# HIV-1, HIV-2 and other Sexually Transmitted Infections in Guinea-Bissau, West Africa

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## and other Sexually Transmitted Infections in Guinea-Bissau, West Africa

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Sign near the 2a esquadra police station in central Bissau, encouraging staff of the Police force to use condoms correctly in order to avoid HIV/AIDS/STIs. Photo by the author.

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*In memoriam*

**Braima Dabo**

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**I.** Månsson F, Alves A, da Silva ZJ, Dias F, Andersson S, Biberfeld G, Fenyo EM and Norrgren H. Trends of HIV-1 and HIV-2 prevalence among pregnant women in Guinea-Bissau, West Africa: possible effect of the civil war 1998-1999. *Sexually Transmitted Infections*. 2007, 83:463-467. © 2007 BMJ publishing group. Reprinted with permission.

**II**. Månsson F, Biague A, da Silva ZJ, Dias F, Nilsson LAF, Andersson S, Fenyo EM and Norrgren H. Prevalence and incidence of HIV-1 and HIV-2 before, during and after a civil war in an occupational cohort in Guinea-Bissau, West Africa. *AIDS*. 2009, 23:1575-82. ©2009 Wolters Kluwer Health/Lippincott Williams and Wilkins. Reprinted with permission.

**III.** Månsson F, Biague A, da Silva ZJ, Isberg P-E, Nowroozalizadeh S, Jansson M, Andersson S, Fenyo EM and Norrgren H. Natural course of seroincident HIV-2 in Guinea-Bissau, West Africa – comparisons with HIV-1 and seroprevalent HIV-2 infection. *Manuscript*

**IV.** Esbjörnsson J*, Månsson F*, Kvist A, Biague A, Nowroozalizadeh S, da Silva ZJ, Jansson M, Fenyö EM, Norrgren H and Medstrand P: Natural inhibition of HIV-1 disease progression by contemporaneous HIV-2 infection. *Manuscript (submitted)* *these authors contributed equally to the work

**V.** Månsson F, Camara C, Biai A, Monteiro M, da Silva ZJ, Dias F, Alves A, Andersson S, Fenyö EM, Norrgren H and Unemo M. High prevalence of HIV-1, HIV-2 and other Sexually Transmitted Infections among women attending two sexual health clinics in Bissau, Guinea-Bissau, West Africa. *International Journal of STD and AIDS.* 2010, 21:631-5. © 2010 Royal Society of Medicine Press, UK. Reprinted with permission.

# Abbreviations

| | |
|---|---|
| Ab | Antibody |
| Aguibef | Associação Guineense para o Bem Estar Familiar (Guinean Association for the well-being of the family) |
| AIDS | Acquired Immunodeficiency Syndrome |
| ART | Antiretroviral Therapy |
| BHP | Projecto de Saúde de Bandim (Bandim Health Project) |
| $CD4^+$ | T lymphocyte bearing Cluster of Differentiation 4-receptor (CD4) |
| $CD8^+$ | T lymphocyte bearing Cluster of Differentiation 8-receptor (CD8) |
| CDC | Centers for Disease Control and Prevention |
| CI | Confidence Interval |
| CMI | Clínica Materno Infantil (Mother and Child Health Clinic) |
| CRF | Circulating Recombinant Form |
| CSW | Commercial Sex Worker |
| CTL | Cytotoxic T lymphocyte |
| CTX | Co-trimoxazole (Trimethoprim-Sulfamethoxazole) |
| CVL | Cervico-vaginal lavage |
| DC | Dendritic Cell |
| DNA | Deoxyribonucleic acid |
| DRC | Democratic Republic of Congo |
| GUD | Genital Ulcer Disease |
| GUM | Genito-Urinary Medicine |
| HIV-1 | Human Immunodefiency Virus type 1 |
| HIV-2 | Human Immunodefiency Virus type 2 |
| HIV-D | HIV-1 and HIV-2 dual infection |
| HPV | Human Papilloma Virus |
| HSV | Herpes Simplex Virus |
| HTLV | Human T-cell Lymphotropic/Leukemia Virus |
| IDU | Intra-venous drug user |
| INH | Isoniazid |
| IPPF | International Planned Parenthood Federation |
| IQR | Inter-quartile range |
| IRR | Incidence Rate Ratio |
| LNSP | Laboratório Nacional de Saúde Pública (National Public Health Laboratory) |
| LTNP | Long-term non-progressor |
| MSM | Men who have sex with men |

| | |
|---|---|
| MTCT | Mother-to-child transmission |
| NK | Natural Killer (Cell) |
| OI | Opportunistic infection |
| PCR | Polymerase Chain Reaction |
| PLWHA | People living with HIV/AIDS |
| PMTCT | Prevention of Mother-to-child transmission |
| PYO | Person-years |
| RNA | Ribonucleic Acid |
| RT | Reverse Transcriptase |
| Sida | Swedish International Development Cooperation Agency |
| SMI | Smittskyddsinstitutet (Swedish Institute for Communicable Disease Control) |
| SMNH | Simão Mendes National Hospital (Bissau) |
| SSA | Sub-Saharan Africa |
| SNLS | Secreteriado Nacional de Luta contra Sida (National Secreteriat of the fight against AIDS), Guinea-Bissau |
| STI | Sexually Transmitted Infection |
| TB | Tuberculosis |
| TPHA | *Treponema pallidum* Hemagglutinin Antigen |
| TPPA | *Treponema pallidum* Particle Antigen |
| UNAIDS | United Nations Programme on HIV/AIDS |
| USA | United States of America |
| VDRL | Venereal Disesase Research Laboratory test |
| VL | (Plasma) Viral Load |
| WA | West Africa |
| WB | Western Blot |
| WHO | World Health Organization |

# Summary

HIV-1 is dominating the global HIV pandemic, while HIV-2 is mainly confined to West Africa. The highest HIV-2 prevalence figures have been reported from Guinea-Bissau. While HIV-1 infection almost invariably leads to progressive immune dysfunction and AIDS, HIV-2 infection is characterised by a smaller proportion of individuals with disease progression to AIDS.

In order to monitor the long-term epidemiological trends of HIV-1 and HIV-2, we performed sentinel studies among pregnant women and followed a cohort of police officers. In the period 1987 to 2004, HIV-2 prevalence declined in pregnant women and during 1990 to 2007 the pattern was the same among police officers. We examined specifically if the civil war 1998 – 1999 had any impact on the HIV epidemic in Guinea-Bissau and found in both groups that HIV-1 prevalence increased sharply after the conflict but then stabilised. In the police cohort, HIV-1 incidence peaked during and shortly after the conflict, but later stabilised at lower levels. Thus, we noticed a short-term effect exerted by the war but no evidence of any long-term effect.

We examined the natural course of HIV-2 infection in seroincident individuals after 20 years of follow-up in the police cohort. As expected, median survival time and disease progression time to AIDS was longer and the CD4+ T cell decline rate was lower compared with HIV-1 infection. There was no significant difference compared with HIV-2 seroprevalent individuals, indicating that earlier studies of HIV-2 seroprevalent individuals were quite representative for HIV-2 infection in general. According to our data, a majority of HIV-2-infected individuals, if followed over longer time, will probably progress to immunodeficiency and clinical disease.

We also investigated the difference in disease course in seroincident HIV-1 infection and the influence of contemporaneous HIV-2 infection. The median time of progression to AIDS was 53% longer in dual HIV-1 and HIV-2 infection than in HIV-1 single infection and the difference was more pronounced in the individuals with a recorded HIV-2 infection preceding seroconversion to HIV-1. The epidemiological results correlated to higher CD4+ T cell counts and lower

genetic HIV-1 diversity among dually infected individuals compared with HIV-1 single-infected.

Finally, we investigated the prevalence of other STIs in women with urogenital problems and found high prevalence of several STIs. The most prevalent were *Trichomonas vaginalis* (20.4%) and *Chlamydia trachomatis* (12.6%). Infections with Herpes Simplex Virus type 2 and *Mycoplasma genitalium* were associated with HIV positivity.

# Resumo em Português (Summary in Portuguese)

O Vírus da Imunodeficiência Humana (VIH) é a causa do Sindroma de Imunodeficiênca Adquirida (SIDA). A epidemia de VIH/SIDA afetou, até agora, mais de 60 millhões de pessoas, e mais de 30 milhões morreram de SIDA. A infeção VIH transmite-se através do sangue, sexualmente, e de mãe para filho na gravidez e amamentação. Na infeção de VIH, o material genético viral é inserido no material genético humano, e a infeção torna-se crónica. As primeiras células alvo do VIH são as células T-auxiliares ($CD4^+$). Quando estas células gradualmente diminuem, o nível de defesa imunológica vai reduzindo e a pessoa infetada pode contrair outras doenças infeciosas (oportunistas) e tumores. Há dois tipos de infeção– o VIH-1 que está presente globalmente e o VIH-2 que está principalmente localizado na Àfrica ocidental e países aí ligados historicamente, como Portugal e França. No início de anos 80, a Guiné-Bissau apresentou os níveis mais altos de VIH-2 no mundo. Porém, a prevalência baixou desde então, tendo simultaneamnete aumentado a prevalência de VIH-1. A infeção pelo VIH-1, não tratada normalmente, leva à morte dentro de 15 anos. A infeção pelo VIH-2 é menos agressiva, levando anteriormente à hipótese de que só 25-30% das pessoas infetadas desenvolviam SIDA. O VIH-2 é também menos transmissível do que o VIH-1. Previamente, especulou–se se o VIH-2 poderia proteger contra a infeção pelo VIH-1 ou atrasar o aparecimento de sintomas do VIH-1.

Neste projeto estudámos três grupos na Guiné-Bissau – mulheres grávidas que, desde 1987, foram testadas anonimamente aquando do parto, mulheres com queixas urogenitais e um grande grupo de polícias. Deste modo, pudemos seguir tendências da prevalência de VIH-1- e VIH-2 na Guiné-Bissau. No grupo de polícias, pudemos calcular também quantos contraíram a infeção pelo VIH de novo, quantos desenvolveram SIDA e/ou faleceram.

Os resultados dos nossos estudos mostram que a prevalência de VIH-2 tem diminuído continuamente desde os anos 80 e que, à volta do ano 2000, a prevalência do VIH-1 ultrapassou a do VIH-2. Estes resultados estão de acordo com os de outros estudos nesse país. A guerra civil na Guiné-Bissau em 1998-1999 parece ter contribuido para um aumento marcado da prevalência do VIH-1

em mulheres grávidas e polícias, assim como para um aumento de casos novos em polícias, durante e depois do conflito. O aumento acabou posteriormente e a prevalência do VIH-1 estabilizou num nível mais alto do que antes do conflito, nos dois grupos.

