is the highest. A large proportion of the HIV-1 infected women in Sweden originate from areas where other subtypes than subtype B predominate. Thus we could expect to find individuals infected with a wide variety of subtypes in our studies made in Sweden. The principal geographical distribution of the different HIV-1 subtypes is depicted in figure 4.

Figure 4. Geographic distribution of the major HIV-1 genetic subtypes and circulating recombinant forms.

**Structure**

HIV-1 is a spherical virus with a diameter of approximately 100 nm. The virion consists of an outer layer, the envelope, built up by a lipid bilayer formed from the host cell membrane, and the glycoproteins gp 41 and gp 120. It also contains some cellular proteins, most notably HLA components. The inside of gp 41 is associated with the matrix protein p17 [16]. The cone-shaped nucleocapsid core is comprised of the major core protein p24 [16], which surrounds two copies of the viral RNA covered by two small core proteins, p9 and p7, as well as the virally encoded enzymes: reverse transcriptase, proteinase and integrase (Figure 5).

**Replication cycle**

The major target for HIV-1 is cells expressing the CD4 surface antigen, although other cell-surface molecules have also been demonstrated to function as receptors in certain cell types [17,18]. These CD4+ host cells are T-helper cells/lymphocytes and cells of the monocyte/macrophage lineage.

The viral life cycle begins when HIV-1 binds to the host cell (figure 6). The entry of HIV-1 into the cell is a multi-step process that is mediated by the viral env protein trimere. When gp120 binds to CD4, conformational changes are induced in the gp120 subunit, which leads to exposure of a previously hidden coreceptor binding site.
A complex is formed between the coreceptor, CD4 and gp120 and the interaction triggers the remaining changes in gp 120 and gp 41. The viral envelope fuses with the lipid bilayer of the target cell enabling the viral core to enter the cell. The nucleocapsid of the virion is then uncoated and released. The process of reverse transcription is initiated in the cytoplasm.

The double-stranded DNA copy which is generated from reverse transcription is translocated into the nucleus and integrated in the host genome. Successfully integrated DNA, which is called a provirus, may remain silent and latent until the host cell is activated [19]. The provirus is used as a template for production of viral genomic RNA and messenger RNA, which are translated into viral proteins. The new viral RNA forms the genetic material of the next generation of viruses.

The viral RNA and viral proteins assemble into intracellular immature particles. These bud from the cell membrane into a new virus. Amongst the viral proteins is HIV protease, which is required to process other HIV proteins into their functional forms. The HIV-1 protease cleaves precursor poly-proteins into the right sizes after virus particles have budded from the cell surface [20]. Thereby, the virus has completed its maturation and can infect another cell.

**HIV-1 coreceptor use**

Whereas CD4 is the major receptor used by HIV-1 for entry into susceptible cells, the chemokine receptors CCR5 and CXCR4 can be used as coreceptors [21]. Our knowledge of HIV-1 coreceptor use originates from 1995-96 when CC-chemokines were found to inhibit HIV-1 replication [22] and CXCR4 was found to be a coreceptor for some strains of HIV-1 [23]. Soon it was demonstrated that the counterpart to CXCR4 for the most frequently transmitted strains of HIV-1 was
Complete absence of the CCR5 receptor, which is seen in homozygosis for the defective ∆32-CCR5 allele, results in strong protection against HIV-1 infection \textit{in vitro} and \textit{in vivo}, while decreased CCR5 expression caused by heterozygosis for the ∆32-CCR5 allele reduces the rate of disease progression in infected individuals [25,26].

Figure 6. Retrovirus replication cycle. Adapted with the kind permission of Access Excellence @ the National Health Museum, Washington, DC, http://www.accessexcellence.org/AB/GG/retrovirus.html.

A unifying nomenclature that classifies HIV-1 according to its coreceptor use has been adopted [27]. HIV-1 isolates can be classified into two major groups based on the ability to use either CCR5 or CXCR4 as coreceptor [21]. The corresponding phenotypes of the viruses are called R5 and X4, respectively, or R5X4 when both coreceptors are used [27]. Earlier, HIV-1 phenotypes were distinguished by their ability to induce syncytia in MT-2 cells [28], which indicated use of the CXCR4 receptor. Experiments later showed that syncytium-inducing virus was synonymous with virus using CXCR4 as coreceptor [29]. The emergence of X4 virus has been associated with disease progression in adults [30] and children [31]. It was not possible, however, to discern whether the appearance of new viral phenotypes preceded disease development or appeared as a consequence [30,31]. On the other hand, the appearance of syncytium-inducing virus has been observed in children as an age-related and late phenomenon [32,33]. Most studies have found a disease progression in children similar to that in adults [33,34], whereas others did not find this correlation [35]. Ability to use different coreceptors has been demonstrated for all HIV-1 subtypes, although subtype C has been reported rarely to use CXCR4 [36,37].
**HIV-1 transmission**

HIV-1 is transmitted through infected blood and body fluids. The routes of transmission are through sexual intercourse (heterosexual and homosexual between men), needle sharing during intravenous drug use, blood products, unsterile healthcare procedures and mother-to-child transmission. HIV-1 transmission in the developing world has traditionally been considered to be mainly heterosexual even though this opinion has been questioned by authors who claim that unsterile healthcare procedures may contribute as much as 20-40% to HIV-1 infection in Sub-Saharan Africa [38]. This theory has not been adopted by most authors [39] and WHO recently estimated that only 5% of HIV-1 infections in the region were caused by unsafe injections [40]. Initially, most HIV-1-infected individuals in the industrialized world were men who had sex with men and illicit drug users, but the panorama in Europe has changed, and an increasing proportion of HIV-1-infected individuals are now immigrants from areas where HIV-1 is endemic.

Sixty percent of the infected individuals in the world are women [41]. Vertical transmission from the mother is the most common route of transmission to children, accounting for at least 90% of paediatric HIV-1 infection worldwide [42].

**Mother-to-child transmission**

Transmission of HIV-1 from an infected mother to her child can occur before, during or after birth. Before the era of prophylactic treatment, the prevalence of mother-to-child transmission was 15-25% in industrialized countries, provided the mothers did not breast-feed [43-45], but studies from breast-feeding settings in Africa have shown transmission rates as high as 40-50% [46,47].

Among non-breast-feeding women, approximately 60-70% of infected children become infected during delivery, and the remaining 30-40% are thought to be infected in utero [48,49]. The vast majority of the children infected in utero are infected during the last months of pregnancy [50-52]. Studies on foetuses have shown that transmission during the first trimester appears to be rare [53]. The infection rate during the second trimester is reported to be 2-5% [54,55].

Several studies have demonstrated HIV-1 transmission through breast-feeding [56,57]. The additional risk of HIV-1 transmission through breast-feeding is approximately 15% [58] and, in some settings more than 40% of mother-to-child transmissions are attributed to breast-feeding [59]. Exclusive breast-feeding has been found to be less associated with HIV-1 transmission than mixed bottle-feeding and breast-feeding [60]. Exclusive breast-feeding for a limited amount of time is an acceptable and feasible option for HIV-1-infected women in settings where breast-feeding cannot be safely replaced by formula-feeding [61].
Risk factors
Maternal viral load and immune status are independent risk factors for mother-to-child transmission [62], and the most important single maternal risk factor is viral load, which is more strongly correlated with transmission than is immune function as measured by the CD4+ absolute count and percentage [63,64]. Low vertical transmission rates have been observed among women with undetectable virus (<500 copies/ml) in late pregnancy when more than 95% of the mothers were treated with one or two antiretroviral agents [63-65], as well as among women receiving combination antiretroviral therapy [66-68]. Indeed, the use of zidovudine or combination antiretroviral therapy appears to have an effect separate from its effect on viral load, in the prevention of mother-to-child transmission [64,69]. No safe HIV-1 RNA level below which transmission does not occur has been identified, although the risk for transmission at levels below 1000 copies/ml is very low [62,70,71]. Virus may however be present in the birth canal but still undetectable in plasma [72]. Several other maternal risk factors for vertical transmission have been identified, including age [73], duration and severity of disease [44] and in all probability, primary infection during pregnancy.

Maternal smoking, illicit drug use and unprotected sexual intercourse with multiple partners during pregnancy are behavioural factors that increase the risk for transmission [74,75] where prophylactic intervention is possible. Chorioamnionitis, premature rupture of the membranes, low birth-weight and preterm delivery are obstetric and foetal factors associated with HIV-1 transmission, which are partly susceptible to intervention.

The role of the mode of delivery has been examined thoroughly. Available data show an advantage of caesarean section before labour and rupture of the membranes, over vaginal delivery, and independently of the maternal viral load [76].

Prevention
Great advances have been made during the last ten years in the prevention of mother-to-child transmission. In the ACTG 076 study [77] from 1994, the transmission rate decreased from 25% to 8% with zidovudine prophylaxis, which was given orally to the mother from gestational week 14-34, intravenously during delivery and orally to the child during the first six weeks of life. Antiretroviral combination treatment of the woman and zidovudine during the first six weeks of life to the child in combination with elective caesarean section has further reduced transmission rates in industrialized countries, which have decreased from 14-25% [45] to less than 2% provided the mothers do not breast-feed [71]. In the USA, elective caesarean section is recommended for women with HIV-1 RNA > 1000 copies/ml near the time of delivery. According to the American College of Obstetricians and Gynecologists, elective caesarean section probably would not provide additional benefit in reduction of transmission if the woman is treated with antiretroviral therapy and the plasma HIV-1 RNA is below 1000 copies/ml [78]. Recent data from the European Collaborative Study do indeed support the conclusion that elective caesarean section further reduces the mother-to-child transmission rate even in women with an undetectable viral load treated with combination antiretroviral therapy [79]. Elective
caesarean section is recommended to all pregnant HIV-infected women in Sweden irrespective of the viral load [80].

