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HIV-1 GENOTYPE AND VERTICAL TRANSMISSION IN NORTHERN VIETNAM

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To my family
ABSTRACT

After 18 years from the beginning of the HIV-1/AIDS epidemic in Vietnam, the number of HIV-1 infected individuals is still increasing. Investigations conducted at two distinct time points in two separate groups of HIV-1 infected intravenous drug users (IDUs) and pregnant women in Northern Vietnam indicated that the HIV-1 CRF01_AE genotype is dominating in Northern Vietnam. Our analysis was based on 399 gp120 V3 env sequences comprising HIV-1 strains studied by us and on previously published Vietnamese sequences and on reference sequences from neighboring countries available in the Los Alamos HIV database. By using different softwares to determine the phylogenetic relationship of the different HIV-1 strains, we showed that the HIV-1 CRF01_AE present in Northern Vietnam is closely related with the HIV-1 CRF01_AE sequences from Southern China, whereas HIV-1 CRF01_AE sequences from Southern Vietnam have a closer link with HIV-1 CRF01_AE Thailand sequences. The genetic analysis of the gp120 V3 env sequences obtained from HIV-1 infected IDUs in 2002 and pregnant women in 2006-2007 revealed that HIV-1 is spreading rapidly in Northern Vietnam. The fast spreading epidemic can be identified by the low level of variation noticed in HIV-1 strains infecting IDUs and pregnant women in Northern Vietnam.

HIV-1 infection in pregnant women is increasing in Vietnam. Several prevention programs have however been started to control HIV-1 vertical transmission in Northern Vietnam. In my study 182 HIV-1 infected mothers and their children were enrolled. The HIV-1 infection in children was confirmed by the presence of the HIV-1 pol gene in blood cell DNA. The PCR was performed from birth until 12 months and HIV-1 serology from 12 to 18 months. The total HIV-1 transmission rate to the children in our study was 6.7% with a rate of pre-partum transmission of 4.2% and intra-partum transmission of 1.5%. About 60% of the HIV-1 infected mothers received one dose of nevirapine at labor. The children were treated with liquid nevirapine within 48 hours from birth. In addition, ARV combination was provided to 11% of the HIV-1 pregnant women for a few weeks prior to delivery and zidovudine was given to the children one week after birth. Through counselling, the women were convinced to not breast-feed their infants. It was documented that there was no evidence of postpartum transmission. The key for the prevention of HIV-1 transmission from mother to child is counselling and this procedure needs to be further implemented in Vietnam.

The different HIV-1 subtypes might have a different response to the anti retroviral treatment. In order to evaluate this possibility we measured the changes of sCD27 levels in plasma in patients infected with HIV-1 subtypes A, or B, or C or D and treated with ART for 12 months. The sCD27 is a marker of immune activation. The data showed that sCD27 is considerably higher (p<0.001) in the HIV-1 infected group than in the control group. After 12 months of treatment, the reduction of sCD27 levels in plasma was significant for all HIV-1 subtypes (p<0.001) with the largest reduction for HIV-1 subtype C. Our results suggest that a significant reduction in immune activation could be measured after 1 year of ART for all the different subtypes; thus sCD27 can be considered as a relevant immune marker to measure response to therapy.
LIST OF PUBLICATIONS

I. **Paper 1:** HIV-1 CRF01_AE in intravenous drug users in Hanoi, Vietnam.
   Tran Thi Thanh Ha, Irina Maljkovic, Sofie Swartling, Phung Dac Cam, 
   Francesca Chiodi and Thomas Leitner.

II. **Paper 2:** Rapid spread of genetically stable HIV-1 CRF01_AE in pregnant 
    women in Northern Vietnam.
    Tran Thi Thanh Ha, Simani Gaseitsiwe, Irina Maljkovic Berry, Pham Viet 
    Hung, Nguyen Mai Anh, Nguyen Huy Bao, Dinh Duy Khang, Nguyen Tran 
    Hien, Phung Dac Cam, Francesca Chiodi, Thomas Leitner, Anneka Ehrnst 
    *Submitted*

III. **Paper 3:** Counselling on formula feeding and antiretroviral prophylaxis, 
    successfully reduced transmission of HIV-1 from mother to child in 
    Northern Vietnam.
    Tran Thi Thanh Ha, Nguyen Mai Anh, Nguyen Huy Bao, Pham Le Tuan, 
    Nguyen Tran Hien, Phung Dac Cam, Francesca Chiodi, Anneka Ehrnst 
    *Submitted*

IV. **Paper 4:** Effects of potent antiretroviral therapy on the immune activation 
    marker soluble CD27 in patients infected with HIV-1 subtypes A-D.
    Ann Atlas, Tran Thi Thanh Ha, Anna Lindström, Anna Nilsson, Annette 
    Alaeus, Francesca Chiodi and Angelo De Milito
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LIST OF ABBREVIATIONS

3TC  Lamivudine is a nucleoside analog reverse transcriptase inhibitor
AIDS  Acquired Immunodeficiency Syndrome
ART  Anti-Retroviral Treatment
ARV  Anti-RetroViral drug
AZT (ZDV)  Zidovudine or azidothymidine is a nucleoside analog reverse transcriptase inhibitor
BEAST  Bayesian Evolutionary Analysis Sampling Tree
CCR2  Chemokine (C-C) receptor 2
CCR3  Chemokine (C-C) receptor 3
CCR5  Chemokine (C-C) receptor 5
CD27  Cluster of Differentiation 27
CD36  Cluster of Differentiation 36
CRF  Circulating Recombinant Form
CSW  Commercial Sex Workers
CXCR4  Chemokine (C-X-C) receptor 4
ECS  Elective Caesarean Section
EDTA  EthyleneDiamineteTraacetic Acid is an anticoagulant for blood
ELISA  Enzyme-linked Immuno Sorbent Assay
Env  Envelope gene
FSU-A  Former Soviet Union A
GFATM  The Global Fund for AIDS, Tuberculosis and Malaria
HIV-1  Human Immunodeficiency Virus type 1
HIV-1 CRF  HIV-1 Recombinant Form
HTLV-III  Human T lymphotropic virus III
IDU  Intravenous Drug User
LAV  Lymphadenopathy virus
LTR  Long Terminal Repeat
MIC  Mother of Infected Child
MOH  Ministry of Health in Vietnam
MTCT  Mother To Child Transmission of HIV
MUC  Mother of Uninfected Child
NK  Natural Killer cell
NVP  Nevirapine (Viramune) is a non-nucleoside reverse transcriptase inhibitor
PBMC  Peripheral Blood Mononuclear Cell
PCR  Polymerase Chain Reaction
PEPFAR  US President’s Emergency Plan for AIDS Relief
PHYML  Phylogenetic Maximum Likelihood
PMTCT  Prevention of Mother To Child Transmission of HIV
sCD27  Soluble CD27
SDF-1  Stromal derived factor-1; the natural ligand for CXCR4 receptor
SIV  Simian Immunodeficiency Virus
SIVcpz  SIV chimpanzee
SIVgor  SIV gorilla
SIVsm  SIV sooty mangabeys
STD  Sexual Transmitted Diseases
TNF  Tumor Necrosis Factor
UNAIDS  The joint United Nations Programme on HIV/AIDS
UNICEF  United Nations Children's Fund
URFs  Unique Recombinant Form
V3 env  Variable region 3 of the HIV-1 envelope gene
VCT  Volunteer Counselling and Testing
WHO  World Health Organization
β2m  β2 microglobulin
1 INTRODUCTION

1.1 THE HIV PANDEMIC

Over 65 million people have been infected with human immunodeficiency virus (HIV) and more than 25 million have died of acquired immunodeficiency syndrome (AIDS) since the beginning of the HIV/AIDS epidemic [1, 2]. The first cases of HIV infection were detected in homosexual men in the United States in 1981 [3]. Two years later, the pathogen was isolated and characterized [4-8]. Originally the virus was named human T lymphotropic virus III (HTLV-III) and lymphadenopathy virus (LAV) and thereafter renamed to HIV. The second type of HIV isolated in 1986 was termed HIV-2 and the first type (previously HTLV-III or LAV) was renamed to HIV-1[9-13]

According to The Joint United Nations Programme on HIV/AIDS (UNAIDS), there were over 33 million people living with HIV-1 in the world in 2007 with 2.5 million new infection in total occurring in 2007, 420,000 new infections in children under 15 and 2.1 million deaths by AIDS [1]. The number of children infected in 2006 was 530,000 of the 4.3 million people newly infected during that year [14]. These figures indicate that much work remains to be done to control HIV general transmission and HIV vertical transmission through different programs.