O curso clínico do VIH-1 é bem conhecido mas, não há muita informação que diz respeito ao VIH-2. A maior parte das investigações sobre o VIH-2 são estudos empessoas seroprevalentes, onde a infeção pelo VIH-2 está estabilizada, sendo desconhecida a duração da infeção (aquando da inclusão nos estudos). Na população de polícias, acompanhados repetidamente, pudemos deterrminar uma data provável de transmissão (seroconversão de anticorpos contra o VIH-1 ou o VIH-2). Foi também possível estudar o curso da doença pelo VIH-2, comparado à causada pelo VIH-1. Pudemos concluir que, aproximadamente 50% das pessoas com VIH-2, desenvolveram SIDA clinicamente, mais do que o previsto, em tempo médio de 15 anos, versus os 8.9 anos nas pessoas com VIH-1. Também observámos uma correlação entre a perda precoce de células T-auxiliares e o desenvolvimento de SIDA, o que reforça a importância de acontecimentos precoces, para o prognóstico.

Nas pessoas com infeção dupla pelo VIH-1 e VIH-2, observámos que a infeção simultânea de VIH-2 parece proteger contra o desenvolvimento da doença causada pelo VIH-1, especialmente quando a infeção de VIH existiu algum tempo antes da seroconversação do VIH-1. Os resultados clínicos relacionaram-se bem com os dos estudos moleculares de evolução do vírus, onde verificámos que os sinais de replicação do VIH-1 foram inibidos pela presença do VIH-2.

Outras doenças sexualmente transmissíveis também foram estudadas. Observámos altos níveis de Trichomonas, herpes e Chlamydia, mas níveis baixos de gonorreia e sífilis. A infeção pelo VIH correlacionou-se com as de herpes e Micoplasma genitalium.

No futuro, será importante continuar a monitorizar a evolução das epidemias de VIH-1 e VIH-2, primeiramente para ver se a transmissão de VIH-1 vai aumentar. É importante também estabelecer melhor vigilância das outras infecões sexualmente transmissíveis, uma vez que devem ser tratadas correa- e atempadamente. Esse controlo vai também prevenir a transmissão do VIH. Os nossos resultados mostram que, apesar das pessoas com VIH-2 desenvolverem doença sintomática em menor escala do que os doentes com VIH-1, a extensão da doença pelo HIV-2 é muito superior à que era antes conhecida. A vigilância da doença clínica e o início do tratamento antirretroviral atempado é particularmente importante/relevante, tendo em conta que os indivíduos com VIH-2 respondem mais tarde ao tratamento do que os com VIH-1. Futuramente seria valioso

possibilitar a medição da carga viral (ARN) de VIH-2, para melhorar o seguimento de pessoas com VIH-2 tratadas com antirretrovirais.

O maior desafio na epidemia de VIH na Guiné-Bissau, atualmente, é o início atempado e manutenção do tratamento antirretroviral necessário para as pessoas com VIH

Em relação aos nossos estudos, o mais interessante será tentar investigar as causas do efeito atenuador do VIH-2 contra o desenvolvimento da doença do VIH-1. Um conhecimento mais profundo desta área poderia ser útil no desenvolvimento duma vacina terapêutica para impedir a perda de imunidade em pessoas com VIH-1.

# Svensk sammanfattning (Summary in Swedish)

Humant immunbristvirus (HIV) orsakar förvärvat immunbristtillstånd (AIDS). HIV/AIDS-epidemin har hittills innefattat över 60 miljoner människor som har infekterats och över 30 miljoner av dessa har avlidit i AIDS. HIV-infektion smittar via blod, sexuellt och från mor till barn vid graviditet eller via bröstmjölk. Vid infektion infogas virusets arvsmassa i den infekterade personens, vilket gör infektionen kronisk. De främsta målcellerna för HIV är en typ av vita blodkroppar, T-hjälpar-cellerna ($CD4^+$-celler). Genom ett gradvis nedbrytande av dessa försvagas den infekterades motståndskraft mot andra sjukdomar och tumörer. HIV föreligger i två varianter – HIV-1 som är globalt förekommande och HIV-2 som huvudsakligen finns i Västafrika och i länder med historiska band dit, framförallt Portugal och Frankrike. Guinea-Bissau har haft de högsta nivåerna av HIV-2-infektion i världen, men förekomsten har varit i sjunkande medan HIV-1-infektion har ökat. HIV-1 leder vanligtvis till AIDS och död inom 10 års tid medan HIV-2 är mindre aggressivt och man har tidigare ansett att endast ca 25-30% leder till AIDS. HIV-2 smittar också i mindre utsträckning än HIV-1. Det har tidigare spekulerats i om HIV-2-infektion kan skydda mot HIV-1-infektion eller fördröja sjukdomsutveckling vid samtidig infektion. Vi har undersökt olika grupper i Guinea-Bissau – gravida kvinnor som sedan 1987 anonymt har provtagits i samband med förlossning, kvinnor som har sökt hälsovård på grund av underlivsbesvär samt en stor grupp poliser. Därmed har vi kunnat följa trender av HIV-1 och HIV-2-förekomst i Guinea-Bissau och hos poliserna även kunnat följa hur många som efter hand har blivit nysmittade av HIV och hur många som har insjuknat i AIDS och avlidit.

Resultaten av våra studier visar att HIV-2-förekomsten stadigt har sjunkit sedan 80-talet och att HIV-1 under senare hälften av 90-talet passerade HIV-2 som den vanligast förekommande typen, resultat som även har påvisats i andra studiepopulationer i landet. Vi såg att det inbördeskrig som förelåg i Guinea-Bissaau 1998-1999 bidrog till en kraftig ökning av HIV-1-förekomst, både hos gravida kvinnor och poliser och bland poliserna kunde vi även konstatera att antalet nyinfekterade ökade markant under och strax efter kriget. Den kraftiga ökningen av HIV-1-infektion var dock inte varaktig utan efter kriget lade sig förekomsten på en jämnt högre nivå i båda grupperna.

De flesta undersökningar av HIV-2 har utgått från individer som redan har varit HIV-2-positiva redan vid studiens begynnelse, där man inte har känt till hur länge personerna har varit smittade. I gruppen av poliser kunde vi genom upprepade undersökningar av alla deltagare fastställa ungefärliga datum för infektionerna och sedan undersöka sjukdomsförloppet hos HIV-2 jämfört med HIV-1. Vi såg då att en något större andel av HIV-2-infekterade individer utvecklade klinisk AIDS (ca 50%) än vad man tidigare trott. Det tog dock nästan dubbelt så lång tid, i medeltal 15 år jämfört med 8,9 år hos HIV-1-positiva individer. Vi såg också en klar koppling mellan tidig förlust av T-hjälparceller och sjukdomsutveckling vilket visar att skeenden tidigt i infektionsförloppet är av betydelse för fortsatt prognos, precis som vid HIV-1-infektion.

Hos individer med HIV-1 och HIV-2 dubbelinfektion såg vi att samtidig HIV-2-infektion verkar skydda mot sjukdomsutveckling av HIV-1, men vi kunde endast klarlägga det i de fall HIV-2-infektion hade förelegat före HIV-1-infektionen inträffade. De kliniska resultaten stämde överens med molekylära studier där vi såg tecken till att nybildning av HIV-1 hämmades i närvaro av HIV-2. Ytterligare insikter i orsakerna till HIV-2:s dämpande inverkan på HIV-1-infektion är viktig för att kunna dra slutsatser som kan användas för framtagande av terapeutiska vacciner som skulle kunna förhindra sjukdomsutveckling hos HIV-1 positiva personer.

Vi undersökte förekomst av andra sexuellt överförbara infektioner förutom HIV. Vi såg höga nivåer av bland annat Trikomonas, herpes- och klamydia-infektion, men låga nivåer av bland annat gonorré och syfilis. HIV-positivitet var kopplad till herpes och *Mycoplasma genitalium*-infektion.

Inför framtiden är det viktigt att fortsätta övervaka trenderna i epidemierna av HIV-1 och HIV-2, framförallt för att snabbt upptäcka om HIV-1-spridning ökar. Det är av betydelse att sätta upp bättre övervakning av sexuellt överförbara infektioner för att kunna behandla dem korrekt och därmed även förhindra HIV-spridning. Våra resultat visar att även om HIV-2 utvecklar sjukdom i mindre utsträckning än HIV-1 så är det i större utsträckning än tidigare känt och vår förhoppning är att vaksamheten för sjukdom och insättning av antiretroviral terapi kommer att ökas för den här gruppen. I framtiden skulle det vara av värde att få till stånd rutinmässig virusmätning av HIV-2 så att behandling av HIV-2 infektion lättare skulle kunna utvärderas, framförallt då HIV-2-infektion regelmässigt svarar långsammare på antiretroviral behandling än HIV-1. Den största utmaningen avseende HIV-epidemin i Guinea-Bissau är att sätta in tillräckligt stor andel patienter på antiretroviral behandling och att hälsovårdssystemet ska klara av att erbjuda den ökande mängden personer fortsatt behandling.

# General Background

## Introduction

As the pandemic caused by the Human Immunodeficiency Virus (HIV) unfolded, it became one of the major health challenges on a global scale. Since the beginning of the epidemic, over 60 million people has become infected and over 30 million has died as the result of Acquired Immunodeficiency syndrome (AIDS). At the end of the year 2010, there were an estimated 34 million people living with HIV/AIDS (PLWHA). During 2010, an estimated 2.7 million new infections and 1.8 million deaths from AIDS occurred [1].

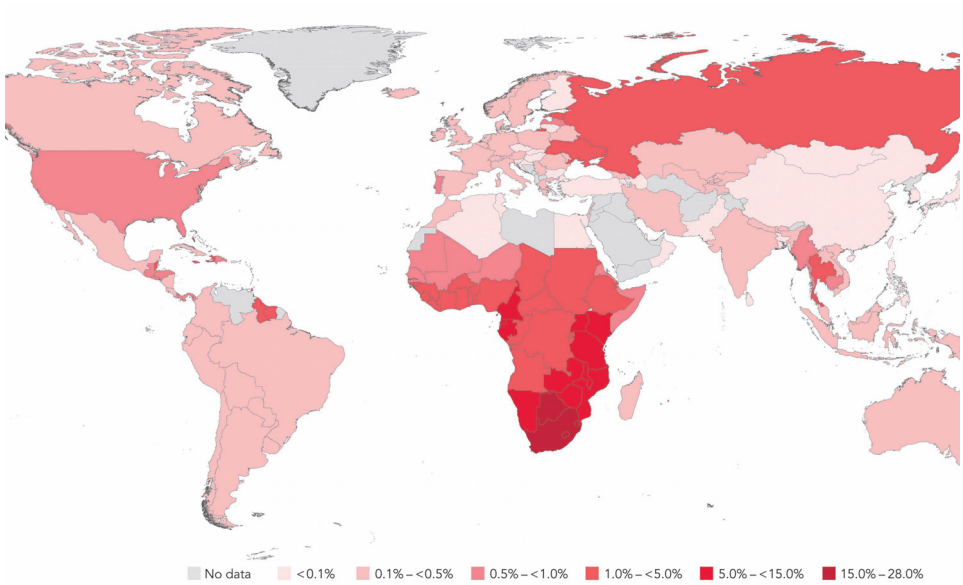
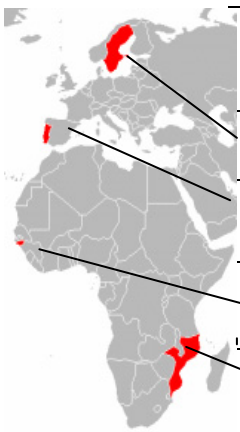



**Figure 1:** Global map of HIV prevalence 2009. From the UNAIDS Report on the Global AIDS Epidemic 2010. Reprinted with permission.