Several short-term regimens of antiretroviral prophylaxis have been tried in resource-limited settings. A single dose of nevirapine to the women before delivery and to the newborn child [81] reduces the transmission rate by 50%, is cheap and can be administrated in resource-limited settings. Unfortunately, postnatal transmission through breast-feeding remains substantial also in children treated with short-term antiretroviral prophylaxis [82].

It is not clear whether infants of women on combination antiretroviral therapy with undetectable virus levels are at risk for transmission of HIV-1 through breast-milk. Current interventions to reduce vertical HIV-1 transmission in industrialized countries include the reduction of the maternal viral load by antiretroviral therapy, the avoidance of exposure to contaminated maternal secretions through delivery by elective caesarean section and avoidance of breast-feeding. Prevention strategies in breast-feeding settings remain a more difficult challenge. In one study, exclusive breast-feeding had advantages over both mixed feeding and bottle-feeding [60] and is an option when formula feeding is not feasible. In Sweden, breast-feeding is prohibited by law when the mother is HIV-infected [83].

**Negative effects of prophylaxis against mother-to-child transmission**

Short-term and incomplete antiretroviral prophylaxis increases the risk for viral resistance to antiretroviral drugs. It is well documented that single-dose nevirapine prophylaxis induces viral resistance [84]. At least 15% of pregnant HIV-1-infected women who receive single-dose nevirapine have one or more detectable non-nucleoside reverse transcriptase mutations [85].

The possible risk of future negative side-effects for the child exposed to antiretroviral drugs in utero is of great concern. The French Perinatal Cohort Study Group described the risk of persistent mitochondrial dysfunction in children exposed to nucleoside analogues during the perinatal period [86,87]. In an analogous retrospective study in the USA, no such dysfunction was observed [88]. However, mitochondrial dysfunction after antiretroviral exposure has recently been reported in infants in two reports from the USA [89,90].

**Pregnancy screening**

Routine voluntary HIV-antibody testing of pregnant women has been widely used in Sweden since 1987 [91]. The acceptance rate has been high, ranging from 90% to 99% in different geographic areas. This national screening programme has contributed substantially to our knowledge of the total number of HIV-infected women who have given birth to children in Sweden and made preventive interventions possible. Continued follow-up of children exposed to antiretroviral drugs in utero, both HIV-infected and uninfected ones, is important.
Diagnosis

Testing for HIV-1 antibodies is of little diagnostic value in early infancy in children born to HIV-1-infected mothers, as maternal IgG antibodies are transferred across the placenta. The median age for their disappearance in uninfected children is 10 months [92], but they can persist until 18 months of age [93]. Methods for detecting p24 antigenaemia were initially insensitive, although immune complex dissociation by acid or heat have increased the sensitivity markedly [94,95]. Virus culture and the polymerase chain reaction (PCR) for the detection of HIV-1 DNA are reliable methods for early diagnosis of HIV infection. Quantitative measurements of HIV-1 RNA have been shown to be as reliable as DNA PCR and virus culture [96,97]. At one week of age, 38% of HIV-infected children have a positive PCR test [48], indicating in utero transmission. In the remaining proportion of vertically HIV-infected children of non-breast-feeding mothers with negative HIV detection tests during the first week of life, tests taken thereafter become successively positive, indicating virus transmission close in time to or during the delivery [48].

A positive PCR test at birth might, however, also be due to contamination by maternal blood [98]. A small number of HIV-exposed seroconverting infants have been reported to have positive cultures and/or PCR tests without being infected [99-101]. Thus, both positive and negative virus detection tests performed early in life might be associated with uncertainty in the diagnosis of HIV-1 infection. In the study by Dunn et al. [48], 271 non-breast-fed perinatally HIV-1-infected children were tested by PCR during the first week of life and followed prospectively. The frequency of PCR-positive results was stable during the first week of life and increased rapidly thereafter. The proportion of exposed infants exhibiting positive HIV-1 DNA and/or RNA tests was found to increase to 96.2% at 4-6 weeks and to 100% at 7 weeks of age [97]. Two negative HIV cultures between months 1 and 6 have been shown to identify uninfected infants with a specificity of more than 99% [102].

Early diagnosis of congenitally or perinatally acquired HIV infection is important. Identified children can then be monitored carefully for immunological, virological and clinical abnormalities, and prophylactic and therapeutic measures can be instituted.

Clinical course of HIV-1 infection

HIV-1-infected adults may exhibit a febrile primary infection with sore throat, rash and enlargement of the lymph nodes [103]. This has not been reported in vertically infected infants.

The symptoms associated with HIV infection in children under 13 years of age are categorized by the Centers for Disease Control and Prevention (CDC) into four clinical categories of varying severity [104]. Symptoms related to AIDS are found in category C, except for lymphoid interstitial pneumonitis (LIP), which is classified in category B (table 1).
<table>
<thead>
<tr>
<th>Category</th>
<th>Clinical Categories</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Category N: Not Symptomatic</strong></td>
<td>Children who have no signs or symptoms considered to be the result of HIV infection or who have only one of the conditions listed in category A.</td>
</tr>
</tbody>
</table>
| **Category A: Mildly Symptomatic** | Children with two or more of the following conditions but none of the conditions listed in categories B and C:  
- Lymphadenopathy ( > 0.5 cm at more than two sites; bilateral = one site)  
- Hepatomegaly  
- Splenomegaly  
- Dermatitis  
- Parotitis  
- Recurrent or persistent upper respiratory infection, sinusitis or otitis media |
| **Category B: Moderately Symptomatic** | Children who have symptomatic conditions, other than those listed for category A or category C, that are attributed to HIV infection. Examples of conditions in clinical category B include, but are not limited to the following:  
- Anaemia (< 80 g/l), neutropenia (< 1000/mm$^3$), or thrombocytopenia (< 100,000/mm$^3$) persisting > 30 days  
- Bacterial meningitis, pneumonia or sepsis (single episode)  
- Candidiasis, oropharyngeal (thrush) persisting for > 2 months in children aged > 6 months  
- Cardiomyopathy  
- Cytomegalovirus infection with onset before age one month  
- Diarrhoea, recurrent or chronic  
- Hepatitis  
- Herpes simplex virus (HSV) stomatitis, recurrent (more than two episodes within one year)  
- HSV bronchitis, pneumonitis or oesophagitis with onset before age 1 month  
- Herpes zoster (shingles) involving at least two distinct episodes or more than one dermatome  
- Leiomyosarcoma  
- Lymphoid interstitial pneumonia (LIP) or pulmonary lymphoid hyperplasia complex  
- Nephropathy  
- Nocardiosis  
- Fever lasting > 1 month  
- Toxoplasmosis with onset before age 1 month  
- Varicella, disseminated (complicated chickenpox) |
| **Category C: Severely Symptomatic** | Children who have any condition listed in the 1987 surveillance case definition for acquired immunodeficiency syndrome, with the exception of LIP (which is a category B condition). |

Early clinical symptoms of HIV infection in infants are seldom diagnostic, often non-specific and may be temporary. Lymphadenopathy, hepatomegaly, splenomegaly, dermatitis, parotitis and recurrent or persistent upper respiratory infection, sinusitis, or otitis media are such symptoms. Recurrent bacterial infection is indeed not an uncommon feature in children not infected by HIV, but HIV-infected children may have common illnesses, including recurrent bacterial infections, that are more severe, frequent and persistent than in other children. For example, the risk for invasive pneumococcal infection was found to be 10-fold higher in HIV-infected children than in HIV-exposed but non-infected children [105]. Two or more systemic bacterial infections (bacteraemia, pneumonia, meningitis, osteomyelitis, septic arthritis, purulent pericarditis) within a two-year period are AIDS-defining in children (table 1).

The most common AIDS-defining illness in children is *pneumocystis carinii* pneumonia (PCP) which accounts for 33% of AIDS-defining conditions in children under 13 years of age. The risk for HIV-infected children not receiving PCP prophylaxis or antiretroviral therapy during the first year of life to acquire PCP is reported to be 20% [106]. PCP occurs most frequently at 4-6 months of age [107] in vertically HIV-infected children, often with an acute onset and a poor prognosis. HIV-infected children under 1 year of age are at risk for PCP even with a CD4+ cell count ≥ 1500 x 10^6 cells/l. Prophylaxis is effective, which emphasizes the importance of knowing the HIV status of delivering mothers.

HIV-1 infected children are also at increased risk for several other opportunistic infections such as disseminated *Mycobacterium avium* complex (MAC), disseminated cytomegalovirus (CMV), *Mycobacterium tuberculosis* and fungal infections.