Among the infected people, 22.5 million are residents in Sub-Saharan Africa [1]. The highest prevalence rate of HIV-1 was reported in the Republic of South Africa. It ranged from 15% in Western Cape to 39% in KwaZulu-Natal by 2006. Prevalence of HIV-1 in Angola (2006) was about 3.7% in the adult population. In Kenya the HIV-1 prevalence rate by 2006 was about 5% in the general population, 4.7% in Côte d’Ivoire, and 3.2% in the Democratic Republic of Congo [1].
HIV-2 was first found in West Africa where monkeys infected with Simian Immunodeficiency Virus sooty mangabeys (SIVsm) live in a wild state [9, 10, 12, 15]. The majority of HIV-2 infections still occur in West Africa. HIV-2 spreads slowly to Europe, Asia and America through immigrants and tourists. In Guinea Bissau by 2006, single HIV-2 infection occurred in 3.9% of the population and double infections with both HIV-1 and HIV-2 occurred in about 0.5% of the population; thus the overall HIV-2 prevalence was 4.4%[16]. Among the HIV infected individuals in Côte d’Ivoire, 94% were infected with HIV-1, 2% with HIV-2 and 4% carried a dual infection in the period 2004-2007 [17]. In Spain, 70% of the HIV-2 infected are African immigrants [18] and 14 out of 16 HIV-2 patients in Northern Italy also originate from Africa [19]. In Asia, 95% of the HIV-2 infected are living in India [20]. HIV-2 infected persons are also found in Korea, Japan, Nepal.
(0.2%) and Philippines [21-24]. No case of HIV-2 infection has so far been reported in Vietnam.

Upon HIV-2 infection the clinical asymptomatic stage persists longer and the disease progression is slower than what observed with HIV-1. In addition, viral load in HIV-2 patients is generally lower than in HIV-1 patient, and the CD4+ T cell count is higher. This may suggest that HIV-2 has a lower pathogenicity and that the human immune system is able to control HIV-2 replication. A different balance between pathogenicity and immune control may explain why HIV-2 infected patients survive longer than HIV-1 patients [25, 26]. HIV-1 and HIV-2 have the same routes of transmission. Gender distribution is not significantly different in HIV-1 and HIV-2 infected patients.

1.2 HUMAN IMMUNODEFICIENCY VIRUS TYPE 1

1.2.1 The genes of HIV-1

HIV-1 belongs to the retrovirus family which genome consists of two positive single stranded RNA. The HIV-1 genome is divided into 3 main genes gag, pol and env, coding for structural proteins and enzymes pivotal for HIV-1 replication. HIV-1 regulatory proteins are coded by the tat and rev genes. Nef, vif, vpr, vpu code for the accessory proteins of HIV-1. In addition to two regions flanking the genome, which are called long terminal repeats (LTRs), a total of 9 genes have been identified in the HIV-1 genome. Based on their different degree of conservation, HIV-1 genes and proteins have been widely used for genotype characterization, drug resistance studies, and diagnosis. The HIV-1 pol gene codes for reverse transcriptase, integrase and protease. It is quite stable throughout all HIV-1 genotypes, with an estimated intra-genotype diversity of about 15%. Mutations related to drug resistance are located in this gene. High priority is given to proteins from the pol gene for HIV-1 diagnostic purposes. The HIV-1 gag gene can have as much as 20% diversity. The p24 gag gene
is very useful for early diagnosis of HIV-1 infection, and the conserved p17 \textit{gag} protein may be consider as a potential targets for immunotherapy of HIV-1 infected cells [27]. The \textit{env} gene is responsible for binding and fusion of HIV to the target cells. A region of particular relevance is the third variable region of gp120 \textit{env} gene called V3 loop. The other regions of \textit{env} as V1 to V5 may modulate to effect of V3 loop [28]. The \textit{env} gene is very variable (30%) and is commonly used for the definition and characterization of genotypes [29-31].

1.2.2 HIV-1 genotypes

AIDS in humans is caused by HIV-1 and HIV-2. HIV-2 evolved from SIVsm in West Africa [10, 12]. HIV-1 is divided into 3 common groups: M (Major), O (Outlier) and N (Non M/Non O). Phylogenetic analysis revealed that groups M and N have their ancestor in SIV which was isolated from the Chimpanze \textit{Pan Troglodytes Troglodytes} (SIVcpz) in South Cameroon [12, 32, 33]. Group O also falls within the SIV phylogenetic radiation, but more closely with viruses in wild gorillas (SIVgor) in Western Africa [32, 34]. The relation between SIVcpz and SIVgor is still unclear [12].

The HIV-1 group M entered the human population along the Congo River in 1959 [32]. However, the traces of these viruses date back to 1940s [35, 36]. Groups N and O are endemic in West Equatorial African countries. Group M dominates the infection globally and is divided in different subtypes designated A, B, C, D, F, G, H, J and K, and at least 37 circulating recombinant form (CRF) of the virus were found so far [32, 33, 37].

The HIV-1 subtypes identified at the beginning of the epidemic have now developed into sub-subtypes. HIV-1 subtypes A and F are examples of this phenomenon, which now can be divided into sub-subtypes A1-A4 and F1-F2, respectively. Inter-subtype recombinant form is the denomination of a virus strain that is a hybrid from more than one CRF. HIV-1 subtype A and HIV-1 CRF02\_AG
dominate in Eastern Africa [38], in addition to the HIV-1 subtype C and D. HIV-1 subtype B is most common in Europe, North America and Australia. HIV-1 subtype C is reported to be largely spread in China, India and Africa [32, 39]. HIV-1 CRF07_BC and HIV-1 CRF08_BC are mixtures between subtypes B and C and were reported to be common in China [40-42]. HIV-1 CRF01_AE is most common in Asian countries and is originated from HIV-1 subtype E that was first detected in Thailand in late 1980s [43, 44]. HIV-1 CRF01_AE is an hybrid between gag gene of HIV-1 subtype A and env gene of HIV-1 subtype E [43, 45].

Most of the circulating recombinant forms are hybrids from 2 HIV-1 subtypes and are called HIV-1 CRF01_AE, 02_AG, 03_AC, AD, 07_BC, 08_BC, BF, BG, CD etc [32, 33, 43]. Some CRFs which are the result of the combination of original subtypes and CRFs have been called second generation recombinant or complex recombinant and designed “cpx” [37]. As an example HIV-1 CRF27_cpx is a recombinant from subtypes A, E, G, H, J and K and a small unclassified fragment found in Democratic Republic of Congo [46]. The recombination of subtypes A, G, J and CRF01_AE has given rise to HIV-1 CRF11_cpx [47]. HIV-1 CRF06_cpx originates from subtypes A, G, K and J. The newest CRF were found in Cameroon in 2007 and are designated as HIV-1 CRF36-cpx and HIV-1 CRF37_cpx [48, 49]. The recombination between group M and O (CRF_DO, CRF_AD and O) was also reported to occur in patients in Cameroon in 1994 [38, 50-52].

The unique recombinant form (URFs) designates an HIV-1 form which is detected in one or many individuals in the same region. URFs have limited transmission to the general population [32]. This URF mosaic virus is labeled with “U” until enough criteria are collected to designate a nomenclature for the virus [37]. In order for a subtype to be designated as a new subtype it needs to be found in at least two individuals in two geographically unrelated regions. The two sequences have to be
similar enough to each other but display enough variation from previously identified sequences throughout their whole genome.

Some specific subtypes are associated with distinct geographic regions as HIV-1 subtype Thai B or Indian C or Former Soviet Union A (FSU-A). HIV-1 CRF01_AE dominates in Asian countries but is rare in Central Africa [44].

1.2.3 HIV-1 co-receptor phenotypes

The main receptor for attachment of HIV-1 to target cell is the CD4 molecule. HIV-1 isolates can also be designated according to the co-receptor that they use to entry into the cell target. HIV-1 viruses which use the CCR5 co-receptor or CXCR4 are called R5 viruses or X4 viruses, respectively. Viruses which can use both co-receptors are called R5X4 or dual tropic viruses [32, 53, 54]. A few HIV-1 strains can also use other chemokine receptors as CCR2 or CCR3 [55].