With 22.5 million individuals infected, over two thirds of the estimated global PLWHA, Sub-Saharan Africa (SSA) has by far been hardest hit by the HIV epidemic (*Figure 1*). Despite these overwhelmingly depressing figures, there is reason for future hope. New infections are believed to have peaked in 1999, and

are since then decreasing. The number of PLWHA receiving appropriate antiretroviral therapy (ART) is increasing. As a result of decreasing prices of antiretroviral medicines and increased investment in HIV-care-related health structures, over 5 million people have now initiated ART in SSA. For comparison, in Europe there were at the end of 2010 an estimated 2.2 million PLWHA. According to official statistics, in Sweden there were 9,400 reported cases of HIV at the end of 2011, whereof 2,346 received a diagnosis of AIDS and 2,182 were reported dead. The estimated number of PLWHA in Sweden at the end of 2010 was 5,300 individuals [2].

If we compare four countries of possible relevance for the reader of the present thesis, Sweden and the Lusophone countries of Portugal, Guinea-Bissau and Mozambique, we see that HIV prevalence levels and consequently the number of PLWHA differ substantially (*Figure 2/Table 1*).



| Country | Total pop. (millions) | Adult HIV prevalence | No of adult PLWHA | Main trans-mission route |
|---|---|---|---|---|
| Sweden | 9.1 | 0.1% | ~5.300 [2] | MSM, hetero-sexual, (IDU). |
| Portugal | 10.6 | 0.6% | ~40.000 [3, 4] | MSM, IDU, heterosexual |
| Guinea-Bissau | 1.5 | 5.8% | ~50.000 [5] | Heterosexual, MTCT |
| Mozam-bique | 22.9 | 11.5% | ~1.200.000 [3] | Heterosexual, MTCT |

**Figure2/Table 1:** Comparison of four countries regarding HIV prevalence (HIV-1 and HIV-2 combined) at the end of 2010 with estimated number of adult PLWHA extrapolated from reported figures of total adult HIV prevalence (data available at end of 2009 from Mozambique).

However, these figures are not making any distinction between the globally predominant HIV-1 and the more localised and less pathogenic HIV-2 infection, which carry different risks of morbidity and mortality [6]. In the figures above the vast majority of PLWHA are HIV-1-infected, with the exception of Guinea-Bissau, where according to the last national survey 53% of the reported adult HIV-infected persons were infected with HIV-1, 30% with HIV-2 and 19% were dually HIV-1 and HIV-2-infected [5].

# The discovery of AIDS and the emergence of the HIV pandemic

The first cases of AIDS were described 1981 in the USA when several cases of *Pneumocystis carinii* pneumonia and other symptoms related to cell-mediated immunodeficiency appeared within clusters in the gay community [7-9]. The disease seemed to be characterised by a progressive loss of immunity leading to opportunistic infections (OIs) and tumours and was named Acquired Immunodeficiency Syndrome (AIDS) [10]. In 1983, the AIDS-causing retrovirus HIV was discovered, then called HTLV-3 or LAV [11]. In 1986, a second retrovirus causing AIDS was discovered in West Africa [12]. This finally led to the present nomenclature of HIV-1 and HIV-2, of which HIV-1 dominates the global HIV pandemic and HIV-2 is mainly confined to West Africa. The earliest reports of AIDS in Africa came from Central and East Africa [13, 14]. Retrospectively, analysis of samples from 1959 and 1960 identified earlier cases of HIV-1 infection in the Democratic Republic of Congo (DRC) since these were tested positive for antibodies (Ab) against HIV-1, tracing the beginning of the HIV-1 epidemic back to the early 20$^{th}$ century in central Africa [15, 16]. While the highest recorded prevalence figures of HIV infection have been reported from Southern and East Africa [17], West Africa has experienced comparatively lower levels of HIV-1 prevalence. In contrast, HIV-2 prevalence was reported to be high in the first survey in several West African countries [18]. HIV-2 has been amplified from a blood sample dating back to 1966 [19] and the HIV-2 epidemic is now believed to have started in the 1930s-40s [20, 21].

# HIV-1 and HIV-2 in West Africa and in Guinea-Bissau

The first survey of HIV-2 was performed on blood samples obtained in 1985-1987 from different subpopulations in six different West African countries. It showed very high HIV-2 prevalence in samples from different groups in Guinea-Bissau, from 1/50 in healthy controls to 25/39 in commercial sex workers (CSW), none displayed antibodies against HIV-1 [18]. Simililarly in a survey in a community-based cohort in Guinea-Bissau performed in 1987, none of the tested individuals was HIV-1 positive but 8.9% of the adult population was HIV-2-positive, with an overrepresentation in 40-65-year-olds [22]. Following studies in Guinea-Bissau and the neighbouring countries confirmed high prevalence levels of HIV-2 [18, 23, 24]. However, in the following decade multiple surveys in the region generally

showed a gradual decline of HIV-2 prevalence, while an emerging epidemic of HIV-1 was noted [25-28]. The pattern of HIV-2 decline and increasing HIV-1 prevalence has continued into the 21st century [29-31].

*Transmission and influencing factors in Guinea-Bissau*

HIV-1 and HIV-2 are transmitted via body fluids such as blood, semen and breast milk. The most common transmission route worldwide is considered to be sexual and in most cases heterosexual, the main others being vertical transmission (in utero, at birth or via breastfeeding), via blood transfusion or other contact with blood as in intra-venous drug use (IDU). Heterosexual transmission is the predominant mode of transmission in Sub-Saharan Africa, followed by mother-to child-transmission [32]. HIV-2 has consistently been shown to be less transmissible both sexually and vertically [6].

Blood transfusions have been controlled since 1987 in Guinea-Bissau [30]. The first programme for Prevention of Mother-to child transmission (PMTCT) was a Nevirapine-based programme, initiated in 2002 by an Non-Governmental Organisation in Bissau [33]. In the capital Bissau access to PMTCT was reinforced as a part of the national ART programme initiated in 2005. There is still not universal access to PMTCT services in Guinea-Bissau but the situation is improving with gradual dissemination of services into the peripheral areas of the country.

The spread of HIV-2 in Guinea-Bissau is considered to have coincided with the prolonged war of liberation against the colonial power Portugal in 1963-74 [21, 34] and there are indications that parenteral rather than sexual transmission was the main route of transmission, possibly by parenteral treatment or female genital excision [34, 35]. The reports of HIV-2 in other former Portuguese colonies including Angola, Mozambique and Goa in India also indicates a Portuguese link in HIV-2 transmission [6].

As Guinea-Bissau experienced both the war of liberation and a civil war in 1998-1999, the question is inevitable of eventual contribution of conflict-related factors to the spread of the HIV-1 and HIV-2 epidemics. HIV transmission has been related to the possibility of mobility [36], although mobility being a wide concept that may not be easily applied in all situations [37]. However, in most conflict situations the presumed immobility of populations have reduced rather than enhanced HIV transmission [38].

Commercial Sex Workers (CSW) are considered to constitute the most important core group in the HIV epidemic in the majority of Sub-Saharan African countries [39]. There are ongoing interventional programmes and studies in Guinea-Bissau.

Men who have sex with men (MSM) were recognised from the beginning of the epidemic in the Western countries as a core group of HIV transmission. Homosexuality is not criminalized in Guinea-Bissau as it is in several other African countries but the stigma is still high leading to secrecy and there are no available data of the group or of eventual impairments regarding access to health care and educational services. In recent years Guinea-Bissau has been recognized as a centre for transportation of narcotics, mainly cocaine from South America, transferred via West Africa to European markets [40]. In addition, heroin is smuggled through West Africa but only to a small extent. There are anecdotal reports of local drug use of crack cocaine in coastal Guinea-Bissau but no reports of IDU.

# Virology of HIV-1 and HIV-2

*The origin of HIV*

The HIV type 1 and 2 belong to the *lentivirinae* genus of the *Retroviridae* family. Lentiviruses also include non-human retroviruses such as the Simian Immunodeficiency Virus (SIV) of primates as well as viruses of cats, cows, horses and sheep, presumably all derived from a common ancestor [41]. HIV-1 and HIV-2 originates from different SIVs, most likely through zoonotic transmission through contact with blood during hunting and handling of meat [42]. Other known human retroviruses are the Human T-Cell Lymphotropic viruses (HTLV) type I and II, of which HTLV-1 is the most common in Africa, causing haematological and neurological disorders [43].

*HIV-1*

For HIV-1, at least four cross-species transmissions have occurred with SIVs related to chimpanzees and gorillas living in central Africa, giving rise to the dominating subgroup M and the much less prevalent subgroups N, O and P [44]. Subgroup M which is dominating the global epidemic is closest related to the $SIV_{cpz}$ of chimpanzees (*Pan troglodytes*) living in central Africa [45]. After zoonotic transmission to humans, subgroup M has then through viral evolution diversified and given rise to subtypes (A-D, F-H, J-K) and circulating recombinant forms (CRFs) [46]. The HIV-1 epidemic in West Africa and Guinea-Bissau is dominated by the CRF named CRF02_AG, which was introduced in Guinea-Bissau in the mid-70's [47].

*HIV-2*

HIV-2 originated from a different SIV, the $SIV_{smm}$ related to the Sooty Mangabey (*Cercocebus atys*) which inhabitates West African forests from Senegal to Côte d'Ivoire. HIV-2 is the result of at least eight cross-species transmissions resulting in the subgroups A-H, but only subgroups A and B have formed epidemics. It has previously been shown that subtype variation in Guinea-Bissau was minimal with predominance of subtype A [48, 49].

*Structure of HIV*

HIV-1 and HIV-2 share around 40-60% genetic homology but have similar virion structures [50]. The virus particles are spherical with a diameter of approximately 100 nm and consist of a lipid envelope, derived from the host cell membrane, enclosing the protein nucleocapsid containing the viral RNA and viral enzymes. The genome consists of approximately 10,000 base pairs, including the structural genes *gag* (encoding the capsid and matrix proteins), *pol* (encoding the viral enzymes) and *env* (encoding the envelope glycoproteins), regulatory genes *tat* and *rev* and accessory genes *nef*, *vif*, *vpr* and *vpu* (HIV-1) or *vpx* (HIV-2) [51].

*Cell tropism, receptors and co-receptors*

HIV uses the Cluster of Differentiation 4 ($CD4^+$) receptor as a primary receptor for entering cells [52] and mainly targets T helper lymphocytes, macrophages and dendritic cells (DCs), though other cell types can also be infected [53]. Cell entry is acquired by interaction between the viral envelope glycoproteins and the CD4 receptor and a co-receptor, most commonly the chemokine receptors C-C chemokine receptor type 5 (CCR5) or C-X-C chemokine receptor type 4 (CXCR4) in HIV-1 [54, 55] while HIV-2 has a broader range of co-receptor usage [56]. Furthermore, a natural 32 base-pair deletion in the CCR5 co-receptor is associated with natural protection against CCR5-using HIV-1 viruses [57].