Before the antiretroviral treatment era, approximately 20% of HIV-1-infected children in Europe developed AIDS before the age of 1 year, and nearly 40% by age 4. Ten per cent died before age 1 year and nearly 30% before 5 years of age [108]. It has been suggested that children infected at or close to birth are the ones who will have the greatest chance to remain asymptomatic for a long period of time, whereas those who are infected in utero will develop symptoms early in life. This hypothesis is supported by some studies [49,109], but others have found no association between the timing of infection and clinical outcome [110,111]. High levels of HIV-1 RNA at birth and during primary viraemia have been associated with an early onset of symptoms and rapid disease progression [112]. In addition, high maternal HIV-1 RNA at or close to delivery have been found to predict disease progression in HIV-1-infected infants, and correlate with the early peak of viraemia in the infected child [113]. Other studies indicate that disease progression in infected infants may be more related to certain characteristics of the virus [114], or the maternal stage of disease [114-116], than to the time of transmission.

A minor proportion of HIV-infected adults, so-called long-term non-progressors, remain healthy and maintain normal CD4+ cell counts for at least 10 years without antiretroviral therapy. Vertically infected long-term non-progressing children have been studied less. In an Italian study conducted before the antiretroviral treatment era only 6% of the children were asymptomatic at 5 years of age [115]. One fifth of the
8–15-year-old children in a cross-sectional study had never had an AIDS-defining condition and had a CD4+ cell count above 500 x 10^6 copies/ml [117]. The majority of these children in the latter study had received single or dual nucleoside reverse transcriptase inhibitor therapy.

**Viral load**

Great advances have been made in the monitoring of HIV-1 infection. Quantitative HIV-1 RNA has been shown to correlate with disease progression and mortality in HIV-1-infected adults [118] and children [34,119-123]. HIV-1 RNA is the most used parameter to measure viral load, although it does not distinguish between infectious and non-infectious virus. Nevertheless, it is often assumed that HIV-1 RNA levels in serum and plasma are direct reflections of infectious virus. RNA in virus particles usually represent infectious virus, but studies from other virus families have shown that it may also be defective [124-126]. DNA in lymphocytes represent latent virus which, under certain conditions, can be activated. This may result in the production of both infectious and non-infectious virus. The details of the initiation and regulation of HIV-1 expression are not well known and the temporal pattern of intracellular and extracellular viral loads has not been thoroughly described.

**Viral dynamics in acute infection**

Acute HIV-1 infection in adults is characterized by the appearance of high levels of HIV-1 RNA in plasma and serum. The high levels spontaneously abate after approximately two months [127] (Figure 7). When we initiated our study of viral dynamics in vertically HIV-1-infected children, the temporal pattern of HIV-1 RNA was not completely clear, but turned out to be different from that seen in adults. High HIV-1 RNA levels are present in plasma and serum at 1.5-3 months of age [120,128]. HIV-1 RNA measurements have shown decreasing levels over time in a cross-sectional study [120] and, in a cohort of children under clinical care [128], to reach minimum levels at 5-6 years of age [120,128,129]. The high viral load in infants during the period of very high numbers of circulating CD4+ lymphocytes [130] has been suggested to result from the replication of HIV within rapidly proliferating target cells in a developing immune system [131-133].

Quantitative measurements of replicating virus could be done in two principally different ways. The sample could be diluted (limiting dilution) before culture or the time to culture positivity could be used as a quantitative measure. A higher viral load could be expected to result in a shorter time to culture positivity. There are some published studies on quantitative measurements of infectious HIV-1 in children, but none on concordant quantitative virus cultures from plasma and peripheral blood mononuclear cells (PBMC) in prospectively followed vertically infected children and none of them compare the amount of infectious virus with the RNA load [134-137]. Ikeda et al. used decreasing numbers of cells in culture and days-to-culture positivity. They found that quantitative measurements of HIV-1 in PBMC were more sensitive as indicators of infection status than quantification of p24 antigen [137]. In another study, end-point dilution cultures of both plasma and cells were assessed in 46 neonates. Plasma and, to a lesser extent, the cell-associated viral burden was
associated with rapid progression of the disease when measured during the first 6 months of life [136].

**Viral load and clinical course**

In HIV-1-infected adults the number of HIV-1 RNA copies in plasma correlates directly with the risk of progression to AIDS and death [118] and is useful in assessing the effect of antiretroviral therapy [138]. Also in infants and children, the plasma HIV-1 RNA load was shown to predict disease progression [34,120-122] quite early on, and this has later been confirmed [123]. However, the interpretation of HIV-1 RNA determinations in children was more difficult than in adults before the temporal pattern of plasma viraemia in non-selected and prospectively followed vertically infected infants and children was known.

![Figure 7](image.png)

**Figure 7.** Typical course of HIV infection in adults, modified from Fauci et al., Ann Intern Med 1996 [139]. Adapted with the kind permission of F. Hoffmann-La Roche AG, Basel, Switzerland.

**Immune status and clinical course**

Absolute numbers of circulating lymphocytes, including CD4+ cells, have been found to be higher in infants and small children than in adults, and to decline over the first 4-5 years of life [130]. The CD4+ percentage is more independent of age and displays less variability. Both the CD4+ cell count and the CD4+ percentage correlate with
disease progression in perinatally HIV-1-infected children and appear to be a better prognostic tool than HIV-1 RNA [123].

For surveillance purposes the CDC developed a system in 1987 for the classification of HIV-infected children according to their clinical and immunological status. This system was revised in 1994 [104] and is widely used for scientific and clinical purposes (tables 1 and 2). Once classified, an HIV-infected child cannot be reclassified into a less severe category even though the child’s condition improves, making this system less suitable nowadays when antiretroviral therapy often results in clinical and immunological improvement.

Table 2. 1994 Revised Human Immunodeficiency Virus Paediatric Classification System: Immune Categories Based on Age-Specific CD4\(^+\) T Cell and Percentage*

<table>
<thead>
<tr>
<th>Immune category</th>
<th>&lt; 12 mos</th>
<th>1-5 yrs</th>
<th>6-12 yrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category 1: No suppression</td>
<td>≥ 1500</td>
<td>≥ 25</td>
<td>≥ 1000</td>
</tr>
<tr>
<td>Category 2: Moderate suppression</td>
<td>750-1499</td>
<td>15-24</td>
<td>500-999</td>
</tr>
<tr>
<td>Category 3: Severe suppression</td>
<td>&lt; 750</td>
<td>&lt; 15</td>
<td>&lt; 500</td>
</tr>
</tbody>
</table>


Social situation and stigma

As a consequence of the decreased mother-to-child transmission rates and the improved therapeutic strategies in the industrialized countries, there is an increasing number of healthy uninfected children born to HIV-infected women [140] and of HIV-infected children surviving on antiretroviral combination therapy. Children born to HIV-1-infected mothers comprise a vulnerable group, whether or not they are infected themselves. Living in families with one or two caregivers carrying a potentially deadly disease means that all these children are affected by HIV in some way. Illness or death of the mother (and/or father) creates a need for social care and support from non-parental sources [114,140-142]. Parental use of illicit drugs and/or financial or immigration problems [140,143-145] also affect the psychosocial environment of the child [146].
Aims of the study

The aims of the study were:

1. to evaluate a possible relation between the age of the child when virus was first detected and the appearance of symptoms and disease progression in vertically HIV-1-infected children. Furthermore, we looked specifically for symptoms compatible with primary HIV-1 infection.

2. to describe the natural history of viraemia over time in vertically infected children by estimating the number of HIV-1 RNA copies/ml in plasma and/or serum at different ages.

3. to document the evolution of coreceptor use of HIV-1 isolates of different subtypes, obtained from both plasma and peripheral blood mononuclear cells (PBMC), in vertically HIV-1-infected children and the relation to disease progression.

4. to investigate the dynamics of HIV-1 infectious viral load in plasma and PBMC and how this relates to HIV-1 RNA in prospectively followed children.

5. to describe the HIV-1 epidemic in mothers and their children in Sweden and explore demographic characteristics and vertical transmission rates over time. Furthermore, we performed a long-term follow-up to evaluate the medical and family situation of children infected or affected by HIV-1.
Patients and methods

Patients
All children in Sweden reliably shown to have been born to an HIV-1-infected woman and the mothers themselves were included. Children who were born in Sweden between 1985 and 31 December 2003 were followed prospectively from birth, if parents/caregivers agreed, and from 1987, they were also enrolled in the European Collaborative Study (ECS) [44,92,108]. Other children were included when they came to our knowledge at a later age if the parents/guardians agreed. Information about the mothers and their children was obtained through our contacts as a national resource centre for HIV-infected pregnant women and children and from the Swedish Institute for Infectious Disease Control (SMI) [9], the main task of which is surveillance of communicable diseases and analyse of the current epidemiological situation in Sweden. All patients in Sweden with an HIV-1 diagnosis are reported mandatorily by code by clinicians and laboratories.

Adopted children from other countries who were antibody-positive at arrival in Sweden and then seroreverted were not included in the study. We are aware of 20 such cases, but there is no reporting system for this group and the actual number in Sweden is not known.

All mothers were counselled not to breast-feed their children, and, furthermore, Swedish law does not allow HIV-infected women to breast-feed [83]. From 1994, mothers and children were recommended zidovudine prophylaxis to reduce vertical transmission according to the ACTG-076 protocol [77]. Elective caesarean section was routinely recommended from late in 1998 and onwards. Combination antiretroviral regimens were recommended and more and more used in pregnancy during the following years.

Paper I
One hundred and seventeen children born in Sweden between June, 1985, and March, 1998, whose mothers were identified as being HIV-1-infected before or at the time of delivery were included in the study and monitored from birth for 18 months or more.