A relation has been reported between the biological phenotype of HIV-1 and the virus transmission and the diseases progression [54-58]. In general, X4 viruses are associated with rapid disease progression; X4 viruses may be transmitted at a very low rate and, if so, selected against during early events of HIV-1 infection. R5 viruses occur early after infection, are more frequently transmitted and dominate until AIDS [59, 60]. In approximately 50% of AIDS patients, the X4 phenotype appears when the number of CD4+ T cells is reduced. It is also possible that X4 viruses may contribute directly to CD4+ T cell killing. Evidence that R5 viruses are more easily transmitted than X4 viruses comes from genetic studies of deficiencies in the CCR5 gene. People lacking the CCR5 gene (homozygous) are rarely infected by HIV-1. Heterozygous individuals get infected at a much lower rate than those who have the wild type CCR5 gene [55, 61, 62]. There is also an influence of the SDF-1 gene, coding for the ligand of CXCR4, on transmission from mother to child of HIV-1 (MTCT) [55, 63].
There is a difference among HIV-1 subtypes with regard to co-receptor use. Approximately 20-50% of the HIV-1 subtype B viruses use CXCR4; this ratio is lower in HIV-1 subtypes A and C whereas subtype D uses CXCR4 more frequently [32, 64, 65]. Thus HIV-1 subtype C uses CCR5 more frequently than the other subtypes [32].

Current research projects are aimed at finding the association between co-receptor use and subtype in order to explain why some subtypes are more easily transmitted or lead to more rapid disease progression than others. Unfortunately, this relationship is still unclear and needs further study. Studies in Uganda showed that HIV-1 subtype D is more often associated with faster progression, higher mortality rate and rapid transmission than other subtypes [59] and HIV-1 subtype B had a lower transmission rate and a rapid progression. Studies conducted in South Africa concluded that HIV-1 subtype C spreads more rapidly than other subtypes. In contrast, no significant differences in disease progression between HIV-1 patients infected with A, B, C and D were observed in a study in Sweden [66].

HIV-2 is so far divided into 7 distinct subtypes A, B, C, D, E, F and G [12]. All subtypes were found in Africa. In addition HIV-2 subtype A dominates in America, Asia and Europe and HIV-2 subtype B can be found in the Middle-East and Europe (Los Alamos HIV database)

1.3 HIV-1 EPIDEMIC IN VIETNAM AND NEIGHBORING COUNTRIES

1.3.1 The HIV-1 epidemic in Vietnam has spread from intravenous drug users to sex workers and to the general heterosexual population

In Vietnam the first HIV/AIDS case was detected in 1990 in Ho Chi Minh city and after 9 years the HIV-1 epidemic had spread rapidly and cases of HIV-1 were reported to occur in all 61/61 provinces. By 1999 there were 13,623 confirmed cases of HIV-1 infection which increased to 126,543 in 2007 [67, 68]. The estimated number of HIV-1 infected people increased by 2007 to over 290,000 HIV-1 cases in the whole country (with
a low estimate of 180,000 cases and a high estimate of 470,000) [69]. Until the end of May 2007, there were 24,788 cases of AIDS, and estimate 20,000 had died from AIDS or related diseases. Ninety percent of the infected HIV-1 people in Vietnam are under 40 years of age [68]. The HIV-1 prevalence among young people (15-24 year old) in 2007 in Vietnam is 0.6% in males and 0.3% in females [69]

Estimated adult HIV prevalence and number of people living with HIV between 1990-2007 in Vietnam


The HIV-1 epidemic in Vietnam is progressing following the pattern of HIV-1 spread in Thailand. In the early phase of the epidemic the majority of HIV-1 infections were detected in intravenous drug user (IDUs) and later on it spread to the commercial sex workers (CSWs) [70]. After infection of CSWs the HIV-1 virus entered into the general population. Young IDUs with multiple sexual partners have been driving the HIV-1 epidemic in Vietnam [71]. During the years 1996, 1999, 2005, HIV-1 infection was found predominantly in IDUs but with decreasing proportion (86%, 65%, 34%)
while the infection in CSWs increased (2.5%, 4%, 6.5% respectively) [67, 71-73]. Differences in HIV-1 prevalence in the different risk groups were observed between the North and the South. In IDUs the HIV-1 prevalence was 56% in the North and 43% in the South. In CSWs it was 17% in the North versus 12% in the South. The HIV-1 epidemic in the North is concentrated to the border provinces Langson, Quangninh and Haiphong, in addition to the capital Hanoi [71].

HIV-1 prevalence in Vietnam is at present 0.53% (low estimate of 0.3% and a high estimate of 0.9%) of the general population which corresponds to 87 millions [68].

1.3.2 The HIV-1 epidemic in neighboring countries

The HIV epidemic in Laos, northwest of Vietnam, started in 1990. By 2007 the adult HIV-1 prevalence in Laos was 0.1% with IDUs and the sexual route as the major causes of transmission [1, 74].

The first HIV-1 case in Cambodia, located southwest of Vietnam, was found in 1991. The HIV-1 prevalence in Cambodia was about 0.9% by 2006 with sexual transmission as the major route of infection [1, 75].

In the North, Vietnam borders with China. In China the HIV-1 epidemic started in 1985. This country is one of the low HIV-1 prevalence countries (0.1% in 2007) with the highest prevalence rate in the IDUs group [1, 76].

Thailand is the most popular country for tourism in Asia. The first HIV-1 case in Thailand was detected in 1984, and the prevalence of HIV-1 in adults was 1.4% in 2007. Around 80% of the HIV-1 infections in Thailand occur through heterosexual contact [1, 77].
1.3.3 The relation of the HIV-1 epidemic in Vietnam to the epidemic in neighboring countries

The first case of HIV-1 infection in Vietnam was a woman, who got infected from her foreign partner with HIV-1 subtype B in 1990 [67]. So far, of the 948 Vietnamese HIV-1 sequences reported in the Los Alamos HIV database, 98% were HIV-1 CRF01_AE, 1.4% were of HIV-1 subtype B and the remaining were of other subtypes. Some studies reported that isolates classified as HIV-1 CRF01_AE in Vietnam show a great similarity with sequences from neighboring countries, especially with Thailand and Southern China (Pingxiang) sequences [67, 73, 78-80].

Map of Vietnam and neighboring countries
This observation highlights the relation of the HIV-1 epidemic to the major heroin shipment routes from the Golden Triangle [Myanmar (Burma), Thailand, and Laos] to Hongkong [78, 81], from Laos into Hanoi and then turning North. In the Chinese provinces of Quangxi and Quangdong, at the border of Northern Vietnam, about 70%-80% of the HIV-1 infected are IDUs [76]. The “open door” policy in Vietnam contributes to fast economic growth. Thus traveling and communication inside and outside Vietnam becomes more frequent. In addition to all good factors generated by the fast growing economy, this becomes also a favorable condition to the import and export of HIV.

1.4 MOTHER TO CHILD TRANSMISSION OF HIV

1.4.1 Mother to child transmission of HIV in the world

According to UNAIDS, at least 90% of HIV-1 infected children were infected through vertical transmission. There is an estimate of about 1,200 new HIV-1 infection a day by MTCT in low and middle income countries [1]. Among the new HIV-1 infections world-wide, more than 10% are in children [1, 82]. The HIV-1 infected neonates and infants develop AIDS more rapidly than infected adults [83]. The rate of HIV-2 transmission from mother to child is much lower than transmission of HIV-1. However, both HIV-1 and HIV-2 viruses can be transmitted from pregnant infected women to their infants.

The rate of MTCT of HIV-1 in the United States and Europe has now been controlled and estimated to be less than 1%, whereas the same figure in developing countries is still around 10% [84]. However these figures can still vary also according to the presence of prevention programs in different countries.

The prevalence of HIV-1 infection in pregnant women in Eastern and Southern Africa is between 15-40% [85]. Prevalence of HIV-1 infection among pregnant women in Botswana was documented as the highest frequency in the world.
(32% by 2006), followed by the Republic of South Africa with 29% by 2006. It was 18% in Zimbabwe, 4.2% in Togo, 1.5% in Benin, and 3.6% in Ghana in 2006. Madagascar is the country with the lowest HIV prevalence among pregnant women in Africa (0.2% in 2005) [1].