*Viral replication and life cycle*

Virus-receptor/co-receptor interaction enables a fusion of the viral and cell membranes, where after the viral capsid is released into the cellular cytoplasm. The capsid breaks up to release its content and the RT enzyme transcribes the viral RNA into double stranded DNA. The RT activity lacks proofreading capacity, hence the high rate of mutations in transcription, which effectively leads to the creation of quasispecies within an individual. Subsequently, the viral DNA is translocated to the nucleus and the viral Integrase enzyme integrates the viral DNA into the host genome. The integrated viral genome, also known as provirus, may remain latent for long time, but following host cell activation virus transcription is initiated making use of the host cell transcription apparatus. Viral

regulatory proteins additionally stimulate replication, assembly and budding of new virus particles [51].

*Viral evolution, diversity and divergence.*

HIV-1 evolution is characterised by high mutation rates, rapid viral turnover and high recombination rates, combined with host selection mechanisms. It can be quantified by diversity (the genetic variation at a given time-point) and divergence (the genetic distance to a reference point, e.g. a founder strain). Several studies have presented evidence of increasing diversity during the asymptomatic stage of infection [58-60]. HIV-1 diversity has also been positively correlated with plasma viral load (VL) and viral fitness [61, 62]. The divergence rate of HIV-1 has most often, though not always, shown to be relatively constant during the asymptomatic stage of infection [59, 63, 64].

*Pathogenesis of HIV-related disease*

The clinical course of HIV infection is usually divided into three stages: the acute, the asymptomatic (or chronic) and the AIDS stage (shown in *Figure 3*). The acute phase of HIV-1 infection is characterised by high viremia and rapid depletion of mucosal $CD4^+$ T cells, mainly in the gut-associated lymph tissue (GALT) [65]. After initiation of immune responses, the viremia decreases and settles to a set point level that often correlates to further prognosis [66]. In the following asymptomatic or chronic phase, the virus is partly controlled. There is constant immune activation, continuous $CD4^+$ T cell depletion and affected innate and adaptive immune responses [51]. In the natural course of the disease, the HIV-positive individual finally enters the AIDS stage where the impaired immune functions no longer are able to counter opportunistic infections and tumours, ultimately leading to death.

# The immune system and HIV infection

The immune system consists of the innate and the adaptive parts. The innate immune system is non-specific in its nature, not giving immunologic memory to the host. It consists of physical anatomical barriers such as skin and epithelium, chemical barriers and phagocytosing cells such as natural killer (NK) cells, macrophages and DCs. The innate immune system also contains chemokines inducing inflammation and complement system which create inflammatory cascades and acts like a bridge to the adaptive immune system [67]. The adaptive immune system consists of the cellular part and the humoral part. The cell-

mediated immune system may eliminate infected cells, mainly by the cytotoxic $CD8^+$ T cells (CTLs), and regulate by cytokine secretion, mainly by the $CD4^+$ T helper cells. The humoral immune system consists mainly of B lymphocytes that produce antibodies (Ab). In HIV infection, Abs are directed towards different HIV proteins, while the neutralising antibodies (Nab) exclusively act towards the envelope glycopropteins. HIV can escape from neutralisation by several different mechanisms [68].

HIV causes dysregulation of the immune system, leading to a multi-faceted picture of functional abnormalities. The main feature of HIV infection is the loss of $CD4^+$ T helper cells, but also functional aspects of both $CD4^+$ and CTLs are affected, including decreased polyfunctional activities and proliferation [69]. However, there is also dysregulation of the innate immune system, resisting control by NK cells and impairing innate regulation of adaptive responses [70]. Also Ab-producing B cells are affected, leading to impaired humoral response against HIV and other agents [71].

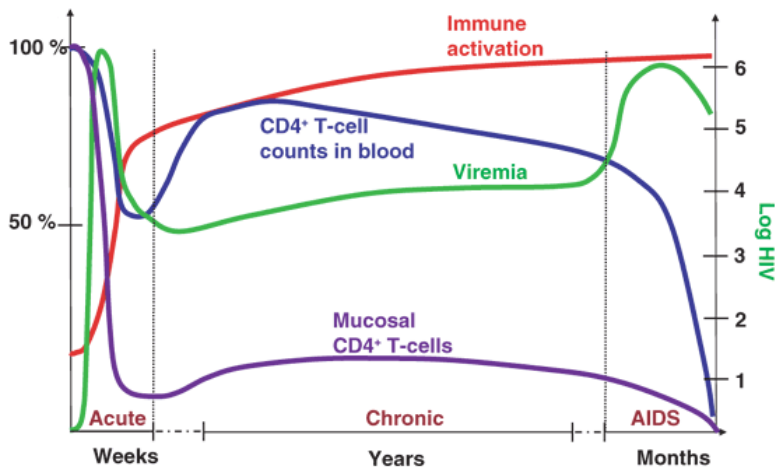



**Figure 3:** The natural course of HIV infection. An early increase of HIV viremia is followed by a decrease to a viral set point, commonly after approximately 1-2 months time. As the $CD4^+$ T cells are gradually depleted, opportunistic infections occur and at the end stage causes AIDS and subsequent death. Excerpt from Grossman et al [72], reprinted with permission from the Nature Publishing Group.

Systemic chronic immune activation during HIV infection has been shown to be an independent marker of disease progression [73]. Prolonged activation leads to gradual depletion and dysregulation of the immune system. The regenerative ability of $CD4^+$ T cells is not immediately impaired but rather gradually progressively exhausted [72] (*Figure 3*). It has been proposed that one

contributing factor to chronic immune activation may be microbial translocation to the blood after destruction of the mucosal barrier in the gastrointestinal canal [74]. Immune activation can be monitored by measuring activation markers expressed on T cells and also by the detection of soluble markers such as serum proteins, including Beta-2-microglobulin, neopterin and immunoglobulin levels [75].

# Laboratory diagnosis of HIV-1 and HIV-2

Serological testing is the base of laboratory diagnosis of HIV-1 and HIV-2. The antibody response can be measured from 1-2 weeks after primary HIV infection [51]. Screening of larger amounts of samples is usually performed with enzyme-linked immunosorbent assays (ELISA) but several different serological rapid tests have been developed and are nowadays frequently used at decentralised testing sites. More recently combined antibody/antigen tests detecting HIV p24 surface antigen have come in routine use, significantly shortening the window period between initial infection and possible diagnosis [76]. Confirmation tests are necessary to rule out unspecific reaction by the initial antibody screening tests. The standard method is the Western blot (WB), detecting specific antibodies against HIV proteins. However, the laborious and expensive nature of the WB assays as well as the risk of cross-reaction between HIV-1 and HIV-2 led WHO/UNAIDS to recommend alternative confirmation strategies [77]. Synthetic indirect ELISAs were shown to be as effective and discriminative as WB in several studies [78, 79]. For diagnosis of HIV-1 and HIV-2 dual infection (HIV-D), the optimal standard today is considered to be Polymerase Chain Reaction (PCR), although low HIV-2 VLs can not always be detected, thus PCR negativity does not completely exclude presence of HIV-2. With this in mind, dual seroreactivity is suggestive of dual infection if carried out with a validated method [80].

# Clinical course of HIV-1 and HIV-2 infection

*The different phases of HIV infection*

Primary HIV infection is characterized by a flu- or mononucleosis-like disease, sometimes accompanied by a mild rash. Symptoms occur in at least 50 % of HIV-1 cases within 1-4 weeks after infection and normally disappear within three weeks, although lymphadenopathy and fatigue may persist longer [51].

Although some persons progress rapidly to AIDS after the primary infection, the majority of HIV-1-positive individuals enters the asymptomatic stage which in average last close to 10 years. While the virus is contained to a relative extent, gradual depletion of the immune system occurs throughout this phase.

The symptomatic stage – AIDS - is the endstage of the natural course of HIV infection in the absence of ART. AIDS is dominated by the onset of opportunistic infections (OI). In Sub-Saharan Africa, Tuberculosis (TB) and Cryptococcal meningitis are the two dominating causes of HIV-related death [81, 82]. Pneumocystis jiroveci pneumonia which is more common in Europe and USA has been less prevalent but a suspected increase in SSA has been reported [83]. Co-infections are important for the disease progression rate as they often induce immune activation [84] and co-infections are important in their own means as they often define the survival time of AIDS in abscense of ART [85].

In HIV-1 infection, the differences in time of progression to clinical disease have prompted the terminology of rapid (or fast), chronic, slow and long-term non-progression (LTNP) groups. Although there is no strict definition, a person is normally considered to be LTNP if clinically asymptomatic for ≥10 years, maintaining normal CD4+ T cell levels [51]. A subgroup of LTNPs are referred to as elite controllers, being capable of long-term control of the plasma VL with undetectable levels [86]. Differences in progression rates have been correlated to subtype, notably with subtype D being considered as more often rendering fast progression [87]. There has not been any evidence of differing progression rates of the most common subtype in Guinea-Bissau and West Africa - CRF02_AG, compared with other subtypes [88]. While VL and $CD4^+$ levels are predictive of outcome, also factors such as age, genetic factors, level of immune activation and concurrent infections play a role in disease progression of HIV-1 [51].

All HIV-2 isolates derived from Guinea-Bissau have exclusively been described as subtype A viruses [89]. Apart from the above mentioned factors for HIV-1, other identified correlates of disease progression in HIV-2 infection have been variations in *gag*-related T cell responses, [90], lack of anti-*tat* antibodies [91], host genetic subtype (HLA-B*1503) [92], viral genetic factors [93] and capside variants [94].

*Definitions of AIDS*

AIDS can be defined according to clinical symptoms but also with a combination of clinical symptoms and laboratory screenings of $CD4^+$ levels. The definition of AIDS by the Centers for Disease Control (CDC) recognises both symptoms (*Table 2*) and laboratory results of $CD4^+$ T cell counts <200 cells/µl or $CD4^+$ T cell percentage <14% count as AIDS-qualifying event [95]. The definition of the

World Health Organization (WHO) is based on a clinical staging system irrespective of laboratory results [96]. The staging system comprises the stages 1 (acute) and 2 (chronic), 3 (symptomatic) and 4 (AIDS-defining, *Table 3*) and has been especially practical in low-resource settings where laboratory diagnostics have not been widely available.