Paper II
Thirty-two infected children were studied. Twenty-one were followed prospectively from birth and included all at that point in time identified vertically HIV-1 infected children born in Sweden between March, 1987, and September, 1997. Eleven children were included when they were identified as being HIV-1-infected at the age of 7-89 (median 61) months and were assumed to be vertically infected because their mothers were HIV-1-infected.

Paper III
Twenty-four vertically HIV-1-infected children born between March, 1987, and September, 1994, were included. Nineteen children were followed prospectively from
birth and five were identified later and assumed to be vertically infected because their mothers were HIV-1-infected.

**Paper IV**

Sixteen consecutive vertically HIV-1-infected children, born between March, 1987, and October, 1993, were followed prospectively from birth. They comprise all vertically HIV-1-infected children born in Sweden during that time, with the exception of one whose mother refused participation.

**Paper V**

Four hundred and nineteen children in Sweden, born between 1982 and 31 December 2003, and reliably shown to have been born to an HIV-1-infected mother, were included.

**Clinical examination and blood sampling (papers I-V)**

Children who were followed from birth were examined at birth and, before 1991, at 3-monthly intervals to 18 months of age and, from 1991, also at 6 weeks and 4.5 months of age [108]. Infected children were then followed at least 3-monthly according to their needs. After 18 months uninfected children were followed once every 1-2 years according to an agreement with their caregiver. Blood samples were collected on these occasions up to 18 months of age, except at 4.5 months of age. After 18 months, blood samples were collected from infected children every three months or according to their needs.

Cord blood was not used. Defibrinated blood (heparin before, and EDTA from, 1990) was used for virus culture from plasma and PBMC, determination of the number of CD4+ and CD8+ lymphocytes and of HIV-1 DNA PCR and quantitative HIV-1 RNA. The transport period from venepuncture to the laboratory did not exceed 24 hours. Serum was used for p24 antigen determination and antibody tests. The remaining plasma was stored at -70° C and serum at -20° C.

**Virus isolation (papers I, III, IV)**

Fresh peripheral venous blood, 5 ml if possible, was collected on each sampling occasion. It is not always easy to obtain blood from young children, for which reason the sample volumes were often smaller. Whenever the volume of blood allowed, virus isolation from both PBMC and plasma was performed in parallel. Plasma volumes between 1 and 5 ml were ultracentrifuged and the virus-containing pellet was added to 3–5-day-old cultures of phytohaemagglutinin (PHA)-stimulated PBMC (20 x 10^6 cells/flask) from blood donors. PBMC from patients (6-14 x 10^6) were added to PHA-stimulated PBMC to a final concentration of 20 x 10^6 cells/flask after Ficoll-Isopaque separation, centrifugation and counting of viable cells. All cultures were maintained for up to 6 weeks. The culture supernatants were assayed once a week for the presence of HIV-1 p24 antigen. Cultures were considered positive when the absorbance level of p24 antigen was higher than the cut-off level on two successive occasions, with increasing values in the weekly analyses, or reached the maximum level (≥ 2.0) at once.
Detection of HIV-1 p24 antigen (papers I, III, IV)

A capture enzyme immunosorbent assay (ELISA) (Abbott, USA) was used for the detection of HIV p24 antigen in serum and in culture supernatants. The specificity was confirmed by an Abbott neutralization assay in the first positive sample.

Quantification of infectious virus (paper IV)

Limiting dilution

Limiting dilution HIV-1 isolation [147,148] was performed in plasma from the sample following a patient's first positive HIV-1 isolation. As the volume of plasma was often small, volumes of 1-3 ml were ultracentrifuged and regarded as undiluted. Irrespective of the starting volume, 0.5 ml, 50 µl, 5 µl and 0.5 µl were used for the ten-fold dilution steps. These volumes were initially added to individual flasks and kept for four weeks. Later the method was changed for economical reasons and these volumes were added in duplicates, as compensation for less time, to a 24-well plate covered with plastic and kept for two weeks without a change of medium or lymphocytes.

Days-to-culture positivity

In positive cultures, the number of days after the start of the culture to the p24 antigen value was higher than the cut-off limit was calculated. This interval was called days-to-culture positivity [149-151]. The values are presented in inverted form as an infectious index, thus ranging from 0 to 1, with a higher value associated with the presence of more infectious virus than a lower one.

Determination of coreceptor use by HIV-1 (paper III, IV)

Blood from all samples obtained for diagnosis and from most samples obtained during the first year was subjected to virus isolation, and thereafter from samples taken every six months or full year. One hundred and fifty-seven HIV-1 isolates from the initial isolation or the first passage were available and used to infect phytohemagglutinin-stimulated PBMC from two healthy blood donors [149].

The coreceptor use of the virus isolates was determined using human astrogliaoma cell lines U87.CD4 expressing CD4 and either of the chemokine receptor CCR1, CCR2b, CCR3, CCR5 or CXCR4 [21] and the human osteosarcoma cell line GHOST(3), expressing CCR5, CXCR4 or the orphan receptor BOB or Bonzo and CD4 [152]. Briefly, different U87.CD4 cells were co-cultured with supernatants of PBMC inoculated with the different primary isolates for 10 days. The cell cultures were observed for cytopathic effects (syncytium formation) indicating coreceptor use. For confirmation, an ELISA was performed to determine the amount of p24 antigen in supernatants. The expression of the green fluorescent protein, GFP, in the GHOST cell line was measured in a fluorescent cell sorter.
Quantitative HIV-1 RNA (paper I-IV)

For quantification of HIV-1 RNA we used the NASBA (Organon Teknika, Boxtel, The Netherlands) method. First, the sample, containing wild type HIV-1 virus, is added to guanidine thiocyanate (GuSCN) lysis buffer. Three RNA internal standards of known concentration are added to the lysis mixture before extraction. The internal standard RNAs only differ from the wild-type RNA and each other by a 20-nucleotide randomized sequence with the same nucleotide composition. Silica particle solution is added, binding all nucleic acid (DNA and RNA) in the lysate. The silica particles are then washed, dried and eluted in a buffer solution. A primer mix is added to the extracted nucleic acids, followed by a short incubation first at 65°C and then at 41 °C, followed by the addition of enzyme mix and incubation at 41°C for 90 minutes. The nucleic acid amplification then occurs using specific primers derived from the gag region of the genome. This cycle is repeated and results in exponential amplification (1 million to 1 billion-times) under isothermal conditions. The amplified RNA is hybridized to capture probes attached to magnetic beads, which are captured on the surface of an electrode by means of a magnet. The amount of nucleic acid representing wild-type virus as well as the internal RNA standards is finally determined directly by electrochemiluminescence.

HIV detection by DNA PCR (paper I)

HIV-1 DNA PCR was run in a semi-nested fashion, employing at least two different sets of primers from the gag, pol and/or env region of the HIV-1 genome [50,153]. The PCR product was visualized after electrophoresis in an agarose gel with ethidium-bromide and photographed using a Polaroid camera. In these cases, the test was defined as positive only if it was positive in at least two primer pairs. However, in retrospective HIV-1 DNA PCR analyse in children whose infectious status was already known, often only primers for the gag gene were used.

Determination of genetic subtypes (papers II-V)

The genetic subtype [154,155] of the virus carried by the infected individuals was determined by sequencing viral DNA from the PBMC [156] or after reverse transcription of patient plasma RNA. In one case, PBMC from a virus culture were used. The HIV-1 V3 region of the envelope gene was amplified by a nested PCR [156] and the PCR product was sequenced with a Big Dye terminator cycle-sequencing Ready Reaction kit (PE Biosystems, Foster City, CA). The sequences were detected with an ABI Prisma 377 DNA sequencer. The genetic subtype was determined by phylogenetic tree analysis, using the programmes DNADIST and NEIGHBOR in the PHYLIP package [157] and recommended reference sequences from the Los Alamos Database [10]. Seven subtypes had been determined previously [158] using a heteroduplex tracking assay (HTA).

Clinical and immunological classification

Clinical and immunological status was classified according to the Centers for Disease Control and Prevention (CDC) classification [104] (tables 1 and 2).
Diagnostic criteria

The children were regarded as infected if HIV-1 antibodies persisted after 18 months of age, or if two HIV-1 virus detection tests were positive on different sampling occasions. Children were regarded as uninfected if they lost the HIV-1-specific antibodies (papers I-IV). For the calculation of transmission rates (paper V), we also defined children as uninfected if they were six months of age or more and had had at least two negative virus detection tests (HIV-1 DNA PCR and/or HIV-1 RNA) on two different sampling occasions after the neonatal period (0-7 days), and the latest at or close to (within one month) six months of age.

Statistical analysis

The statistical methods used are indicated in each paper. The analyses were performed using the JMP and SAS 8.2 software packages from SAS Institute, Inc. (Cary, NC, USA).