In China the frequency of HIV-1 infection in pregnant women was reported to be 0.4-1.4% in 2003. The first case of MTCT was reported in 1995 and increased overtime from 0.1% in 1997 to 0.4% in 2002 [86]. In Cambodia a frequency of HIV-1 infected mothers of 1.7%-2.8%-4.1% was reported in 1996, 2002, 2006 respectively [1, 87, 88]. In Thailand, 2.35% pregnant women were HIV-1 infected in 1995 and this figure decreased to 1.18% in 2003 [1, 77, 89].

MTCT can occur in utero, during delivery and at breast-feeding and all these events have a cumulative effect over time. In fact, the cumulative HIV-1 transmission is around 25-45% with 5-10% of infections occurring in utero, about 10-15% at intra-partum and 10-20% through breast-feeding and increase with prolonged breast feeding [58, 82-84, 90-94]. With no breast-feeding, the overall transmission is 15-25% divided to 5-10% in utero and 10-15% in intra-partum [83, 84]. Approximately 40% of the infections occur in utero and 60% of the infected children acquire the infection at the time of delivery [95].

1.4.2 Intervention programs to reduce HIV-1 transmission from mother to child

The rate of vertical HIV-1 transmission can be reduced by intervention with antiretroviral prophylaxis, elective caesarean section (ECS) and no breastfeeding [94, 96-101]. Combination of all these interventions has reduced the rate of transmission in industrialized countries to ≤1% [102]. But these procedures are difficult to apply in developing countries because of the economic and social conditions, ethical factors and poor knowledge. The intervention package recommended by WHO contains
counselling, testing, antiretroviral drugs, ECS and non breast-feeding [100, 103]. These procedures are applied and standardized in industrialized countries but still need to be further implemented in developing countries [95].

According to WHO, antiretroviral treatment (ART) is recommended for pregnant women for prevention of mother to child transmission of HIV (PMTCT). ART will not only contribute to the well-being of the mothers but also significantly reduce HIV transmission to their infants. Additional consideration is the need of well being of the fetus. Drug regimens for the mothers depend on clinical stage and CD4+ T cell count [82, 103]. In developing countries, consideration regarding cost, safety and simplicity of administration are important factors. For the pregnant women who are eligible for ART, regimens start as soon as possible with zidovudine (AZT) + lamivudine (3TC) + nevirapine (NVP) twice daily. For the pregnant women who are not eligible for ART, treatment should start with AZT from 28 weeks of gestation. Both groups are then provided AZT+3TC+NVP intra-partum and AZT for one week postpartum. For the infants NVP 2mg/kg is provided within 48 hours and AZT for one week [82].

Forty-one percent reduction of the risk of transmission was seen in NVP treated groups, as compared to AZT treated groups in recent studies [104-106]. These procedures have markedly reduced the rate of MTCT but contributed to increased drug resistance of HIV-1. HIV-1 genome mutations associated with NVP resistance could be found in 15-69.5% of HIV-1 infected women [96].

The presence of HIV-1 in breast milk was confirmed and its level was related with plasma viral load. Formula feeding is not commonly used in developing country since it is expensive. Otherwise formula feeding is recommended in places where access to clean water and understanding of sanity is good, thereby fulfilling the requirements of formula feeding being acceptable, feasible, and safe [107-109]. That
HIV-1 can be transmitted through breast feeding is well known and exclusive breast feeding is one efficacy intervention in PMTCT [94, 98, 100, 110-112].

HIV-1 transmission from mother to child occurs through the transfusion of blood from the mother to the fetus, cervical contraction, infection after the rupture of membranes, or direct contact of the fetus with secretions and blood from the maternal genital tract [113]. Vaginal delivery is not recommended for the HIV-1 pregnant women since the presence of HIV-1 in the vaginal secretions was demonstrated. Therefore ECS was recommended for PMTCT [100, 113, 114]. For medical workers, the fear to become infected by cutting themselves during the cesarean section provides a barrier for the general application of this procedure in developing countries. However the efficacy and safety of ECS for PMTCT were demonstrated in recent studies [97, 113, 115]. The rate of MTCT in the ECS groups is significantly lower than in the vaginal delivery group and emergency caesarean section groups as it was concluded in previous studies [97, 115].

One more intervention that has a great impact on PMTCT is counselling. Counselling can be an important motor to bring the HIV-1 pregnant women to the PMTCT services. Through counselling HIV infected patients improved their knowledge, changed their attitude, behavior and practices. However, many of the HIV pregnant women have refused to attend counselling because of stigma and discrimination [116]. The target for PMTCT for 2010 is to ensure that 80% of HIV pregnant women will have antenatal care, counselling, testing and access to treatment with antiretroviral drug (ARV) in order to reduce of 50% the proportion of infants newly infected with HIV-1 [103].

1.4.3 HIV-1 transmission from mother to child in Vietnam

The number of HIV-1 infected women in Vietnam is still increasing. While there is some decline in HIV-1 prevalence in neighboring countries like Thailand
and Cambodia, the epidemic is growing in Vietnam [1]. The number of HIV-1 infected women reported in Vietnam probably only represents 20% of the real number [117]. The first HIV-1 pregnant woman in Vietnam was detected in 1993. Then the proportion of HIV-1 infected pregnant women increased from 0.03% in 1994 up to 0.37% in 2005 and 0.38% in 2006 [1, 71, 117, 118]. The number of HIV-1 infected children increased proportionally. When taking into account the high estimates of HIV-1 infection among pregnant women, the proportion of HIV-1 infected women in Vietnam may have reached 0.7% [119].

<table>
<thead>
<tr>
<th>HIV-1 prevalence in pregnant women in Vietnam</th>
</tr>
</thead>
<tbody>
<tr>
<td>% HIV prevalence</td>
</tr>
<tr>
<td>Year</td>
</tr>
<tr>
<td>0.00</td>
</tr>
</tbody>
</table>

Source: Ministry of Health, Sentinel Surveillance Survey data

According to World Health Organization (WHO), an HIV/AIDS free new generation cannot be achieved without universal access to high quality PMTCT [109]. Since 2005, Vietnam receives support from the US President’s Emergency Plan for AIDS Relief (PEPFAR), the Global Fund for AIDS, Tuberculosis and Malaria (GFATM), UNAIDS and UNICEF. The National PMTCT program was directly guided by the Vietnamese Ministry of Health (MOH) and collected efforts were given to counselling, HIV-1 testing, ARV, non-breastfeeding and implementation of formula
feeding. The success of the counselling and testing mostly depends on the health care system that is well organized in cities and big provinces but still poor in rural areas in Vietnam. Also, ARV has become available in Vietnam mostly in the big cities and provinces. By law and policy, a single dose of NVP was offered free of charge to all HIV-1 pregnant women. However, this procedure failed due to the weakness of the health system. Only 25% of the HIV-1 pregnant women received NVP in Vietnam, most of them in the cities or big provinces [118]. In most cases, vaginal delivery was always given priority, while intervention with the caesarean section depended on the situation of the labor and delivery.

Following the recommendation by WHO, the use of the formula feeding was also recommended for all HIV-1 mothers in Vietnam but this implementation is introduced very slowly because of economic and social factors. In Vietnam, cow milk is available on the market, but the use of this product instead of the breast milk is considered to be expensive, especially for the rural citizens. In addition when respecting the right of the woman not to talk openly about her HIV-1 status, an explanation to the other family members about why she does not breast-feed becomes difficult. Therefore the rate of HIV-1 women who did not receive any intervention in Vietnam was estimated to be about 1/3. Nearly 1/5 got comprehensive intervention [118].

In order to reduce the HIV-1 prevalence, the Vietnam National HIV/AIDS prevention program received an estimate of 9.4 million from the country’s budget and donations from foreign organizations [120, 121]. An estimate of about 10.000-12.000 HIV-1 patients will be provided with ARV treatment. Free condoms and laboratory testing and counselling will also be provided when possible [120, 121].
1.4.4 Factors and markers related with vertical transmission of HIV-1

There are obviously a number of factors and markers that through the years have been shown to be associated with MTCT. Although the analysis of these factors has not been part of my present work it is important to list their relevance here.

MTCT is depending on the duration of delivery time, the mode of delivery, the presence of sexually transmitted infection and premature delivery [83, 84, 93]. The high-risk transmission of HIV-1 from mother to child also relates to high maternal viral load, low CD4+ T cell count and the presence of co-infections [83, 122]. Mothers who are co-infected with Hepatitis C virus have an increased risk of transmitting HIV-1 to their children and the same trend is also reported for Hepatitis B virus, Malaria, Tuberculosis and all agents which cause sexual transmitted diseases (STD) [123-125]. In contrast, HIV-1 infected mothers co-infected with Trypanosoma cruzi or GB virus C have been noticed to have a reduced risk to transmit HIV-1 to their children [126, 127].