**Table 2:** The CDC classification of AIDS-defining symptoms (category C), 1993 revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults.

| **Category C AIDS-Indicator Conditions (CDC)** |
|---|
| Bacterial pneumonia, recurrent (two or more episodes in 12 months) |
| Candidiasis of the bronchi, trachea, or lungs |
| Candidiasis, esophageal |
| Cervical carcinoma, invasive, confirmed by biopsy |
| Coccidioidomycosis, disseminated or extrapulmonary |
| Cryptococcosis, extrapulmonary |
| Cryptosporidiosis, chronic intestinal (>1 month in duration) |
| Cytomegalovirus disease (other than liver, spleen, or nodes) |
| Encephalopathy, HIV-related |
| Herpes simplex: chronic ulcers (>1 month in duration), or bronchitis, pneumonitis, or esophagitis |
| Histoplasmosis, disseminated or extrapulmonary |
| Isosporiasis, chronic intestinal (>1-month duration) |
| Kaposi sarcoma |
| Lymphoma, Burkitt, immunoblastic, or primary central nervous system |
| *Mycobacterium avium* complex (MAC) or *Mycobacterium kansasii*, disseminated or extrapulmonary |
| *Mycobacterium tuberculosis*, pulmonary or extrapulmonary |
| *Mycobacterium*, other species or unidentified species, disseminated or extrapulmonary |
| *Pneumocystis jiroveci* (formerly *carinii*) pneumonia (PCP) |
| Progressive multifocal leukoencephalopathy (PML) |
| *Salmonella* septicemia, recurrent (nontyphoid) |
| Toxoplasmosis of brain |
| Wasting syndrome caused by HIV (involuntary weight loss >10% of baseline body weight) associated with either chronic diarrhea (two or more loose stools per day for ≥1 month) or chronic weakness and documented fever for ≥1 month |

**Table 3:** The WHO classification of AIDS-defining symptoms (clinical stage 4) 1994 version revised in 2007.

| **WHO clinical stage 4, 1994 version revised in 2007** |
|---|
| HIV wasting syndrome, as defined by the CDC (see Table 2, above) |
| Pneumocystis pneumonia |
| Recurrent severe bacterial pneumonia |
| Chronic herpes simplex infection (orolabial, genital, or anorectal site for >1 month or visceral herpes at any site) |
| Esophageal candidiasis (or candidiasis of trachea, bronchi, or lungs) |
| Extrapulmonary tuberculosis |
| Kaposi sarcoma |
| Cytomegalovirus infection (retinitis or infection of other organs) |
| Central nervous system toxoplasmosis |
| HIV encephalopathy |
| Cryptococcosis, extrapulmonary (including meningitis) |
| Disseminated nontuberculosis mycobacteria infection |
| Progressive multifocal leukoencephalopathy |
| Candida of the trachea, bronchi, or lungs |
| Chronic cryptosporidiosis (with diarrhea) |
| Chronic isosporiasis |
| Disseminated mycosis (e.g., histoplasmosis, coccidioidomycosis, penicilliosis) |
| Recurrent nontyphoidal *Salmonella* bacteremia |
| Lymphoma (cerebral or B-cell non-Hodgkin) |
| Invasive cervical carcinoma |
| Atypical disseminated leishmaniasis |
| Symptomatic HIV-associated nephropathy |
| Symptomatic HIV-associated cardiomyopathy |
| Reactivation of American trypanosomiasis (meningoencephalitis or myocarditis) |

The diagnostic possibilities of most symptoms listed above are limited in Guinea-Bissau and similar low-resource settings, thus a diagnosis is most often decided upon only clinical findings.

*Natural course of disease*

The natural course of HIV-1 is well described from mainly high-income countries, but there are also descriptions from Africa. In Uganda, two studies reported median survival time of 9.8 and 9.0 years among HIV-1 seroincident individuals, which is consistent with reports from USA and Western Europe in the pre-ART

era [97, 98]. So far there was no report of natural course of seroincident HIV-2 infection from Africa and estimations were based on seroprevalent individuals [99].

*Clinical and Immunological differences in the disease course of HIV-1 and HIV-2*

The first studies of HIV-2 seroprevalent individuals observed a reduced rate of disease compared with HIV-1 among CSWs in Senegal [100] and a mortality only twice that of the HIV negative population in urban Guinea-Bissau [101]. The mortality rates of HIV-2-infected have in later investigations remained two- to five-fold those of uninfected individuals in Guinea-Bissau [102-104], in sharp contrast with higher figures from HIV-1 in East Africa [105]. HIV-2 displays a longer asymptomatic phase than HIV-1, but once reaching low $CD4^+$ T cell levels, HIV-1 and HIV-2 share a similar clinical spectrum in individuals reaching AIDS, maybe, with the exception of a lower incidence of Kaposi Sarcoma (KS) [106, 107]. Overall, HIV-2 infection has been considered to progress to clinical disease in approximately 25% of cases, compared to almost all HIV-1-infected individuals [108]. An overview of differences between HIV-1 and HIV-2 are shown in *Table 4*.

The reasons for slower disease progression in HIV-2 compared with HIV-1-positive individuals are still not clear but both host and viral factors seem to influence the course of disease [108]. Previous studies has reported a lower replication capacity of HIV-2 in comparison to HIV-1 [109]. HIV-2 infected individuals display around 10 times lower plasma VL than HIV-1 infected individuals when matched for $CD4^+$ T cell count, despite the fact that proviral load is similar in the two types of infections [110, 111]. In The Gambia and Senegal it was shown that HIV-2 disease progression was correlated to lower VL and slower $CD4^+$ T cell decline, however if reaching high VL as in HIV-1 infection, there was no difference in prognosis [112-114]. Slower $CD4^+$ T cell decline rates were later also shown in HIV-2 patients in France [115]. Better immune control has also been reported with neutralizing antibodies as a factor of protection against disease progression [116] and in comparison with HIV-1, HIV-2 displays structures that favour triggering of potent neutralizing antibodies [117]. T cell function is better preserved in HIV-2 than in HIV-1 infection [118] and despite low levels of viral replication, there is an HIV-2 specific response by maintenance of $CD8^+$ T cells in early differentiation phase [119]. HIV-2 seems to be more susceptible than HIV-1 to restricting host factors [120]. Natural killer cell response is stronger in HIV-2 than in HIV-1, but the difference disappears with $CD4^+$ T cell decline [121]. A lower level of immune activation is correlated to non-progression of disease and immune activation seems to be directly correlated to VL in HIV-2 [122], however

not in all reports [123]. The level of microbial translocation correlates more to the severity of disease rather than to type of HIV infection, HIV-1 or HIV-2 [124], as does certain dysregulation of innate immunity [125].

**Table 4**: Differences between HIV-1 and HIV-2 infections.

| | **HIV-1** | **HIV-2** | **Reference** |
|---|---|---|---|
| Origin | SIV of chimpanzee | SIV of sooty mangabey | [126, 127] |
| Distribution | Pandemic | West Africa and links, especially Portuguese | [6] |
| Plasma RNA VL | High | Low | [128] |
| Genital viral shedding | Higher | Lower | [129, 130] |
| Vertical transmission | 20-25% | <5% | [6, 131] |
| Co-receptor usage | CXCR4 and CCR5 | Broader range of co-receptors | [56] |
| Immune response | Reduced NK cell function, higher degree of immune activation | Polyfunctional T cells, Preserved NK cell function, broad and potent NAb response, lower degree of immune activation | [117, 119, 121, 132, 133] |
| CD4+ T cell decline | High | Low | [112] |
| Disease progression | Median survival ~10 ys | Majority LTNPs | [100] |
| Mortality | 10-15 x gen pop. | 2-5 x gen pop. | [6, 99] |
| Age peak | Young, middle-age | Older | [134, 135] |

# Interactions between HIV-1 and HIV-2

The geographic co-existence of HIV-1 and HIV-2 infection in West Africa enables encounters between the two infections. HIV-D infection has been reported from Mali, Ivory Coast, Senegal, The Gambia and Guinea-Bissau [80]. The prevalence have varied greatly with the highest level of 24% reported in female CSWs in Côte d'Ivoire [136]. Outside West Africa, mainly Zimbabwe [137, 138] and India [139] have reported HIV-D infection.

In 1995, there was a report from a cohort of CSWs in Senegal, suggesting a possible preventive effect against HIV-1 infection exerted by HIV-2 [140]. This could however not be reproduced in other cohorts from Guinea-Bissau [25, 141], the Gambia [142] or Ivory Coast [143] and overall it was later concluded that HIV-2 rather constituted a risk factor for HIV-1 infection [144]. However, in vitro data has suggested that HIV-2 infection was protective against disease progression from SIV and SIV/HIV recombinants (SHIV) in macaques [145, 146], leading to questions of an eventual mitigating effect of HIV-2 against HIV-1-induced

disease. Several West African studies have investigated different populations for comparison of immunological changes, disease progression and survival of HIV-1, HIV-2 and HIV-D groups. Apart from one study in Guinea-Bissau that reported lower VL in HIV-D than in HIV-1 patients [128], studies have reported similar VL levels and immunological markers, both in the Gambia [147] and in Côte d'Ivoire [136, 148]. Reports from several countries of mortality in HIV-D have consequently showed similar mortality in HIV-D as in HIV-1 [103, 149-152]. However, all these studies were observing seroprevalent participants without knowledge of seroconversion date or of the sequential order of infection, though in most cases one would presume that HIV-2 was present first. CTL-responses in HIV-2 that were cross-reactive against HIV-1 have been seen in patients with some HLA variants, though they were not inhibiting HIV-1 replication in HIV-D infection [147].

# Treatment and prevention of HIV-1 and HIV-2

*Antiretroviral therapy*

Even though specific HIV therapy commenced with the introduction of the first antiretroviral agent Zidovudine in 1987, it was not until 1996 with the arrival of effective combination therapy - (Highly active) anti-retroviral therapy – (HAART, ART) – that HIV could be controlled satisfactory. The world has seen tremendous efforts in the last decade to expand treatment programmes of ART, also in SSA where over 5 million PLWHA have initiated ART. Newer simplified regimens with fixed-combination tablets (FCT) and heat-stable capsule versions have facilitated the roll-out of treatment services. ART in South Africa has resulted in survival on the same level as HIV negative persons [153]. There have been major obstacles on the continent though, related to fragile health infrastructures and substantial mortality has been observed among individuals lost to follow-up [154]. The national ART programme in Guinea-Bissau was launched in 2005. At the end of 2010, officially 3,632 individuals were initiated on ART but there has been repeated problems of treatment stock-outs with subsequent diffuculties in retention of patients [155].

Normally treatment is initiated after detailed information and education of the patient about common side effects and the necessity of adherence to therapy. The treatment of HIV-1 normally consists of a combination of two nucleoside reverse-transcriptase inhibitors (NRTI) and a non-nucleoside reverse-transcriptase inhibitor (NNRTI) or a protease inhibitor (PI). There are other classes of therapeutic agents, such as fusion inhibitors, integrase inhibitors and co-receptor

antagonists, though in most circumstances too highly priced for being generally available in Sub-Saharan Africa at present. The treatment of HIV-2 and HIV-D needs taking into account that HIV-2 is not sensitive to the NNRTI, in Africa most often necessitating treatment with PIs or a less preferred three-NRTI combination. HIV-2 and HIV-D patients have been shown to experience a slower recovery after initiation of ART [156].

The WHO treatment guidelines for ART was most recently revised in 2010. The main revisions were earlier time point of ART initiation at $CD4^+$ T cell level $\leq$350 (previously $\leq$200) cells/µl or at WHO clinical stage 3 or 4 (previously stage 4), recommendations of other drugs with less side effects and earlier initiation of therapy in co-infections with Tuberculosis and Hepatitis B Virus [157].

In 2011, the results of a multi-centre study revealed that early ART in discordant couples gave a 96% reduction in new transmissions, indicating that prevention could be enhanced if initiating therapy even earlier than the current guidelines [158]. This can be seen as a parallel to HIV-2 infection, where lower HIV-2 VL is associated with lower transmission rates.

*HIV Latency*

Initial hopes of potential eradication of HIV-containing cells using HAART were soon dashed as it turned out that HIV persists in viral reservoirs in the central nervous system, genital region and in the GALT [159]. Viral replication may persist as ART is halted [160].