Ethical consideration

The study was approved by the Ethical Committee of Karolinska Institutet, Stockholm, Sweden (nos. 89:264, 98-184 and 03-299).
Results and discussion

HIV-1 detection in perinatally HIV-1-exposed children (paper I)

Twenty-five per cent of the HIV-1-infected infants (4 out of 16), in whom a virus culture was performed at or close to their birth (0-4 days of age), had a positive test result, as well as 33 % (4 out of 12) of those in whom an HIV-1 PCR test was performed during the same period. For logistic reasons, we used the range 0-4 days even though a positive HIV-1 detection test within 48 hours from birth has been proposed to identify an infant with early (in utero) infection [159]. On the other hand, Dunn et al. found a positive HIV-1 PCR rate of 38% on the day of, or the day after, birth. No major change in the virus detection rate was apparent over the first week of life, whereupon the detection rate rose rapidly in the second week of life to 93%. This may indicate that one week corresponds to the incubation period for infection at or close to delivery. Our finding of 33% virus detection during days 0-4 is in accord with the results of previous studies where the virus detection rate during the first week of life was 30-50% in perinatally HIV-1-infected children [48,102,160,161] and may illustrate the relative distribution between infection in utero and infection at or close to the time of birth [48,159,162].

At 4–8 weeks of age, all cultures, HIV-1 DNA PCR assays and quantitative HIV-1 RNA tests performed in infected infants in our study were positive, with the exception of one viral culture from lymphocytes. This might indicate a high viral load at this time, which is in accord with other studies [121] and supports studies [96,102] showing the highest virus detection rate using virus culture and/or DNA PCR at this age in perinatally acquired HIV-1 infection.

In two children, the time to the first virus detection was remarkably long, 8-9 months, despite repeated virus detection tests before this age. Indeed, there was a striking covariance between the appearance of viraemia, affection of the immune status and, in one child, the debut of clinical symptoms. Postpartum transmission through breast-feeding was unlikely as the children were closely followed, bottle-fed and there were no indications that they were given breast milk. On considering the very large number of children followed prospectively and reported in the literature, it becomes obvious that these two children were diagnosed extremely late. It is now generally accepted that HIV-1 infection in perinatally exposed children can be excluded by 6 months of age if adequate sampling and testing is instituted and the children are not breast-fed.

HIV-1 detection tests were positive in two children eventually identified as uninfected. One of 338 (0.3%) plasma cultures and 1 of 316 (0.3%) HIV-1 DNA PCR tests from two different children were positive in this group. From one of these children, blood samples were not taken within the first 4 days of life. Virus culture at week 2 was positive in plasma but negative in PBMC. The virus isolation test on plasma became positive after 1 month of culture. However, the culture became contaminated by bacteria and was discarded without storage of the remaining material. HIV-1 DNA was demonstrable in PBMC with primers for the env gene, but
not with primers specific to the \textit{gag} and \textit{pol} genes. Subsequent cultures and PCR tests were negative. However, IgM antibodies against HIV-1 were detected at months 6 and 9, and IgA at month 6. Seroreversion had occurred at 11.5 months of age. The second child had a positive HIV-1 DNA PCR (two primers: \textit{gag} and \textit{pol}) on day 1. HIV-1 DNA was detected by one primer (\textit{pol}) but not by another (\textit{gag}) at 7 weeks. HIV cultures were negative at day 1 and 7 weeks. Subsequent cultures and PCR tests were negative and seroreversion had occurred at 21 months of age. Both children had a normal clinical and immunological status.

The possibility of clearance of HIV has been the subject of debate [99-101]. One explanation might be that the uninfected children with detectable HIV-1 had been exposed to maternal HIV without being truly infected. In our study, HIV detection in uninfected children occurred only during the first 2 months of life, which may possibly support this theory, but false-positive results cannot be ruled out. Frenkel et al. [163] analysed all the reports in the literature and found no evidence of a true clearance of HIV infection, and another study identified mislabelling of samples as the most likely factor in unexplained positive results [162]. The accepted criteria for HIV infection in perinatally exposed infants younger than 18 months of age are two positive virus detection tests on different sampling occasions which exclude these children in whom virus detection tests are positive but who are uninfected. The study elucidates the importance of repeated sampling during the first months of life and of a thorough follow-up of HIV-1-exposed infants.

**Primary HIV-related symptoms in perinatally HIV-1-infected children (paper I)**

Two children developed HIV-1-related symptoms at the time of appearance of viraemia suggestive of primary HIV-1 infection. These cases are of interest because primary infection with symptoms resembling those seen in adult primary HIV-1 infection has not been reported previously in vertically infected children. Whether or not the presence of a symptomatic primary infection predicts a more rapid disease progression and a severe clinical outcome in infants, as in adults [164], is not known. One of these two children died at 5 months of age with a causation unrelated to HIV. The other child developed AIDS at the age of 4.2 years.

**Disease progression in perinatally HIV-1-infected children (paper I)**

Three of the four children with demonstrable virus by culture and PCR within the first 4 days of life presented with clinical symptoms before the age of 12 months compared to 6 of the 12 children with negative virus detection tests at birth. At the end of the follow-up period, the infection in all four children with detectable virus within the first 4 days of life had reached CDC immunological class 3 compared to 6 of the children who were negative at birth. Two of the children with detectable virus at birth progressed to AIDS, and one of them died. Eight of the 14 children with at least one negative virus culture and/or negative HIV-1 DNA PCR preceding the first positive virus detection test remained asymptomatic without treatment during the follow-up period between 3 and 11 years, with the exception of a temporarily swollen parotid gland in one child. We found no statistically significant relationship between
the time of the first virus detection and either disease progression or the appearance of early symptoms, which may be due to the small sample size.

Eleven of all 24 HIV-1-infected children exhibited HIV-related symptoms during the first year of life. Ten of these 11 children progressed to immunological class 3 during the follow-up and nine progressed to AIDS or death. This was significantly different from the 13 children who remained asymptomatic during the first year of life, five of whom progressed to immunological class 3 and two to AIDS or death. The children with HIV-related symptoms during the first year of life had a more rapid progression of both clinical and immunological deterioration. All children in this study were followed up prospectively from birth and were monitored closely for clinical symptoms, although not all were sampled from birth. Therefore, probably very few symptoms were overlooked.

Although small, this is a national cohort study of perinatally HIV-1 infected children, followed prospectively from birth. Since Sweden has a nationwide antenatal screening programme dating as far back as 1987, the children were born to an unselected and comprehensive group of HIV-1-infected childbearing women and not only HIV-1-infected women in medical care. Therefore, this study adds to our knowledge about the natural course of events in perinatally HIV-1-infected children.

Natural history of HIV-1 RNA load in perinatally infected children (paper II)

In this study the highest median HIV-1 RNA levels were seen at 1.5-3 months of age. The HIV-1 RNA numbers then decreased over several years and reached a minimum at 5-8 years of age. This pattern was not always seen in individual children. The time to the first HIV-1 RNA peak value in each child ranged from 1.5 months to more than 2 years. In adults the HIV-1 RNA copy numbers rise quickly to high levels in symptomatic primary infection, decline over a period of weeks [165] and, in some individuals, reach a steady state level [166]. In contrast, also in other studies than ours, the numbers of HIV-1 RNA copies in perinatally infected children have been found to decline slowly until five to six years of age [120,128,129], although very few individual long-term data have been published previously [129]. However, as mentioned before, the slope of the linear regression line in the analysis of the viral load in the last available sample after one year of age differed significantly from zero. This slope reflects either a true decrease in HIV-1 RNA over time or it might be biased by samples taken from possibly healthier children at older ages. The stable CD4⁺ cell count in the analysis of the last available sample from each child might support this explanation. On the other hand, exclusion from the study was due either to death or to the initiation of more advanced antiretroviral treatment than zidovudine monotherapy, and thus a relatively high amount of HIV-1 RNA and a low CD4⁺ count can be presumed.

Another suggested explanation for the slowly declining HIV-1 RNA levels in children is the simultaneous decrease in the number of CD4⁺ cells normally occurring during the first years of life, so that the number of cells available for the virus to reproduce in is reduced[128]. However, although individual CD4⁺ cell curves went
down significantly over time in our study, the slope of the curve of the last available
number of CD4+ lymphocytes did not differ from zero.

Previous studies have shown higher mean HIV DNA copy numbers in PBMC in
symptomatic children than in asymptomatic children [109]. Infants with a rapid
disease progression have also displayed higher peak HIV-1 RNA levels in the first
two months of life, as well as higher geometric mean values during the first year of
life compared to those with a slow progression [121,128]. We could not detect a
significant correlation between disease progression and mean viral load during the
first year of life. However, children presenting with early HIV-1-associated
symptoms had a higher mean viral load between one and four years of age compared
to children who were asymptomatic during the first year of life.

Children who were asymptomatic until 5 years of age or more were analysed
separately. These children also had relatively high HIV-1 RNA copy numbers during
several years. Fluctuations occurred, making prediction of the course of disease
difficult from single viral-load determinations. This is in contrast to adult
asymptomatic patients, who have been shown to have a fairly stable viral load [167].

The results of the study of the PENTA group [129] showed that viral load in
vertically HIV-1-infected children decreased until 6 years of age before it increased
again. Our study supports this model, although, in our quadratic curve model, the
HIV-1 RNA level reached its nadir at approximately 8 years of age. The increasing
viral load beyond the age of 5 – 8 years may predict disease progression, although 6
out of 7 children in our study with their last HIV-1 RNA sample taken after 8 years of
age were still asymptomatic and had stable CD4+ cell counts.

We found no difference with regard to viral load between children on zidovudine and
untreated children at most ages except for a higher HIV-1 RNA level in zidovudine-
treated children at 12-18 months and 3-4 years of age. One explanation could be that
the children who received zidovudine had more advanced disease. Other reports also
indicate that zidovudine monotherapy have little, if any, influence on the decrease in
HIV-1 RNA numbers with increasing age [121,128,129].