The transmission rate of HIV-1 from mothers to child increased from 15% to 43% in mother with CD4+ T cell counts >600/μl and <200 cell/μl respectively [99]. The risk of vertical transmission of HIV-1 is directly proportional to plasma viral load [128-130]. In fact, maternal HIV-1 RNA levels were highly predictive of perinatal transmission risk suggesting that the levels of virus late in gestation and/or during labor and delivery are associated with risk of transmission [131].

The relation between the properties of HIV-1 isolates and vertical transmission of HIV-1 was demonstrated. The R5 viruses are more commonly associated with vertical transmission than X4 viruses [56, 60, 64, 132-135]. X4 viruses can be transmitted to infants but less frequently than R5 viruses [56, 64, 132-134]. Today, it is believed that an homogeneous R5 virus population is transmitted to infants, which later may develop into an heterogeneous virus population [132, 136]. The individuals carrying the CCR5 variant 32bp gene (CCR5Δ32) were less susceptible to infection
with R5 viruses and had a slow disease progression upon infection [61, 62, 134]. SDF-1, the ligand for the CXCR4 receptor, has been shown to display genetic polymorphism. The relationship of SDF-1 genetic polymorphism with the vertical transmission of HIV-1 was also studied and it was shown that the maternal heterozygous SDF1 3’A mutation may have increased the risk of HIV-1 vertical transmission [61-63].

To find additional immunological markers associated with vertical transmission of HIV-1 to predict the transmission risk is very important in prevention projects. Therefore some immune markers were investigated such as p24 antigenemia, β2 microglobulin (β2m), neopterin and CD36. The majority of these markers were found to be elevated in HIV-1 infected persons as compared to HIV-1 negative individuals. The association with diseases progression or ART was found for p24 antigenemia [137-139]. Also β2m and neopterin may be useful for early diagnosis in HIV-1 infected children [140]. CD36 is expressed at high levels in children who got HIV-1 infected through vertical transmission; the relationship of this marker with vertical transmission has yet not been studied [141].

1.5 PLASMA SOLUBLE CD27 LEVEL

A vaccine would be the most optimal way for HIV-1 prevention. Research on this topic should be intensified in spite of the fact that many vaccine projects for HIV-1 have not been successful. Utilizing the results from a previous study [142], we focused on the relation between plasma soluble CD27 (sCD27) and the outcome of ARV therapy. We also studied how this marker related to the risk of MTCT.

CD27 molecule is a transmembrane protein which belongs to the TNF receptor family, is expressed on NK, T and memory B cells [142, 143] and is also a surface marker of memory B cells [144]. In peripheral blood and tonsils,
approximately 30% of B cells and 75% of T cells express CD27 [145, 146]. The sCD27 is released by activated T cells through cleavage by metallo-proteases [147].

The size of sCD27 is 28-32 kDa. The sCD27 is found in plasma samples from healthy individuals [148] and high levels of sCD27 have been found in biological fluids from patients with autoimmune disorders, including Lupus Erythematosus, Rheumatoid Arthritis and Multiple sclerosis [142, 147]. Plasma levels of sCD27 have been used as a marker in acute and chronic B cell malignancy [148-150] and may also represent a simple marker of immune activation during ART in HIV-1 infected subjects [142]. The mechanism leading to an increased release of sCD27 during HIV-1 infection is unknown.
2 AIMS OF THE THESIS

a. To characterize the genotypes of HIV-1 in Northern Vietnam in HIV-1 infected IDUs and pregnant women.

b. To follow the evolution of the HIV-1 epidemic in Northern Vietnam and in relation to neighboring countries according to HIV-1 sequences.

c. To study HIV-1 transmission from mother-to-child in the North of Vietnam.

d. To study the relation of sCD27 with Antiretroviral Therapy in patients infected with different HIV-1 subtypes.

e. To investigate the association of plasma sCD27 with HIV-1 transmission from mother to child.
3 MATERIAL AND METHODS

3.1 STUDY POPULATIONS

During 2002 we collected specimens from 17 HIV-1 IDUs from a reformatory in Hanoi to investigate which HIV-1 phenotypes were circulating in IDUs in Hanoi at that time. The IDUs had not met prior to the admission to a reformatory for treatment of IDUs; they lived in different regions around Hanoi (Paper 1).

From 2005-2007, my colleague, Dr Anh, and myself met 234 HIV-1 pregnant mothers of whom 182 mothers and their children were enrolled in our study, 147 from Hanoi (my patients) and 35 from Haiphong (my colleague’s patients). Samples were collected to identify their HIV-1 status. This material was also used to define the HIV-1 genotype in this population. The HIV-1 mother status was tested when they attended the Obstetric and Gynecology hospitals in Hanoi and Haiphong for check up and delivery. To monitor for the infection in the children, we followed them from birth up to 12 or 18 moths of life (Papers 2 and 3).

Blood was collected from 64 HIV-1 infected individuals attending the Infectious Diseases Clinic at the Karolinska Hospital in Stockholm. A control group was also collected, matching the HIV-1 infected patients in age and length of permanence in Sweden (paper 4).

Venous blood was collected in EDTA (Papers 1, 2, 3 and 4). The venous blood from children was collected at birth and at 1, 3, 6 and 12 or 18 months after birth. Blood samples from mothers were taken after delivery (papers 2, 3). Blood samples in Stockholm were taken before initiation of ART and 12 months after ART (paper 4)

3.2 DNA AND RNA PREPARATION (PAPERS 1, 2, 3 AND 4)

From the blood samples, peripheral blood mononuclear cells (PBMCs) were isolated by Lymphoprep (Axis-Shield PoC AS, Oslo, Norway) and plasma was
collected as well. DNA was prepared from 2x10^6 PBMCs, lysed in 0.2 ml Lysis buffer pH 9.0 (10 mmol/L Tris-HCl pH 9.0, 1 mmol/L EDTA, 0.5% NP-40, 0.5% Tween-20), containing proteinase K 20mg/ml and detergent. The solution was incubated overnight at 37°C, followed by inactivation of enzymes by heating at 95°C for 10 minutes. RNA was extracted from 140μl plasma by RNA QIAamp mini kit (QIAGEN). Viral RNA was used as template to run cDNA synthesis by the Ominiscript kit (QIAGEN). The remaining cells and plasma were stored at -20°C.

3.3 NESTED PCR (PAPERS 1, 2, 3, 4)

The polymerase chain reaction (PCR) technique was used for detection of HIV-1 pol gene in the sample. A child was identified as HIV-1 infected if the PCR result was positive at 2 different occasions or at least once after birth and by serology at 18 months of age. The child was confirmed to be non HIV-1 infected, if serology was negative at 12 and/or 18 months. We used two sets of primers from the pol gene. One set comprised the outer primers JA79, JA82 and the inner primers JA 80, JA81. The other set comprised the outer primers JA171, JA174 and the inner primer JA172, JA173 [151] (paper 3).

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>JA79</td>
<td>5’-ACAGGAGCAGATGATACAGTATTAG-3’</td>
</tr>
<tr>
<td>JA82</td>
<td>5’-CCTGGCTTTAATTTTACTGGTACAG-3’</td>
</tr>
<tr>
<td>JA80</td>
<td>5’-GAAGATGGAACCCAAAATGTAGG-3’</td>
</tr>
<tr>
<td>JA81</td>
<td>5’-CAATTATGTGACAGGTAGTCC-3’</td>
</tr>
<tr>
<td>JA171</td>
<td>5’-CCCCAACGTCAAGGAGTAGAAGAA-3’</td>
</tr>
<tr>
<td>JA174</td>
<td>5’-TACTACTGCCCCTTCACCTTTCCA-3’</td>
</tr>
<tr>
<td>JA172</td>
<td>5’-CTTAAAGACAGCAGTAGCAAAATGAGCAG-3’</td>
</tr>
<tr>
<td>JA173</td>
<td>5’-TGCTGTCCCTGTAAATAACCCGAA-3’</td>
</tr>
</tbody>
</table>
In papers 1, 2 and 4 we ran nested PCR, amplifying the V3 loop region of the HIV-1 gene. The outer primers were JA167 and JA 170 and inner primers JA168 and JA169 [132, 152, 153].