*The prospect of an HIV vaccine*

Despite vast investment in basic research and clinical trials, until now it has not been possible to produce basis for a preventive vaccine. A recent trial in Thailand managed to lower infection rate by 26% [161], which is the most successful attempt so far of creating a preventive vaccine. In order to manage a fully preventive vaccine, it will most probably be necessary to elicit a broad immune reaction including both cellular and humoral activity.

*Other interventions for prevention of transmission*

In recent years several trials of adult male circumcision has showed reductions in male-to-female HIV transmission rates of 50-60% [162-164]. This has led the WHO to point it out as a focus area for intervention, mainly in Southern and East Africa. Another prevention approach is the notion of pre-exposure prophylaxis with antiretroviral agents. There have been successful trials with a 39% reduction of HIV-1 acquisition among women when using a vaginal Tenofovir-containing gel [165] and a 44% reduction in HIV-1 acquisition among MSM using daily

Tenofovir/Emtricitabine prophylaxis [166]. Several large-scale studies are now running to elucidate on these results.

# Other Sexually Transmitted Infections

*Burden of disease and the relation to HIV*

In 1999, the combined incidence of four sexually transmitted infections (STI), syphilis, gonorrhoea, genital Chlamydia and Trichomoniasis, was estimated by WHO to be over 340 million new cases per year and increasing [167]. STIs other than HIV are known risk factors for HIV transmission, both in ulcerative and non-ulcerative forms [168]. It has also been shown that interventional STI treatment can reduce HIV incidence [169]. STIs can enhance HIV transmission by an increase of HIV shedding in the genital tract, by recruitment of inflammatory cells that act as target cells for HIV infection and by breach of mucosal barriers [168]. In later years, focus has especially been directed towards HSV-2 infection which has emerged as the probably most important co-infection in regard to HIV transmission [170]. HSV is one of the causes of genital ulcer disease (GUD), others being bacterial *Treponema pallidum* (syphilis), *Haemophilus ducreyi*, *Chlamydia trachomatis* serovar L1-3 and *Klebsiella granulomatis* infections. Non-ulcerative STIs includes *Neisseria gonorrhoeae* (gonorrhoea), *Chlamydia trachomatis* D-K and *Mycoplasma genitalium*, pathogens which cause urethritis, cervicitis and pelvic inflammatory disease, the long-term effects in women being risk of fallopian tube obstruction causing with subsequent risk of extra uterine pregnancies and infertility. Other STIs include Human Papilloma Virus (HPV) with different serotypes causing condyloma, cervical neoplasia and invasive cervical cancer with increased risk in HIV-positive individuals. According to WHO the most common STI with an estimated 170 million infections per year is the the protozoa *Trichimonas vaginalis*, causing vaginitis and urethritis. Apart from genital infections, other STIs cause non-genital diseases such as HTLV with neurological and haematological disorders and Hepatitis viruses B and C (HBV, HCV) causing acute and chronic hepatitis with long-term effects of liver cirrhosis and cancer.

*Diagnosis and treatment*

Laboratory diagnosis of different STIs includes traditional direct microscopy, culture, serology, PCR methods and increasing use of rapid detection tests. In low-resource settings, there is often a lack of appropriate diagnostics.

Effective prevention and treatment of STIs has been seen as one of the fundamentals in HIV prevention. In resource-limited settings, usually a syndromic

management approach based on clinical symptoms is used for treatment, where treatment is given directly at first visit while waiting for laboratory results (if laboratory diagnostics are available). In the current WHO treatment guideline of STIs, there are recommendations of syndromic management, although acknowledging the limitations and with an emphasis on the need of local adaptation [171]. It is also important to combine the antimicrobial interventions with good counselling, behaviour change promotion and additional services like condom distribution.

*Reports from the subregion: Guinea-Bissau, The Gambia and Senegal*

Only few previous studies have described the situation of STIs other than HIV in Guinea-Bissau. In a report from 1993, there was a significant correlation between HIV-2 infection and previous syphilitic infection [172]. A survey from 2001 indicated Chlamydia trachomatis prevalence of 4 % and Neisseria gonorrhoeae prevalence of 17% among women with vaginal discharge [173]. In 2002, screenings of pregnant women showed prevalence rates of 6.2% of *Mycoplasma genitalium* and 3.9% of active syphilis. [174, 175]. Investigations of Hepatis C concluding predominantly sexual transmission was reported in 2007 [176]. There have been reports of decrease in HTLV-1 in urban and rural Guinea-Bissau [177, 178].

Reports of STIs have been more frequent from the neighbouring West African countries of The Gambia and Senegal. In The Gambia, an early report in 1984 of CSWs showed prevalence of *Trichimonas vaginalis* of 32%, *Neisseria gonorrhoeae* of 6.9% and *Chlamydia trachomatis* of 6.7 % while *Treponema pallidum* of 1%. Reports from Senegal of STI prevalence among CSW:s have repeatedly shown prevalence above 20% for all these four pathogens [179, 180]. Recent reports demonstrated high levels of HSV-1 and/or HSV-2 DNA in cervico-vaginal lavage (CVL) among 39% of attendees at a genito-urinary medicine (GUM) clinic [181] and 88% HSV-2 seroprevalence of in CSWs [182]. There were also a report of *Haemophilus ducreyi*, *Treponema pallidum* and HSV DNA in 56, 15 and 13 % of GUD specimens [183]. Reports from Senegal have also included high STI prevalence among male patients attending a GUM clinic [184] and STIs among MSM [185].

*Importance of continuous interventions*

Comparisons of these studies are difficult due to differences in diagnostic methods and study groups. However, there is evidence of subregional high STI prevalence that constitutes potentially enhanced risks of HIV transmission. It is interesting to note that despite high levels of HIV and other STIs in risk groups, the HIV

epidemic has been contained at a comparatively low level in the general population in Senegal while the HIV-1 prevalence at the same time increased in Guinea-Bissau. Local preventive interventions can be successful and there is importance of continuous active prevention in identified risk groups also in Guinea-Bissau.

# Setting – Guinea-Bissau

## Geography, demography and history

The republic of Guinea-Bissau is situated in West Africa, bordering Senegal in the North, Guinea-Conakry in the South-East and the Atlantic Ocean to the west (*Figure 4*). It is also closely situated to The Gambia in the north. Guinea-Bissau has an area of 36,125 square kilometres. According to the latest census in 2009, 1.5 million inhabitants, whereof 0.3 million in the capital Bissau. 43% were under 15 years of age [186].

The climate is tropical with a rainy season in June to November and a dry season from December to May. The rural areas are predominantly covered with dense or semi-dense forest. The economy is dominated by fishing and agriculture with cashew nuts being the main export product.

Guinea-Bissau was a former Portuguese colony that gained independence in 1974 after a war of liberation lasting more than 10 years which left the country at a very basic economic, administrative, educational, and healthcare level. A civil war took place in 1998-1999, again causing substantial disruption to national economy and infrastructure. After the civil war there has been an additional major coup d'etat and several other politically destabilising incidents.

While there are more than 20 different languages spoken to this day in Guinea-Bissau, the official language is Portuguese and the commonly used *lingua franca* is Kriol, a mainly Portuguese-based creol language. The population is diverse with a multitude of different ethnic groups, the major ones being Balanta, Fula, Mandinga, Mandjaco, Papel, Mancanha and Biafada. Intermarriage is common. Religions are traditional animist beliefs, Islam and different Christian denominations.

Guinea-Bissau has regularly been ranked among the world's least developed countries - in the latest United Nations Development Programme Report of 2011 as country number 176 out of 187. The estimated Gross National Income (GNI) per capita was 994 $ (aggregated income converted to international dollar by purchasing power parity index). In comparison, the GNI/capita of Sweden was

35.837 $ and of Portugal 20,573 $. The mean life expectancy at birth is 48.1 years and the maternal mortality rate is 1000 per 100,000 births and the under-5 years mortality is 193 per 1000 live births. The adult literacy rate is 52 % [187].


**Figure 4:** Map of Guine-Bissau, no 4063, June 2004, United Nations, Department of Peacekeeping operations, cartographic section. Reprinted with permission.

# LNSP

Sweden was a major bilateral aid donor to Guinea-Bissau soon after independence, up until 1997. The National Public Health Laboratory (Laboratório Nacional de Saúde Pública - LNSP) in Bissau was constructed in 1987 with finance from the Swedish International Development Cooperation Agency (Sida) and with technical assistance from the Swedish Institute for Communicable Disease Control (SMI). The establishment included development of routine diagnostics for HIV and basic epidemiological surveillance. Research projects were initiated with support from

the Swedish Agency for Research Cooperation (SAREC). The major part of HIV testing in Guinea-Bissau was performed at the LNSP until the dissemination of use of rapid tests that occurred after 2005. Still the serology unit at LNSP functions as a national reference of serosurveillance and confirmatory testing as well as a centre for research collaborations.

# A short summary of the civil war 1998-1999

The conflict in 1998 started abruptly on the 7th of June with a military uprising against the regime and ended with the ousting of President Vieira. Troops from Senegal and Guinea-Conakry intervened in the early part of the fighting but were withdrawn according to a peace agreement, after which deployment of peace-keeping troops from Benin, Gambia, Niger, and Togo took place.

The initial fighting was especially intense in the capital Bissau and forced the vast majority of the inhabitants to leave, mainly for interior parts of the country with comparatively few people crossing the borders to neighbouring Senegal and Guinea-Conakry. People returning to the city had too flee repeated times amid renewed outbreaks of fighting. During the conflict there was a general decline in public services, such as health institutions [188-190] and the function in the country has not yet fully reached pre-conflict levels.

At the end of the conflict, LNSP in Bissau was unfortunately hit by a missile which burnt down almost the whole building. Miraculously some things escaped the fire, including the flow cytometer and it continued to work several years after the conflict.

# Aims and objectives of the studies

*The aims of the studies in this doctoral dissertation were:*

To investigate the long-term epidemiological trends of HIV-1 and HIV-2 prevalence and incidence in Guinea-Bissau

To investigate the relative impact of the civil war in Guinea-Bissau in 1998-1999 on HIV transmission

To investigate the natural course of HIV-2 seroincident infection and compare with HIV-1 and seroprevalent HIV-2 study participants

To investigate the effect of simultaneous HIV-2 infection on the clinical course of seroincident HIV-1 infection

To investigate the level of co-infection and possible correlation between HIV and other Sexually Transmitted Infections

# Study populations and study designs

## Pregnant women (I)

Within the national sentinel surveillance system, since 1987 there has been ongoing sentinel HIV surveillance studies on women giving birth at the Simão Mendes National Hospital (SMNH) in Bissau. It is the only general hospital in Bissau, catering to the whole population and acting as a national reference hospital. The anonymous screening has been performed every year or every second year between August and December. For the study in paper I, a total of 20,422 women were tested, and in addition a further 4505 women have been tested in the years 2006 to 2010. There are no reports of any exact proportion of women givning birth at the SMNH from the start of the study, though it has been reported to be the majority of the inhabitants of Bissau [175]. However, since the year 2000, the location of birth was registered in the epidemiological surveillance of the Bandim Health Project (BHP), comprising 25-30% of the total population of the capital Bissau. Over the years 2000-2011 there were roughly 50% giving birth at the hospital rather than at health posts or at home, with no significant changes over these years (Personal communication, Dr Ane Fisker, BHP). It should probably be representative for the whole of Bissau. The proportion of pregnant women from outside the city of Bissau seeking to deliver at the maternity unit is unknown.