HIV-1 RNA numbers may thus vary considerably over time in individual perinatally
infected children. The lack of an obvious steady state and the relatively high levels in
both symptomatic and long-term asymptomatic children make HIV-1 RNA more
difficult to use than in adults as the only tool for predicting disease and initiating
therapy. Also in adults, the practise has evolved during recent years to base treatment
initiation decisions on clinical and immunological parameters, rather than on viral
load measurements.

**Importance of blood fraction, specimen collection, processing and storage on
viral load detection (papers I-IV)**

Viral load can be measured in both blood plasma and serum. The studies in this thesis
include samples collected over a period of more than 20 years. Methods for
measuring HIV-1 RNA, for example, were not available when the collection of
samples was initiated and we could not redesign the studies afterwards to achieve optimal conditions. Comparative studies measuring the HIV-1 concentration found a high correlation between paired blood plasma and serum measurements when specimens were processed and stored similarly [168-171]. Viral load values determined from plasma samples were somewhat higher, although the differences between plasma and serum viral load were small or insignificant [169,170,172]. We were not able to compare paired samples, but we did not observe any difference between plasma and serum HIV-1 RNA levels on a group level. The HIV-1 RNA measurements from serum were carried out mainly with samples stored at -20°C, which might have influenced the levels of HIV-1 RNA [169].

The choice of anticoagulant used in blood collection tubes may significantly alter viral load results. The virion decay rate is significantly faster in heparin-treated blood than in sodium EDTA-treated blood [168,170]. Plasma treated with sodium heparin is not appropriate for the Amplicor HIV-1 Monitor assay because heparin is a potent inhibitor of PCR. We used heparin before, and EDTA from, 1990 and no heparin-defibrinated samples were analysed with the Amplicor HIV-1 Monitor assay.

Rapid degradation of HIV-1 in unprocessed, whole blood in vitro has been reported, with the highest rate occurring within the first 6-8 hours after collection [168,171]. Other studies, however, report minimal decay during this period [169,173]. Most plasma processing protocols require separation of cells and plasma within 6 hours. In situations where this is difficult or impossible, loss of plasma HIV-1 is minimal when plasma separator tubes (CPT, PPT) are used, centrifuged immediately, and stored at 4°C for up to 30 hours until long-term storage at -80°C can occur [168]. As we collaborated with hospitals all over Sweden and samples were sent to one laboratory, the time to processing sometimes exceeded 6 hours but no sample was processed more than 24 hours after sampling.

Coreceptor use in perinatally infected children (paper III)

To further understand the role of coreceptors in disease development in vertically infected children, we determined the coreceptor use of 143 virus isolates, 86 from PBMC and 57 from plasma received throughout the course of the infection in 24 children. Their mothers came from many different areas of the world and could therefore be expected to carry different HIV-1 subtypes.

Accordingly, we found many HIV-1 genetic subtypes (A, B, C, D, G, and CRF01_AE). In the majority of the children (83%), HIV-1 used only CCR5 (85% of the isolates) throughout the observation period. Still, there was a statistically significant, but not reciprocal, relation between the change from R5 virus to X4 virus and immune deficiency and severity of disease. However, the evolution of X4 from R5 virus occurred after the onset of immune deficiency. Only CCR5-using viruses were present in all 41 specimens sampled during the first year of life. This included eight samples from children displaying a rapid disease progression. The appearance of virus with the syncytium-inducing phenotype has been reported not to occur until after the first year of life, even in children with early symptomatic infection [32,33]. Conceivably, the X4 phenotype may emerge more frequently with increasing time.
after infection, similar to what was previously described regarding the syncytium-
inducing phenotype [32,33]. In our study, there were four children of the same ages as
the four who displayed a change in virus phenotype. They showed no signs of
disease progression and had only R5 viruses. Others have demonstrated that the R5
virus is the only phenotype present early on after sexual, parenteral and vertical
transmission [174]. A selection of either R5 or X4 viruses may occur during the virus
isolation procedure, so that the true proportion of R5 or X4 variants is concealed. If
the R5 viruses were favoured, the results strengthen the suggestion of a consecutive
switch from R5 to X4. If the X4 phenotype was favoured, which may be more likely,
the results suggest that the infection of the children started with the R5 phenotype.

The coreceptor use of paired primary isolates from plasma and PBMC was highly
concordant. All but 2 of the 54 pairs displayed identical coreceptor use. This finding
was not entirely expected, because it has been reported that plasma virus may mirror
the actively replicating virus population, whereas PBMC virus may represent an
archive of older virus variants [175]. However, genetic studies of viruses in both
PBMC and plasma will include all HIV-1 quasispecies, whether they are infectious or
not, whereas the phenotypes of coreceptor use reflect infectious virus only.

It has been reported that a switch to the X4 phenotype triggers clinical and
immunological deterioration in adults and in children [31-33,176,177]. However, the
studies of 3-8 children were small and involved analysis of only a few sequential
isolates before the phenotypic switch. We believe that our study allowed a better
resolution of the exact temporal relationship between the change from CCR5 to
CXCR4-using virus and CD4+ cell decline. Accordingly, our data suggest that
emergence of the X4 virus was a consequence, rather than a cause, of
immunodeficiency in our children. However, it cannot be ruled out that CXCR4-
using virus is present in other organs than the blood and exerts its destructive effect
but cannot be isolated.

**Infectious viral load in children (paper IV)**

The amount of infectious virus in plasma declined markedly after an initially high
level. However, the amount of infectious virus in PBMC as well as the HIV-1 RNA
in plasma and serum remained at relatively high levels during the first 5 years of life.
This indicated that, later on during the infection, a considerable proportion of HIV-1
RNA was not infectious and perhaps defective. There was also a significant
correlation between infectious virus in plasma and disease progression.

We used two methods of measuring infectious virus, i.e. days-to-culture positivity
[149] and limiting dilution virus isolation [147,148]. The limiting dilution technique,
which is the most widely accepted way of measuring infectious virus, could not be
used on all samples. However, there was a good correlation between limiting dilution
and the infectious index in plasma.

HIV-1 isolation from PBMC yields a positive outcome in a large proportion of
patients at any stage of disease [149,151]. This qualitative isolation requires only a
few cells containing HIV-1 which can be induced to produce numerous infectious
virus particles per cell after stimulation to cell division. On the other hand, the 
outcome of the qualitative isolation from plasma is directly related to the number 
of infectious particles. The amount may be insufficient if it does not exceed a critical 
level. This may be one reason why plasma has not been used as frequently as PBMC 
for HIV-1 isolation. The results of plasma isolation may also vary more between 
laboratories [147,149,178-181].

The relationship between quantitative isolations from PBMC and plasma is the 
reverse. Accurate dilution of cells is more difficult than that of plasma since the cells 
often sediment during the process. Furthermore, each cell may contain more than one 
HIV-1 copy and the number of infectious proviruses per cell may vary and this may 
interfere with the reproducibility. The number of HIV-1 provirus-carrying cells that 
go into cell division and hence will produce infectious virus is also unpredictable. 
The outcome of dilution or volume reduction of plasma should, however, be a direct 
reflection of the original concentration of the infectious virus. The decrease in the 
median plasma infectious index after the initial plateau level contrasted with the more 
stable pattern of the median PBMC infectious index from approximately 3-4 months 
of age.

There are some published studies on quantitative measurements of infectious HIV-1 
in children, but none of them compare the amount of infectious virus with the RNA 
load [134-137]. Ikeda et al. used decreasing numbers of cells in culture and days-to- 
culture positivity. They found that quantitative measurements of HIV-1 in PBMC 
were more sensitive as indicators of infection status than quantification of p24 
antigen [137]. In another study, end-point dilution cultures of both plasma and cells 
were assessed in 46 neonates. Plasma and, to a lesser extent, cell-associated viral 
burden were associated with rapid progression of the disease when measured during 
the first 6 months of life [136].

We observed a relatively rapid decline of plasma viraemia after approximately two 
years of age, which was not reflected by a similar decline in the cellular infectious 
 viral load or in plasma RNA levels. But, there seemed to be an association between 
infectious plasma load and the presence of p24 antigen. In addition, five children 
displayed a second peak of infectious virus in plasma, which occurred in connection 
with a change in coreceptor use in two of them.

We found higher median values of the plasma infectious index in children who 
developed AIDS than in those who did not, but this difference was not found with 
regard to HIV-1 RNA. We also found higher initial peak values of the plasma 
infectious index in children who progressed to CDC clinical class B or C than in 
those who did not progress clinically. Similarly, HIV-1 RNA peaks also differed 
between these two groups.

The decrease of infectious HIV-1 in plasma in primary infections in adults has been 
interpreted to be related to the development of neutralizing antibodies [182,183] and 
cellular immunity. Dianzani and coworkers found that HIV RNA was associated with 
IgG and concluded that the discrepancy between infectiousness of plasma and HIV-1 
RNA load was related to immune complexes formed by anti-HIV-1 antibodies and
HIV-1 RNA containing particles. The continued presence of infectious virus could be dependent on immune escape of virus mutants [182]. As IgG antibodies in children appear before infectious virus in plasma disappears, the presence of neutralizing antibodies cannot fully explain the phenomenon in the children observed by us.