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>JA167 Outer</td>
<td>5′-TATCTTTTGAGCCAATTCCTATACA-3′</td>
</tr>
<tr>
<td>JA 170 Outer</td>
<td>5′-GTGATGTATTRCATAGAAAAATTC-3′</td>
</tr>
<tr>
<td>JA 168 inner</td>
<td>5′-CAATG(C/T)ACACATGGAATTA(A/G)GCCA-3′</td>
</tr>
<tr>
<td>JA 169 Inner</td>
<td>5′-AGAAAAATTC(C/T)CCTC(C/T)ACAATTAAA-3′</td>
</tr>
</tbody>
</table>

**PCR1**: Ten μl DNA were amplified in a final volume of 50μl containing 5μl MgCl2 25mmol/l, 5μl PCR buffer, 1μl dNTP 2.5mmol/l, 0.2μl Taq and 0.5μl of each primer (10μmol/l) and H2O. PCR was run for 30 cycles of 92°C/30sec, 50°C/30sec and 72°C/30sec with the denaturation at 92°C/1min and incubation at 72°C/1min.

**PCR 2**: Two μl of DNA producted from PCR 1 were amplified in a final volume of 50μl containing 5μl MgCl2 25mmol/l, 5μl PCR buffer, 1μl dNTP 2.5mmol/l, 0.2μl Taq and 0.5μl of each primer 10μmol/l and H2O. PCR was run for 30 cycles at 92°C/30sec; 55°C/30sec, 72°C/30sec with the denaturation at 92°C/1min and incubation at 72°C/1min.

The PCR product was visualized in 1.5 % agarose gel after ethidium bromide reaction with DNA. The PCR was regarded as positive if both primer sets were positive and the correct size of the amplified product was visualized in the gel (JA79-82 = 129 base pairs; JA171-174 = 178 base pairs). When both bands were negative the sample was regarded as negative.

### 3.4 Determination of HIV-1 Genotypes (Papers 1, 2 and 4)

Purification of DNA from 100μl PCR product was performed by QIA quick PCR purification kit (QIAGEN company). The staining of the nucleotides was
performed by Big Dye Terminator 3.1 (AB Applied Biosystems). The free nucleotides were removed by Dye Ex 2.0 spin kit (QIAGEN company). The product was directly run and sequenced on the ABI PRIMS 3700 DNA sequencer (Applied Biosystems, Foster City, CA). The sequence chromatograms were evaluated by Sequencher 4.1 program (Gene Codes, Ann Arbor, MI). BioEdit was used for editing the nucleotide sequences by hand. Sequences alignment was performed by MAFFT version 6 program. Phylogenetic trees were constructed by the TreeView 1.6.6 program. The reference sequences were available at the Los Alamos HIV database.

3.5 CREATION OF PHYLOGENETIC TREES (PAPER 2)

Phylogenetic analyses of genetic material (DNA, RNA and proteins) are a method to study epidemiologically important relations and differences. It has proven valuable in the characterization of the HIV epidemic both geographically and with regard to changes over time and of differences in different risk groups. It forms the bases for the classification of HIV-1 into types, subtypes and recombinant forms.

In this thesis it was used to characterize the subtype of HIV-1 in IDUs and pregnant women as well as in the analysis of changes of HIV-1 genome over time. We used the PHYML (Phylogenetic Maximum Likelihood) program available at http://atgc.lirmm.fr/phyml/binaries.html to create the phylogenetic trees.

3.6 CALCULATION OF THE EVOLUTION OF HIV EPIDEMIC (PAPER 2)

The evolution of the HIV-1 epidemic in Vietnam was calculated using BEAST version 1.4.8 (Bayesian Evolutionary Analysis Sampling Trees), available at http://beast.bio.ed.ac.uk. In order to get the most correct values, all sequences needed to come from the same site from at least two different time points. The distance in time between the collected samples should be at least 3 or 4 years. A low evolutionary rate of change of HIV-1 implies that the epidemic spreads fast. A high evolutionary rate is associated with a slow spread of the epidemic at that site.
3.7 QUANTIFICATION OF PLASMA sCD27 (PAPER 4)

The levels of sCD27 in plasma were measured by Enzyme-Linked Immuno Sorbent Assay (ELISA). This is a ‘sandwich – type” ELISA. We used Human sCD27 ELISA kit from CLB (Amsterdam). The assay was performed in 96 well micro titer plates that were coated overnight with a monoclonal CD27 antibody. This monoclonal captured sCD27 present in a sample. The non-bound material was removed by washing. A biotinylated secondary antibody bound the anti-CD27-sCD27 complex. Horseadish peroxidase (HRP) conjugated streptavidin was then added. The combination between biotin and streptavidin created the color reaction. The analysis was completed by adding a substrate, which modulated the color that can be read in a spectrophotometer at 450 nm wave length absorbance. The color is formed in proportion to the amount of sCD27 originally present in the sample.
4 RESULTS AND DISCUSSION

4.1 HIV-1 CRF01-AE IS THE MAIN HIV-1 GENOTYPE IN NORTHERN VIETNAM (PAPERS 1, 2)

In paper 1, which was carried out in 2002, we investigated the HIV-1 genotype in 17 IDUs in Hanoi. The HIV-1 genotype was determined, based on the amino acid sequences of the V3 env region and part of the p17 gag protein.

All but one of the IDUs were infected with HIV-1 CRF01_AE despite that they lived in different regions and had no social relationship with one another. In one of the IDUs, sequences from both HIV-1 CRF01_AE and HIV-1 subtype C could be found, leading to the possibility that this individual may have been infected with two distinct viruses (HIV-1 CRF01_AE and HIV-1 subtype C genotypes) or with a recombinant virus between HIV-1 CRF01_AE and HIV-1 subtype C genotypes.

In paper 2, the HIV-1 genotype in 37 HIV-1 pregnant women (15 from Haiphong and 22 from Hanoi) were determined basing on the sequences of the V3 env region alone. The HIV-1 CRF01_AE was found in all of them.

4.2 THE EPIDEMIC OF HIV-1 STRAIN IN VIETNAM AND RELATION TO NEIGHBORING COUNTRIES (PAPER 2)

In order to establish a comparison for the sequences derived in our studies, 399 gp120 V3 env HIV-1 sequences from Asia (including Vietnamese sequences previously characterized) were retrieved from the Los Alamos HIV database.

Up to now, there are 387 phylogeny packages available to analyze the relationship of both nucleotide and amino acid sequences, of which 52 can be used free of charge.

Using the PHYML program, the HIV-1 CRF01_AE sequences retrieved from the Los Alamos HIV database, the 37 maternal sequences, and the sequences from 14
IDUs were divided into four phylogenetic areas. The majority of sequences in sections I and II were represented by HIV-1 CRF01_AE sequences from Thailand (67% and 79% respectively). No sequences from Northern Vietnam appeared in these sections (I and II), a few occurred in section III and the largest number (68%) was present in cluster IV. The HIV-1 CRF01_AE sequences from Southern China had a similar distribution as the Northern Vietnamese sequences: 8%, 6%, 3% and 30% in clusters I, II, III and IV, respectively.

The sequences from Southern Vietnam appeared rarely (16%, 8%) in clusters I and II, but were highly represented in cluster III (60%) and almost absent in cluster IV (2%). This phylogenetic analysis illustrated the difference between Northern – Southern Vietnam and also the relationship between sequences from Thailand and Southern Vietnam, and between Southern China and Northern Vietnam. The close link between the heroin traffic and HIV-1 in the Northern Vietnam and South China is well known. The link between South Vietnam and Thailand may be associated with movement of both IDUs and CSWs across Cambodia [154].

Five of our maternal sequences and one IDU sequence from Hanoi were located in the cluster III, dominated by sequences from Southern Vietnam and Thailand. The remaining sequences from 32 mothers and 13 IDUs belonged to the cluster IV, also gathering with sequences from Northern Vietnam and Pingxiang in Southern China. This result was similar to what found in previous studies, illustrating that HIV-1 sequences from Northern Vietnam are phylogenetically related to sequences from Southern China [67, 73, 78-80, 155-157].

The North and South of Vietnam are connected through a long and narrow piece of land. It is possible that the IDUs and CSWs in the North and South have different ties to neighboring countries. While it is easy for the general population to travel between the North and the South, HIV-1 risk groups may remain in their own
milieu. Most of CSWs have a lower education level than the national level, they often live with male IDUs for sharing money and drug [154]. Since the CSWs and IDUs economy is always problematic, local traveling is mostly preferred than long distance travel.