## The police cohort (II, III and IV)

In 1990 an occupational cohort of police officers was initiated. The reason why police officers were chosen was the nature of permanent employment ensuring possibility of follow-up. The cohort has been an open prospective cohort with continuing new recruitment until the civil war broke out in June 1998. Regular follow-up visits were resumed in the year 2000 and new recruitment to the cohort was re-initiated in the year 2003. For these studies, the longest follow-up was until February 2011. By then, 4820 police officers were enrolled, whereof 4817 had a recorded HIV test result. 12.9 % were women. 76.1% of all participants returned

for a second visit, with a mean follow-up time of 8.2 (SD 5.6) years and median follow-up time of 5.9 years (IQR 3.5-13.0). For more details of follow-up times, see the supplementary discussion in paper IV.

The cohort has been physically managed from a health post at the main (*2ª esquadra)* police station in the capital Bissau. The follow-up team has consisted of one physician and 2-3 nurses as well as 3-4 auxiliary staff. Regular visits were made to police stations in the capital as well as in the interior regions of the country. All police officers were invited to participate in the study which has been voluntary, over 98% of staff present at police stations when the team was visiting were willing to participate. Follow-up visits were scheduled to every 12-18 months.

Each individual was registered with a study number and for identification at follow-up visits, the participants were identified using an identification system based on name, gender, age, birth place, ethnical group, religion, civil status and educational level. This was done both for avoiding double registration of new study participants and for decreasing risk of confusion of already registered participants and their blood samples.

At each individual visit, a questionnaire with demographic information and symptoms related to HIV and sexually transmitted infections (STIs) was filled in. Body weight was measured and a physical examination performed. Symptoms were later classified according to the WHO clinical staging system [96]. Pre-and post-test councelling for HIV was given. Blood samples for HIV and *Treponema pallidum* serology were collected at inclusion and follow-up visits. Baseline serology for HTLV was also performed. For HIV-positive individuals $CD4^+$ T cell counts were performed since 1993.

At all visits, information about HIV and STIs were given and condoms were distributed. Basic healthcare was provided with medicines free of charge. Antiretroviral drugs were provided under the umbrella of the national ART programme, initiated in 2005, commencing in the Police cohort in January 2006. In addition, Co-Trimoxazole (CTX) prophylaxis was provided as well as Isoniazid (INH) prophylaxis to HIV-positive study participants, according to WHO guidelines [191]. In the analyses of survival and disease progression (paper III and IV), participants who initiated ART were censored from date of initiation of treatment. No censoring was performed of participants who started CTX prohylaxis as adherence was difficult to monitor and probably several un-registered more or less irregular treatments occurred, outside and inside the cohort. Also, participants who initiated INH were not censored from the analyses as we believed there was a high risk of re-infection with high TB incidence in Guinea-

Bissau [192] and also that the INH treatment was initiated in 2005 and only in HIV-positive individuals with positive tuberculin skin test (TST) according to WHO guidelines at the time [191].

Mortality reports were collected from the Interior Ministry, symptoms related to death was recorded by help of family members, police registers and from questionnaires if reported in reasonably short time prior to death.

As the police officers often are transferred between different units in the country, the follow-up situation has been more difficult than it normally is in a community-based cohort. Date of last visit has been set as the actual follow-up time. A discussion on the question of loss to follow-up is present in the supplementary discussion of paper IV.

## Women with urogenital problems (V)

The study was conducted in two clinics for female health in Bissau – the Clínica Materno Infantil (CMI), affilitated with the SMNH and the Aguibef clinic – run by the International Planned Parenthood Federation (IPPF). Women seeking health care for urogenital problems were invited to participate in the study. After consent, study participants answered a questionnaire about demographic and STI-related issues. They received pre-test counselling, after which a gynecologial examination was performed. Cervical samples as well as wet mount for microscopy were taken. Genital ulcers were sampled when visible. The patients recieved results of wet mount microscopy and HIV test after one week, and post-test counselling was given. If the HIV test was positive, the patients were referred to one out of three treatment centres in Bissau. PCR and culture results of other STIs were delivered after 2-3 weeks. Except for HIV and HTLV, the women received treatment within the study for all STIs diagnosed as well as genital Candidiasis and occasional condylomas, and if partners turned up they received treatment as well.

# Methods

## Definitions of AIDS

In study III we defined AIDS according to the WHO clinical staging system (where AIDS is clinical stage 4). Analyses were however also carried out according to the CDC staging system including $CD4^+$ T cell levels, but were not reported in the article due to large size (see the results and discussion section).

In study IV we used a wider definition of AIDS as being in WHO clinical stage 4 or in CDC stage C – practically meaning that also pulmonary tuberculosis was included as an AIDS-defnining diagnosis – and also included $CD4^+$ T cell absolute count $\leq$200 and/or $CD4^+$ T cell % $\leq$14.

## Laboratory methods

The samples used in these studies were analysed for serology, PCR for STIs other than HIV and culture diagnostics at the LNSP of Guinea-Bissau, except for direct microscopy which was performed locally at clinics in the capital Bissau. Additional analyses of soluble immune markers, HIV PCR and sequencing were performed at SMI or at Lund University, Sweden after shipping of frozen samples. PCR confirmation of Mycoplasma genitalium was performed at Örebro University, Sweden.

*Serological determination of HIV-1 and HIV-2 infection*

Samples were screened with Behring Enzygnost HIV-1+2 ELISA from 1987 to 1994 and with Behring Enzygnost Plus Elisa from 1995 (Behring, Marburg, Germany). Confirmation was performed until 1997 with Western Blot analysis using Diagnostic Biotechnology HIV Blot 2.2 (Diagnostic Biotechnology, Science Park, Singapore) and an in-house anti-HIV-2 Western Blot assay [193]. Dually reactive HIV-1 and HIV-2 sera were then further tested with a synthetic peptide ELISA for discrimination between HIV-1 and HIV-2 (Peptilav, Sanofi Diagnostic Pasteur, Marnes.la.Coquette, France). From 1999, a different confirmation strategy

was used with Capillus HIV-1/HIV-2 (Cambridge Biotech Limited, Galway, Ireland) and Immunocomb II HIV-1 and 2 BiSpot RST (Orgenics, Yavne, Israel) [79].

*Serology for Treponema pallidum, HTLV-1 and HTLV-2*

In the Police cohort, sera were screened with Treponema Pallidum Hemagglutinin Antigen (TPHA, Fujirebio, Tokyo, Japan). Active disease was confirmed with Venereal Disesase Research Laboratory test (VDRL, Sypal, Diagast, Lille Cedex, France). The TPHA test had been replaced around the year 2000 by Treponema pallidum Particle Antigen (TPPA, Fujurebio, Japan), as an upgrade. In study V, screening was performed with VDRL and confirmed with TPPA. Screening of sera for HTLV was performed with Murex HTLV 1+2 (Murex Biotech Ltd, Dartford, UK) and positive sera were confirmed using Inno-Lia HTLV I/II Score (Innogenetics, Gent, Belgium).

*Determination of T lymphocyte subsets*

From the initiation of the studies of T lymphocytes in 1993 at the LNSP in Guinea-Bissau, T lymphocyte subsets were determined by conventional flow cytometry. Until 2005 determinations were performed with FACStrak (Becton Dickinson, San Jose, CA), and Leukocyte counts were performed with a cell counter (Coulter Counter CBC5; Coulter Electronics Ltd, Luton, England). From 2006 and onwards T lymphocyte subsets were measured by CyFlow (Partec, Münster, Germany). The replacement was due to several reasons: the FACStrak needed to be replaced and there was also a need to lower cost per sample in order to cope with increasing number of tests after initiation of the national ART programme. We performed an evaluation where we compared the two tests in parallel during the last 4 months in 2005. There was very good correlation between the two modalitites regarding $CD4^+$ T cell percentage and satisfactory correlation regarding $CD4^+$ T cell absolute counts (*Figure 5*). An advantage with the FACSTtrak was that also CD8% levels were obtained. An advantage with The CyFlow, apart from the cost was that T cell counts are directly generated, avoiding the uncertainty of incorporating an additional calculation of leucocytes into the algorithm.

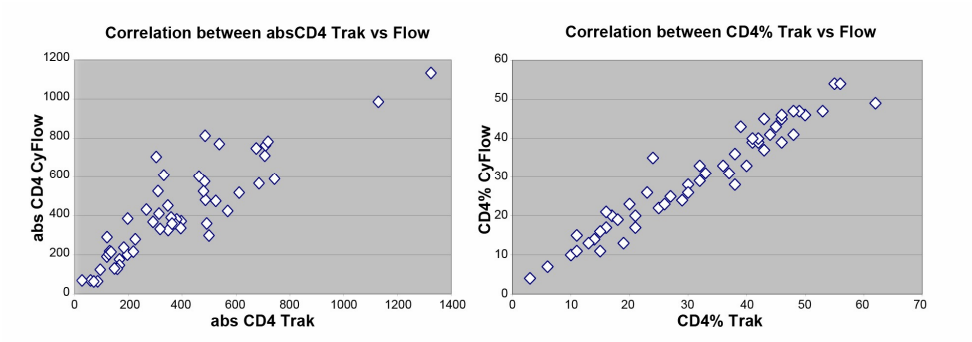



**Figure 5:** Comparison between Becton Dickinson FacsTrac and Partec CyFlow. Evaluation of $CD4^+$ T cell absolute count (Spearman rank 0.85, $p<0.001$) and $CD4^+$ T cell % counts (Spearman rank 0.97, $p<0.001$) using the two different flow cytometry instruments.

*Quantitation of HIV-1 and HIV-2viral load*

Quantitative HIV-1 VL has not been routinely carried out at the LNSP. However, for study III we had access to measurements from two time periods. In the first period, 1993-1995, HIV-1 plasma VL was measured by a commercial assay for HIV-1 VL (Amplicor HIV-1 Monitor Assay, version 1.5; Roche Diagnostic Systems, Branchburg, NJ, USA) and HIV-2 plasma VL was measured by a prototype assay developed by Roche Diagnostic Systems, based on the same principles as the Amplicor HIV-1 Monitor assay. The detection level for HIV-2 VL was 50 copies/µl (in our study 125 copies/µl after dilution of samples) [128]. In the period 2004-2006, total HIV plasma VL was analysed by measuring reverse transcriptase (RT) activity using the CAVIDI ExaVir Load kit (Cavidi Tech AB, Uppsala, Sweden) according to the manufacturer's instructions, and presented as RNA copy numbers/ml equivalents. The lower detection limit for this test was set to 1000 copies/µl after dilution of samples.