Most single-stranded RNA viruses have the ability to produce particles containing defective genomes. The occurrence of defective interfering particles, accumulating over time, has been reported regarding different RNA viruses such as measles virus, polio virus, and vesicular stomatitis virus [124,125]. These particles contain viral genomes that are defective in one or more functions and lead to slower virus growth and reduced cell destruction [125]. The available methods for HIV-1 RNA quantification do not distinguish between infectious and uninfectious virus. The activation of latent HIV-1 DNA in lymphocytes may result in the production of both infectious and uninfectious virus. The increasing divergence between the RNA levels and the levels of infectious virus determined by quantitative plasma virus isolation in the present study could be explained by an increasing proportion of defective virus. This could explain the possible effect of zidovudine on the infectious viral load but not on the RNA load.

Quantification of HIV-1 RNA in children infected with HIV-1 of different subtypes (paper II-V)

Most genetic subtypes of HIV-1 have entered Sweden [184]. Six different subtypes were present in this cohort. The NASBA test as well as the Amplicor Monitor 1.0 test have not been able to accurately quantify HIV-1 RNA in plasma samples from many subtype A- (and probably CRF01_AE-) infected individuals [185]. In addition, the NASBA test has been inadequate in quantification of subtype G HIV-1 RNA [186]. In one subtype A- and two CRF01_AE-infected children we found high levels of HIV-1 RNA and no incapacity of the NASBA test to quantify HIV-1 RNA in plasma or serum, which does not exclude errors in other children whose virus were not subtyped (paper II). No subtype-specific difference in peak HIV-1 RNA or infectious index peak values was seen. A tendency towards lower peak values of HIV-1 RNA in children infected with virus of subtype B could not be explained by inadequacy of the method used (paper IV).

Kanki et al. found that HIV-infected individuals with subtype A had slower disease progression than those with non-subtype A infection [187]. We have no reason to believe that there were so many children with subtype A in our study that our results would be affected (4 out of the 17 who were subtyped carried virus of subtype A) (paper II).

Zidovudine monotherapy and viral load (paper I-IV)

In previous studies, zidovudine monotherapy had no significant effect on HIV-1 RNA levels [121,128,129], but decreased the amount of infectious HIV-1 in PBMC [135] and plasma [134,135]. There was a possible influence of zidovudine monotherapy on the infectious index in plasma in our study. However, several children, including some of those reaching CDC immunological class 3, displayed a spontaneously
decreasing plasma infectious index over time. No influence of zidovudine on the infectious index in PBMC or on the HIV-1 RNA load was seen.

**HIV-1 genetic subtype and coreceptor use (paper III)**

Subtype-specific in vitro differences between phenotypes of HIV-1 regarding the use of chemokine receptors [36,188] and syncytium induction [37] have been reported. The role of HIV-1 subtype differences in vivo is ambiguous [187,189-192]. Syncytium-inducing virus is rare among isolates of subtype C [188,193], even in AIDS patients [188,193,194]. Our series was too small to allow a statistical analysis of the genetic subtype and coreceptor use. Also, the differences in the clinical stage of the HIV-1 infection may have influenced any relation between coreceptor use and phenotype. Nevertheless, the finding that virus with the X4 phenotype could be isolated from children carrying subtypes A, D or CRF01_AE, but not subtype C, is in agreement with earlier studies.

**HIV-1-infected women in Sweden (paper V)**

The demographic characteristics of HIV-1-infected women have changed moderately during the period studied. There was a high proportion of mothers originating from Sub-Saharan Africa already early on in the epidemic, in contrast to the situation in other European countries [92] at that point in time. This proportion tended to increase over time but it was not statistically significant. Simultaneously, the proportion of women born in Sweden, 44% of whom were infected through intravenous drug use or blood products, decreased. The background of the mothers adds immigrant problems to the burden of HIV-1 infection, but makes problems related to intravenous drug use more infrequent than in cohort studies from other countries in Europe [73,140,195,196].

**Mother-to-child HIV-1 transmission in Sweden during 1985-2003 (paper V)**

Three hundred and fifty-five children were prospectively followed from birth and the infection status was determined in 314. Twenty-seven (8.6%) children were infected. The mother-to-child transmission rate was 24.7% during 1985-93 before the antiretroviral prophylactic era. There was a significant decrease in the mother-to-child transmission rate to 5.7% during 1994-98, which reflects the gradual introduction of the ACTG 076 protocol late in 1994 and the reduction rate is similar to what was seen in the ACTG 076 study [77]. Only one infected child (0.7%) was born in Sweden during 1999-2003, reflecting the effect of the increasing use of antiretroviral combination therapy by pregnant women and the routine use of elective caesarean section. The proportion of children born after antiretroviral treatment of the mother during pregnancy and/or prophylactic antiretroviral intervention increased from 2.3% during 1985-93 to 88.0% in 1994-98 and 91.5% during 1999-2003, and the proportion of elective caesarean section deliveries increased from 8.0% to 43.5% and 80.1%, respectively, during the same period.

Although these transmission figures are results from routine care, the infection rate is as low as in intervention studies [71]. National guidelines on the management of
HIV-1-infected pregnant women formulated by the Swedish Reference Group for Antiviral Therapy (RAV) [197] and the Swedish Medical Products Agency and the high acceptance rate in the pregnancy screening programme are factors that probably contributed to the low transmission rate. We believe that the multidisciplinary teamwork in the clinics caring for the pregnant women and their children-to-be is of great importance for the adherence to treatment and care and thereby for the low transmission rate.

Six infected children were born after prophylactic intervention according to the ACTG 076 protocol. Two mothers who were delivered by caesarean section were identified as HIV-1-infected in gestational week 37 and hence the prophylactic treatment period was short. One mother who delivered vaginally did not receive the intravenous part of the zidovudine treatment. In two cases, the mother had been previously treated with zidovudine monotherapy during pregnancy and zidovudine resistance cannot be excluded in these cases. Thus, in five out of six cases with transmission despite prophylactic intervention, the prophylaxis was not optimal.

Even though the acceptance rate for the Swedish antenatal screening programme is high, four children were vertically infected by HIV-1-infected women who were not diagnosed before delivery. One child was born in 1985 before antenatal screening was introduced and one woman had a primary HIV-1 infection during pregnancy after having tested negative in the screening programme. HIV-1 infection might possibly have been avoided in two children if their mothers had been tested during pregnancy, but this was not done because they had previously tested negative. This emphasizes the importance of continued high antenatal testing rates even in low HIV-1 prevalence countries like Sweden.

No child was infected when the mother received prophylaxis with two or more antiretroviral agents. The viral load was not known in all these women, but it was probably low. Low vertical transmission rates have been observed among women with undetectable virus (< 500 copies/ml) in late pregnancy during which more than 95% of the mothers were treated with one or two antiretroviral agents [63-65], as well as among women receiving combination antiretroviral therapy [66-68]. Whether the low transmission rates with combination therapy are only due to a reduction of HIV-1 RNA or whether some other mechanism contributes to the protection is not known. In the USA, elective caesarean section is recommended for women with HIV-1 RNA > 1000 copies/ml near the time of delivery. According to the American College of Obstetricians and Gynecologists, elective caesarean section probably would not provide any additional benefit in the reduction of transmission if the woman is treated with antiretroviral therapy and the plasma HIV-1 RNA is below 1000-copies/ml [78]. Our study confirms the low transmission rate when combination antiretroviral combination therapy is used and we could not detect any difference between groups according to the mode of delivery, but recent data from the European Collaborative Study do indeed support that elective caesarean section further reduce the mother-to-child transmission rate even in women with an undetectable viral load treated with combination antiretroviral therapy [79].
The question has recently been raised as to whether girls are more at risk of being vertically HIV-infected than boys [198], especially those delivered by elective caesarean section [199]. Our numbers are too small to explore this issue and we did not see such a tendency among the prospectively followed children in our study, who, in most cases, are also included in the ECS, from which this gender difference has been reported [199].

**HIV-1-infected children in Sweden (paper V)**

Seventy-two HIV-1-infected children were identified. They most probably comprise a majority of the HIV-1-infected children who have lived in Sweden. Thirty-one of these 72 children were born in Sweden and 41 abroad. Ten children left Sweden and 11 died. One child died in 1982 [7] and ten died between 1987 and 1997. Ten children died of AIDS and one of an HIV-1-unrelated condition. One infected child died in 1982 [7] and ten children died between 1987 and 1997. The survival of HIV-1-infected children has improved dramatically since antiretroviral combination therapy was introduced in 1996. No child died of HIV after 1997, even though 10 children have had AIDS-defining diagnoses. Thus, the number of children living with HIV and AIDS is increasing. Fifty-one vertically HIV-1-infected children were known to be living in Sweden at the end of 2003. Ten children were categorised in CDC immunological class 1, 16 in class 2 and 22 in class 3. Fifteen children were in clinical category N, 9 progressed to category A, 14 to category B and 10 to category C.

**AIDS-defining diagnosis**

Ten children acquired AIDS. The AIDS-defining diagnoses were five *Pneumocystis carinii* infections, two *Mycobacterium avium* complex infections, one lymphoma, one wasting syndrome, and one lymphoid interstitial pneumonia (LIP).