Using the BEAST method to estimate the evolutionary rate of the epidemic in Northern Vietnam, we observed a very low rate of evolution of the HIV-1 V3 env region, measured to $2.378 \times 10^{-3}$ substitution site$^{-1}$ year$^{-1}$ (95%CI; $3.837 \times 10^{-4} - 4.218 \times 10^{-3}$), whereas changes have previously been measured to range from $2.3 \times 10^{-3}$ - $6.7 \times 10^{-3}$ substitution site$^{-1}$ year$^{-1}$ [158]. Since the sequences were carried out at sufficiently different time points, the result can be interpreted so that the spread of the HIV-1 epidemic in the North of Vietnam is very rapid, not only in IDUs but also in different risk groups, including HIV-1 infected pregnant women.

The evolutionary rate of HIV-1 has been reported to differ according to subtypes and risk behavior group and in relation to geography [158]. As an example it can be mentioned that the evolution rate of HIV-1 subtype A1 in Former Soviet Union is faster than HIV-1 subtype A1 in Africa (rate of $2.02 \times 10^{-3}$ vs $16.9 \times 10^{-3}$) [158]. It is also relevant to point-out that the HIV-1 CRF01_AE in the whole Southeast Asia shows a slower spread than what was found in Vietnam with an evolution rate of about $8.32 \times 10^{-3}$ [158]

4.3 COUNSELLING OF HIV-1 INFECTED MOTHERS IN NORTHERN VIETNAM (PAPER 3)

In Vietnam, the HIV screening test is a routine examination for every pregnant woman, who is charged with a minimal fee refundable by health insurance. This test is free of charge in most countries [159]. Five years ago, the Ministry of Health in Vietnam developed a Volunteer Counselling and Testing (VCT) program in each district, city and province. As a result of this program all Vietnamese citizens can get
counselling, testing for HIV and condoms free of charge without reporting. This policy was expected to result in the early detection of HIV-1 infection in the population. In the hospital, counselling and ARVs were provided free of charge for every HIV infected person. Following the WHO treatment guideline, the counselling and testing of HIV pregnant women were set-up and run in the Obstetric and Gynecology hospitals in Hanoi and Haiphong. The HIV screening test was applied to all pregnant women at 7-8 months gestation in Vietnam [117].

At the end of December 2003 the workshop “Mother to child transmission of HIV” was organized by Anneka Ehrnstr and Francesca Chiodi and held at the Obstetric and Gynecology hospital in Hanoi, Vietnam, funded from the SIDA Swedish Link program. During this workshop, we had important and interesting discussions between Vietnamese medical doctors and Swedish experts on different aspects of HIV-1 mother to child transmission. During a second visit to Hanoi, by Anneka Ehrnstr, Kerstin Lagerström (sociologist and career manager at Karolinska Institutet) and Annika Lidfeldt (medical social worker at Karolinska University Hospital) focus was given to communication between profession, with patients, in creating means of multidirectional influence in an HIV perspective.

We knew from the Swedish experience and from other countries that counselling is the first step to approach the HIV infected patient and has a key role in the PMTCT. By counselling, the HIV patients become confident with the doctor and this is an important step to change their behavior. With this knowledge in mind, I and my collaborators set-up to initiate a study on MTCT of HIV-1 in Northern Vietnam, funded by a grant from SIDA to Vietnam. In Sweden I have learnt the procedure of counselling of HIV-1 infected pregnant women and also the importance of follow-up of the children with adequate sampling (from my co-supervisor Anneka Ehrnstr) at birth, 1-2 months after birth, and at the late time point of 12 -18 months for a final
determination of the HIV-1 status in the child. This consecutive sampling also allows the determination of the time of transmission in infected children.

Among 234 HIV-1 infected pregnant women, who attended the delivery hospitals in Hanoi and Haiphong, 182 mothers agreed to enroll in our study. This group also included 50 mothers (27.5%) who could not get counselling before delivery, since they attended the hospital only for delivery. This group was given counselling after delivery.

A previous study conducted in Vietnam aimed at studying the behavior of HIV-1 infected women, reported that 22/52 (42%) HIV-1 infected pregnant women received no counselling at all [118]. This difference may be due to the fact that the field work of Nguyen and collaborators was more extensive than mine and it included urban and sub-urban areas where the health system is still poor and the stigma and discrimination are very strong social constrains [95, 116, 159].

In my study, counselling was offered to all HIV-1 pregnant women regardless of the time of enrolment in the study. In addition, a 18 month follow up of their children was also offered, not only concerning the HIV status but also the general health situation of the child. If there was no time for a meeting before delivery, we collaborated with the doctor and nurse in charge of labor to provide ARV to the pregnant woman on time and also to protect the health care worker. A meeting was arranged with the mother as soon as possible to inform her about the HIV-1 status. The mother was recommended not to start breastfeeding.

Normally we spent 3 hours on the first meeting, which took place in my office at the Obstetric and Gynecology Hospital in Hanoi. My colleague, Dr Mai Anh, met the pregnant women at the Obstetric and Gynecology Hospital in Haiphong. We tested the knowledge of the mother about HIV/AIDS and focused on information on how to live with HIV-1 and nutrition facts for the baby. The women were informed about the
opportunity to try to avoid transmission of HIV-1 to the child by NVP treatment of themselves and their baby, by formula feeding, and the importance of following up the child for early diagnosis of the virus infection.

All the mothers were advised to bring up their children by formula feeding after ensuring that they could follow this recommendation and apply it safely. Exclusive breast feeding is uncommon in Vietnam [160]. One more difficult aspect for the mother was how to explain why they would not use breast-feeding without informing about their HIV status. To resolve this aspect we provided them with plausible explanations that were not directly related to HIV/AIDS.

Questions on how they contracted HIV-1 infection were not asked to the mothers, in order to focus on creating a situation of confidence and trust with the women included in the program.

4.4 MOTHER TO CHILD TRANSMISSION OF HIV-1 IN NORTHERN VIETNAM (PAPER 3)

The ARV was given free of charge to every HIV pregnant woman under the supervision of the National program for PMTCT. We followed the following regimen: if the HIV-1-infection was detected during delivery, the woman was provided with one dose of 200 mg NVP at delivery or at least two hours before caesarean section. If the HIV-1-infection was detected during gestation, the women were provided with AZT + 3TC + nelfenavir (300mg+150mg+1250mg twice per day, respectively). NVP was provided in case the maternal level of CD4+ T cells was less than 200 cells/μl, while nelfenavir was preferred for CD4+ T cells of more than 200 cells/μl. Their children were given 2 mg of NVP liquid/kg weight within the first 48 hours of life (before 2005), and AZT liquid was continued for a week (from 2006).

In my study, 182 children were followed up to 18 months of life; for 135 children there were stringent criteria to identify their HIV-1 status. Of the children, 9 of
135 (6.7%) contracted infection from their mothers. The data of MTCT in Vietnam is not available yet, since many projects aimed at reducing MTCT in Vietnam were interrupted due to the loss of many patients because of fear of stigma and lack of capacity for follow-up [161]. In China, few HIV-1 infected children related to MTCT were reported, but high prevalence of MTCT was found (30-38%) in certain areas as the Henan province [86, 162]. The rate of MTCT in Thailand was also documented to be reduced from 10.2% in 2003 to 8.1% in 2005 through the use of different means of prevention [89, 163].

Seven infected children of 167 had a positive PCR test at birth, as evidence of intrauterine transmission in 4.2%. Two children were negative at birth but positive at one month, suggesting that these 2 children were exposed to intra-partum transmission, which had then occurred in 1.48%. There was no sign of late transmission through breast feeding, as all 135 children were tested 1-3 months after birth and later, with no new infection detected later than at 1-3 months. The frequency of intra-uterine and intra-partum transmission in our group of mothers can be compared with the frequency of 4.4% and 3.8% in Thailand in 2005 [163].

For the majority of the HIV-1 pregnant women in my study the HIV-1 status became known late in pregnancy and therefore the full course of ART could not be provided. This problem was also reported in a previous study [161]. Nearly 60% of HIV-1 pregnant women in my study received one dose NVP at delivery which could not have affected in utero transmission. This probably explains the higher rate of intrauterine transmission. Fifteen of the mothers (11%) got triple ARV treatment (AZT + 3TC + nelfinavir) for a few weeks before delivery and their children got more AZT liquid for one week after delivery. Two of these children were infected with HIV-1 at birth, which provides evidence of intrauterine infection. Since ARV was provided to the mothers only 4 weeks before delivery, these children could have been infected
before ARV treatment was started. On the other hand, 13/15 of the mothers, who got triple ARV treatment, gave birth to a child who was not infected, demonstrating the usefulness of triple ARV treatment also in a shorter perspective.

The association between low CD4$^{+}$ T cell counts and HIV-1 transmission from mothers was observed when dividing the HIV-1 infected mothers in two groups with CD4$^{+}$ T cell counts $<200 \times 10^{6}$/L and CD4 cells $\geq 200 \times 10^{6}$/L ($p<0.0001$), which is a cut-off value used to define AIDS. Five of 38 children born by emergency caesarean section got infected (13.1%) compared with 4/97 (4.1%) infected children who were naturally delivered. This difference was not statistically significant ($p=0.12$). The use of emergency caesarean section was approved if the labor took too long time. Emergency cesarean section often occurs after rupture of the membranes, increasing the exposure of the child to HIV, while ECS decreases this exposure. This scenario probably explains why emergency caesarean section might have led to an increased risk of MTCT similarly to the conclusion from some previous studies [100, 113, 115].

4.5 LEVELS OF CD27 CAN BE USED AS A TOOL TO MONITOR THE CHANGE IN IMMUNE ACTIVATION FOLLOWING ART (PAPER 4)

In the paper 4, the levels of sCD27 were significantly higher in the HIV-1 infected group than in the uninfected group (522 U/ml vs 285 U/ml with $p<0.001$). After one year of ART, the reduction of sCD27 was seen in HIV-1 patients infected with either subtypes A, B, C and D. The level of sCD27 decline was however different in the different groups. The greatest reduction was observed in HIV-1 subtype C patient group after one year on ARV therapy (683 U/ml vs 319 U/ml with $p<0.001$) while for HIV-1 subtype A patient group the levels went from 428 U/ml to 273 U/ml with $p=0.001$. In the group of patients infected with HIV-1 subtype B, the levels of sCD27 were reduced from 454 U/ml to 412 U/ml ($p=0.021$) and decreased from 502 U/ml to 205 U/ml ($p=0.016$) in HIV-1 subtype D patient group.
In previous studies it was suggested that plasma sCD27 may be an optimal marker to follow immune activation during HIV-1 infection [142, 164]. The high levels of sCD27 found in plasma of HIV-1 subjects infected with subtype C, and the significant reduction noticed after 12 month ARV treatment in these individuals, may suggest that HIV-1 subtype C induced immune activation may be stronger than for other HIV-1 subtypes. It was previously suggested that HIV-1 subtype C may be related with the rapid disease progression and increased drug resistance [39]. The fact that the levels of sCD27 had changed significantly after treatment in all subtypes and had been reduced to normal after 12 month of treatment, points to the possibility that sCD27 may be useful as an immune parameter to monitor response to therapy. Similar results were shown in an additional study in 1999 [165]. The first published study on this subject showed that a correlation exists between sCD27 and levels of T cells [166]. Then a study conducted in Ethiopia in 2001 also concluded that sCD27 inversely correlated with CD4 T cell counts reinforcing the predictive value of this measurement in relation to worsening of immunological conditions during HIV-1 infection [164].

4.6 RISK OF HIV-1 TRANSMISSION FROM MOTHER TO CHILD IN RELATION TO PLASMA LEVELS OF MATERNAL SCD27

Inspired from the results obtained in paper 4, we investigated if the levels of sCD27 can be predictive for the risk of HIV-1 transmission from mother to child. The blood samples from HIV-1 infected mothers (n= 52) and uninfected mothers (n = 49) were taken at one day after delivery at the Obstetric and Gynecology hospitals in Hanoi and Haiphong. The measurement of the levels of sCD27 was performed by ELISA method. The result showed that the levels of sCD27 in plasma in the HIV-1 infected group was significantly higher than in the uninfected group (803 U/ml vs 494 U/ml with p<0.001), similarly to what already reported in paper 4 and the above mentioned studies. To investigate the relation between sCD27 with the risk of HIV-1 transmission
from mother to child we compared the levels of plasma sCD27 in 11 mothers infected child (MIC) and 41 mothers uninfected child (MUC). Using the Student’s t test, we could not measure any statistical significant difference in the levels of sCD27 between MIC and MUC (721 vs 825 with p=0.418). These results indicated that plasma sCD27 levels can not be used as a possible marker to predict if HIV-1 will be transmitted from mother to child.

**Plasma sCD27 in samples from Vietnamese HIV-1 infected and control**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Plasma sCD27 (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIC (n=11)</td>
<td>0</td>
</tr>
<tr>
<td>MUC (n=41)</td>
<td>500</td>
</tr>
<tr>
<td>Control (n=49)</td>
<td>1000</td>
</tr>
<tr>
<td>HIV-1(+) (n=52)</td>
<td>1500</td>
</tr>
</tbody>
</table>
5 CONCLUSION

Hopefully my thesis helps to draw the picture of the HIV-1 epidemic in Northern Vietnam, in respect to genotype spread and MTCT.

The HIV-1 CRF01_AE genotype is dominating in Northern Vietnam and this feature of the HIV-1 epidemic in Vietnam has not been changing significantly over time. In order to get the full picture of HIV-1 epidemic in Vietnam it would be important to extend this study to some additional provinces and to other HIV-1 risk groups, e.g. CSWs. To obtain these results could be useful for HIV-1 vaccine and prevention studies.

In Vietnam, IDUs and CSWs act as a bridge to spread HIV to the general population. To limit the spread of HIV, programs for IDUs to use clean needles and free condoms for the general population were carried out in some provinces; these programs however did not completely succeed since the participation was low, due to fear of stigma and discrimination. Hopefully, several prevention programs will be run in the future and we will improve on how to encourage participation.

Programs for PMTCT are now running in Vietnam, but the national PMTCT program needs to be further developed and shaped to fit Vietnamese culture. ART for PMTCT is now available in Vietnam, but only few HIV-1 pregnant women have received it, because the identification of their HIV-1 status occurs late during pregnancy.

The efficacy and safety of formula feeding for PMTCT was reported in my study. Non breast feeding should be recommended for all HIV-1 mothers. The financial support for formula feeding should be provided for at least 6 months to 1 year of age of life in infants. Financial support for formula feeding should be added to PMTCT programs in Vietnam since it can increase significantly the effectiveness of PMTCT.
All HIV-1 infected women included in the study who received counselling accepted to exclude breast feeding; in addition these mothers and their children accepted to return to the doctor thus implying that the counselling which was carried out in my study and which I have learnt from the Swedish colleagues was of good quality. This counselling strategy should be further implemented in Vietnam.

In Vietnam, counselling was performed by medical doctors and this is good because they have a good medical knowledge; but the disadvantage is that they have limited time to follow up the children up to 18 months and also they have incomplete knowledge on how to impact on societal rules and family structure. Therefore the best scenario would be that counselling should be assisted by social workers, but this profession is still poorly represented in Vietnam. Another group of people who could contribute to counselling are volunteer HIV patients who have gone themselves through counselling.

The early diagnosis of HIV-1 infection in children is very important in order to decide in relation to ART. ART is of great benefit for HIV-1 infected patients but it can also be harmful due to toxicity. All children in our study were tested with PCR from birth and additionally by serology at one year of age or beyond. Nine children were reproducibly positive with PCR and also had a positive serology and therefore were diagnosed to be HIV-1 infected. None of the uninfected children was ever positive by PCR or serology. PCR technique should be applied as a golden standard technique for the early HIV-1 diagnosis of children born from HIV-1 infected mothers. Hopefully this technique should be standardized and set-up in several hospitals and laboratories in Vietnam.

None of the HIV-1 pregnant women in my study received ECS. However its safety for mothers and efficacy to prevent HIV-1 infection to infants were elucidated in other studies. This procedure is very important to apply since the rate of HIV-1
transmission in late pregnancy and during labor is high. Unfortunately, ECS has not yet become a routine procedure when assisting the delivery of HIV-1 infected mothers in Vietnam and other developing countries. The impact of ECS in Vietnam should be clarified in future studies.

The hope is there that an effective HIV vaccine will be available soon; in the meantime we have to try our best to limit the spread of HIV-1 by applying all means of efficient and safe intervention.
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7 REFERENCES