**Antiretroviral treatment**

The policies for therapeutic management of vertically HIV-infected children were recently reported for different centres in the ECS collaboration [200]. The proportion of untreated children in Europe has been reported to be 9-11% [200,201] compared to 27.4% (15 out of 51) in this study. There have been and are still differences in therapeutic tradition, as reflected in treatment guidelines, between Europe and the USA, where earlier and more aggressive treatment has been recommended in the USA. Most clinicians in Sweden follow the European tradition of postponing antiretroviral treatment until clinical symptoms and/or immune deterioration appears [202] and may have been even more restrictive than in many other European countries, for example, France, where a high proportion of infected children receive early treatment. The present trend in treatment guidelines, in both Europe and the USA, is towards a more watchful waiting approach [203-205].

All the children with AIDS are being treated with antiretroviral combination therapy and even though five out of eight children with reported therapy do not have optimal virus suppression, in most cases they have regained acceptable CD4+ cell counts, reflecting the effective immune reconstitution in children [206]. The clinical
improvement is remarkable as well and only one of these children who previously had AIDS defining conditions, had HIV-related symptoms at the latest follow-up.

**Caregiver**
Nine infected children lived with both and 28 with one of their parents. Eleven children lived with none of their biological parents. Seven of them were adopted or in care organized by social authorities and three were taken care of by relatives. Eleven children lost their mother and, in six known cases, their father to AIDS. Three children lost both their parents to AIDS.

**Psychosocial support and disclosure**
In a recent study, Mellins et al. found a high prevalence of behavioural problems among HIV-infected children, but concluded that neither HIV infection nor prenatal drug exposure was the underlying cause. They suggest that other biological and environmental factors are more likely contributors to poor behavioural outcomes [207]. We found an increase in reported minor developmental delay and/or behavioral problems in HIV-1-infected children, as compared to uninfected children, which may indicate a need for psychosocial support for these children. This difference might be biased by the more regular follow-up and thorough examination of infected children than uninfected ones.

In this study, the median age for disclosure of the HIV-1 infection to the child was 10 years (7-13 years) which is similar to what was found in a study by Thorne et al. [201]. It is important that the children be given information about their HIV infection before puberty in order to enable them to work on and integrate the information and to acquire an adequate knowledge of what it means to live with HIV before their sexual debut. At our centre we organized an “HIV school” over a long weekend twice a year for adequately informed HIV-infected children and teenagers from the whole country to provide support and further education and to enable them to meet other children and teenagers with HIV [208]. With more effective treatment strategies and increased survival, an increasing number of HIV-infected children are becoming young adults. Thus, this kind of support and education is of increasing importance in the care given to HIV-infected children.
Conclusions and future perspectives

We followed 419 perinatally HIV-1-exposed children in Sweden, 355 of them prospectively from birth. The mother-to-child-transmission rate in Sweden decreased markedly during the studied period and is at present less than 1%. No infected child is known to have been born in Sweden since 1999. This can be attributed to the high acceptance rate for the pregnancy screening programme for HIV-1 combined with the availability of antiretroviral prophylaxis and elective caesarean section. Since knowledge of the infection status of the mother is crucial to being able to reduce mother-to-child transmission of HIV-1, continued routine testing and counselling are important. In addition to the epidemiological and follow-up report on children to HIV-1-infected mothers we plan to complete the project with a report on HIV-infected mothers.

There is concern about the children who have been exposed to antiretroviral substances in utero, for example regarding the risk for mitochondrial toxicity, and further follow-up of these children is important. It is important to follow also the uninfected children, with regard to possible side effects on metabolic and/or other functions. If no, or very few, negative effects attributable to antiretroviral exposure will be found in the future, this too, is very important and reassuring knowledge for HIV-infected individuals making reproductive plans.

The number of children living with HIV-1 in Sweden is still increasing as a result of prolonged survival and immigration. We followed 72 perinatally HIV-1 infected children, 51 of whom lived in Sweden at the end of 2003. No death of a child attributable to HIV-1 has occurred in Sweden since 1997. Most children have good immune function and a low viral load, even those who previously had AIDS-defining conditions. This result of the access to antiretroviral treatment makes it possible for HIV-1-infected children to live almost normal lives and to reach adulthood. In that perspective the long-term negative effects of antiretroviral therapy, such as lipodystrophy and the metabolic syndrome, are of great concern, and strategies for prevention of antiretroviral side-effects are important.

Psychosocial support and education are of great importance for HIV-1-infected children and their families to help them cope with their HIV status. Further work on how to support HIV-infected children and their parents/caregivers in the process of disclosure is needed.

The natural history of viral load, with the median HIV-1 RNA level being the highest at 1.5-3 months of age and decreasing between 1 and 8 years of age is valuable knowledge when making therapy decisions, although the importance of HIV-1 RNA levels for such decisions has decreased in favour of clinical and immunological parameters.

The HIV-1-infected children carried virus belonging to many subtypes reflecting a diverse origin of their mothers. The phenotypic change of the virus and its association to disease progression and immunological deterioration is incompletely explored and deserves further studies. This cohort of mother-child pairs infected with various
subtypes will provide future possibilities for studies of subtype-specific and phenotypic aspects of HIV-1 infection.

Our studies of the correlation between early symptoms, time of virus detection, viral load, immunological deterioration and disease progression, of the coreceptor use and of the natural course of viral dynamics in perinatally infected children are all performed on samples collected before the antiretroviral era. As a majority of children with HIV-1-related symptoms or severe immune deterioration will be treated with antiretroviral drugs, it will not be possible to study these tasks in industrialized countries in the future.
Acknowledgements

I wish to express my sincere gratitude to:

Ann-Britt Bohlin, my supervisor, teacher and friend, for introducing me to the fascinating and engaging field of paediatric HIV, for sharing your great knowledge and guiding me so excellently through all phases of this thesis – always busy but with a high degree of presence in every situation.

Anneka Ehrnst, my co-supervisor and research companion, for generously sharing your extensive knowledge of viruses in general and HIV in particular, with your free thinking constructive, and, in a positive sense, unconventional theories.

Erik Belfrage and Susanne Lindgren, for sharing your extensive knowledge of HIV, for your good friendship and close scientific and clinical collaboration regarding HIV in children and childbearing women.

Charlotte Casper for constructive collaboration regarding coreceptors.

My co-authors, Jan Albert, Malin Arneborn, Jonas Blomberg, Bertil Christensson, Peter Clevestig, Eva Maria Fenyö, Marianne Forsgren, Magnus Gisslén, Katarina Gyllensten, Thomas Leitner, Gunilla Lidin-Janson, Knut Lidman, Rolf Ljung and Anders Sönnerborg for fruitful collaboration.

Agne Larsson, former head of the Division of Paediatrics at the Institution of Clinical Science, for creating an atmosphere were research is appreciated and stimulated.

Kerstin Andreasson, Robert Fredriksson and others at MTC, for your kindness and support when I was trying to learn laboratory work.

The biological analysts Gun Sundin, Ulla Lips and Vanja Sundberg for your careful performance of the virus isolations.

All collaborators in the paediatric HIV team, Johanna Crafoord, Anna Hjälmdahl-Trygg, Ann-Christine Lindahl, Britta Lindqvist, Anneli Mikaelsson, Maja Nanneson, Christina Ralsgård, Johanna Rubin and Lotta Rydström for your professional care and concern of the HIV-infected children and the HIV-affected families.

All collaborators regarding pregnant HIV-infected women and their children at “Familiesociala mottagningen”, Department of Paediatrics, Department of Obstetrics and Gynaecology and Department of Infectious Diseases, Ulla Backe-Nylund, Ann-Britt Bengtsson, Inger Ekman, Kristina Elfgren, Lena Harland, Ann-Britt Hjelm, Anneli Kaldma, Inger Lindgren, Christina Ottenblad and PehrOlov Pehrson.

All colleagues at Departments of Paediatrics and Infectious Diseases all over Sweden for collaboration in collecting data about children and mothers.
Mats Blennow, head of the Neonatal Unit at Childrens Hospital, Huddinge, for creating pleasant working conditions, and together with Kari Arhimaa, Kajsa Bohlin, Martino Flanby, Boubou Hallberg and Ihsan Sarman, my other colleagues and friends at the neonatal unit, for doing all the work that someone had to do when I was busy doing research somewhere else.

All friends and colleagues at the Department of Paediatrics at Karolinska University Hospital Huddinge.

Mr Isaac Austin, for revising the language.

My father Nils-Arne, my mother Wiola and my sisters Carina and Marie with families for your kind and warm support.

Ylva, Jojje and Nora for being you.

This study was supported by grants from the Swedish Medical Research Council, the Swedish Research Council, the Research Foundations of the Karolinska Institutet, the Swedish Physicians against AIDS Research Foundations, the Sven Jerring Foundation, the Samariten Research Foundation, the General Maternity Foundation and Freemason’s in Stockholm Foundation for Childrens Welfare.
References


99. Rudin C, Senn HP, Berger R, Kuhne T, Erb P. Repeated polymerase chain reaction complementary to other conventional methods for early detection of


120. Palumbo PE, Raskino C, Fiscus S, Pahwa S, Fowler MG, Spector SA, Englund JA, Baker CJ. Predictive value of quantitative plasma HIV RNA and


132. Rich KC, Siegel JN, Jennings C, Rydman RJ, Landay AL. Function and phenotype of immature CD4+ lymphocytes in healthy infants and early


194. Morris L, Cilliers T, Bredell H, Phoswa M, Martin DJ. CCR5 is the major coreceptor used by HIV-1 subtype C isolates from patients with active tuberculosis. AIDS Res Hum Retroviruses 2001;17:697-701.