*PCR for Chlamydia trachomatis, Mycoplasma genitalium, Haemophilus ducreyi and Herpes Simplex virus type 1 and 2*

The PCR methodology was set up at LNSP in 2005. In study V, DNA was extracted from cervical samples using the E.Z.N.A. tissue DNA kit (Omega Bio-tek, Doraville, USA). Conventional PCRs were developed using previously described primers, which utilised the glycoprotein D gene for the diagnosis of HSV-1 and the Glycoprotein G gene for HSV-2 [194, 195]. *H ducreyi* was identified by 16S rRNA gene [196], *M genitalium* by 16S rRNA gene [197] and the mgpB gene for confirmation [198] and *C trachomatis* by the okpA gene [199].

*Culture of Neisseria gonorrhoeae*

Cervical samples were cultured for 48-72 hours on modified Thayer-Martin medium. Confirmation was performed with rapid oxidase production and identification of Gram-negative diplococci on microscopy and use of PhadeBact Monoclonal GC test (Bactus, Stockholm, Sweden).

*Examination of Trichomonas vaginalis*

Immediatly after sampling, wet mount microscopy of vaginal samples were examined for living trophozoites.

*Analysis of soluble immune markers, HIV-1 RNA amplification and sequencing for analysis of viral evolution, diversity and divergence analyses.*

Paper IV was a joint effort with many persons involved in different techniques performed at Lund University and at SMI in Sweden. The analyses were performed on a subset of samples of single HIV-1 infections and HIV-D infections and the analyses of immune markers and the HIV RNA amplification and sequencing procedures are thoroughly described in the supplementary methods in paper IV. Briefly, concentrations of beta-2- microglobulin (b2m) and neopterin in plasma were determined using ELISA kits. Viral RNA was reverse transcribed using gene-specific primers, and the HIV-1 *env* V1-V3 region was amplified by PCR and thereafter cloned. Twelve colonies were purified from each sample. Sequencing of the genetic material was carried out and recombination analysis performed in order to construct the phylogenetic trees. Diversity was calculated by averaging tree distances from individual specific samples, divided by elapsed time. In analysis of divergence, the most recent common ancestor (MRCA) was determined, the mean number of base-pair substitutions between the clones and the MRCA was calculated and the difference in substitution rate was divided by elapsed time.

# Statistical analysis

Calculations of total HIV-1 and HIV-2 prevalence included HIV-D-positive individuals in each group where not otherwise indicated. Trends of prevalence were evaluated by Chi2-test for trend (I) and linear-by-linear association test with adjustment for sex and age (II). Date of estimated seroconversion was set to mid-point between the last negative and the first positive sample. Negative person-years were contributed until the day of estimated seroconversion. Incidence rates and ratios (II) were analysed using Poisson regression. In the incidence trend

analysis, only seroconverters to either HIV-1 or HIV-2 were included while seroconversions to HIV-D were excluded. Study participants contributed to respective age group and shifted to higher age-groups as they grew older during the study period. Dually reactive HIV-D samples were censored from the last single-positive time-point. Age was reported as means (I, V) when normally distributed or as medians with interquartile ranges (IQR) (II, III, IV). $CD4^+$ T cell counts were given as medians with IQR. $CD4^+$ T cell decline rates and $CD8^+$ T cell increase rates were analysed using a mixed linear regression model with time since seroconversion or seroprevalent study inclusion (III, IV). Survival analysis for mortality and progression to AIDS was performed with Kaplan-Meier analysis, followed by Cox proportional hazards model adjusting for sex and age where proportional hazards assumption was graphically controlled for (III, IV). Control for loss to follow-up was performed with reverse Kaplan-Meier estimation and control for selection bias of long-term non-progressors was performed with simulations described in the supplementary discussion of paper IV. Correlations of STIs were analysed with Odds Ratios (OR) generated by logistic regression (V). Generally, 95% Confidence intervals were analysed, parametric and non-parametric tests were used when appropriate. 2-sided p-values were used with a significance level of $<0.05$. EpiInfo was used for database handling and SPSS (PASW), R and Stata were used for statistical analysis.

# Ethical considerations

Participants in all studies received oral information prior to inclusion and participation was voluntary.

For the pregnant women (I), the surveillance testing was anonymous and no counselling or test result were administered to the participants. After the initiation of PMTCT within the national ART programme, the women were in addition offered counselling and testing outside the scope of our study.

For the Police cohort (II, III and IV), participants were offered pre-test counselling before the HIV test. It was voluntary to receive test results. After the initiation of the national ART programme, the interest among study participants to receive HIV test results increased substantially and with few exceptions, everyone are nowadays receiving their results. Follow-up and treatment was performed at a health post at the main police station in Bissau.

For the women seeking health care for urogenital problems (V), pre-test and post-test councelling was included in the inclusion interview and follow-up visit of the

study. Participants receiving a positive HIV test result were referred to a treatment site in Bissau.

The studies were approved by the Research Ethical Committee at the Karolinska Institute, Stockholm (study I-IV), by the Ministry of Health of Guinea-Bissau (I-V), by the Interior Ministry of Guinea-Bissau (II-IV) and by the Research Ethics Committee at Lund University (V).

# Results and discussion

## Epidemiological trends of HIV-1 and HIV-2 (I, II)

In early 2006 anonymous HIV sentinel surveillance had been performed since 1987 to 2004 among the delivering women at the Simão Mendes National Hospital in Bissau. We compiled the data and sought out to especially examine the possible impact of the civil war in 1998-1999.

The trend of HIV-2 prevalence was by then already known to be declining and HIV-1 was increasingly being recorded in Guinea-Bissau. [25, 26, 141].

During the study period, 20,422 women were tested for HIV. There was a significant decline of HIV-2 prevalence from 8.3% in 1987 to 2.5% in 2004. The decline in HIV-2 prevalence has shown to be consistent with studies published later, from a community-based study in the capital Bissau where HIV-2 prevalence declined from 8.9% in 1987 to 4.4% in 1996 [30] and a community-based study in rural north-western Guinea-Bissau where HIV2- prevalence declined from 8.3% in 1990 to 4.7% in 2007 [31].

HIV-1 prevalence increased during the early 90's until 1997 In June 1998, the civil war broke out. There was no scheduled sentinel in 1998 but it would anyway probably not have been possible to perform during the conflict. In the first survey after the civil war in 1999, the HIV-1 prevalence increased significantly from 2.5 to 5.2%. The increase did not continue however, and the HIV-1 prevalence stabilised around 5% in the subsequent years, a level corresponding to the overall 4.6% obtained in a community-based survey in urban Bissau in 2006 [30].

Since the publication of the study, surveillance has continued in the years 2006, 2008 and 2010. The results have shown a continuous decline of HIV-2, to 1.5% in 2010. HIV-1 prevalence increased in 2008 and remained on a higher level in 2010 compared to the years 2000-2006 (*Figure 6*). This could possibly be influenced by the PMTCT programme that have caused women to seek to the Hospital in a higher extent. It was also shown that the increase was solely observed among women aged 25-49, indicating that HIV-1 (and HIV-2) prevalence did not increase among younger women in the capital. In the 2010 survey, 59.3% of the HIV-positive women had taken an earlier HIV test compared with 51.8% in the seronegative group. The total HIV positivity was 7.2%, as compared with the 2010 figures from the Prevention of Mother-to-Child Transmission (PMTCT) programme at the maternity ward, normally testing only HIV negative women, which showed 5.5% total HIV positivity (though performed with different rapid tests than our testing strategy). It indicates that a substantial part of the increased number of HIV-positive women attended the maternity for access to PMTCT. The next planned sentinel study in 2012 will hopefully add more information of the direction of the HIV-1 and HIV-2 epidemics.

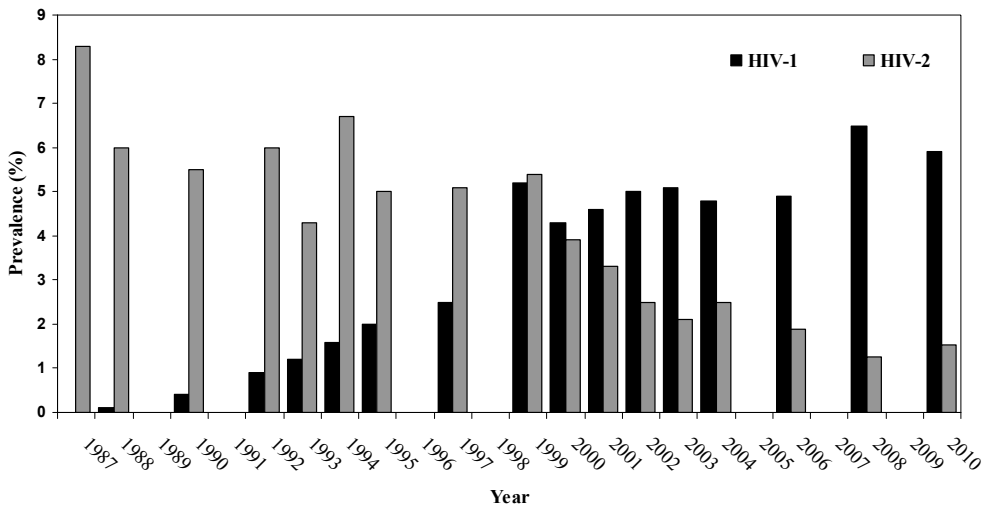



**Figure 6:** Total HIV-1 and HIV-2 prevalence among pregnant women at the Simão Mendes National Hospital in Bissau. Dual HIV-1 and HIV-2 infections were each added into each group.

In the police cohort, HIV1- and HIV-2 prevalence of police officers newly recruited to the study followed the same pattern, with HIV-2 prevalence of 13.4% in 1990 that later declined while HIV-1 prevalence increased over the study period (*Figure 7*). As among the pregnant women, after the civil war in 1998-1999 there was a significant increase in HIV-1 prevalence reaching 9.4% in 2003-2004 when new inclusions started in the cohort. HIV-2 prevalence continued to decrease after the war but later stabilized around 6%.

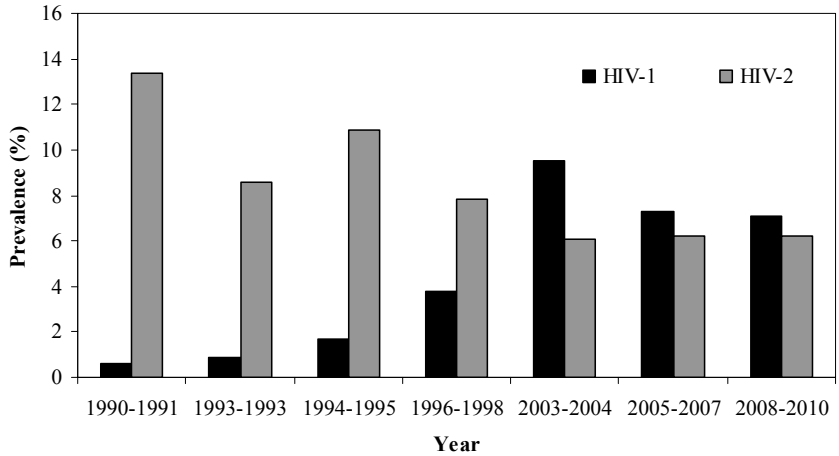



**Figure 7:** HIV-1 and HIV-2 prevalence 1990-2010 in police officers. Dual HIV-1 and HIV-2 infections were each added into each group.

The prevalence figures were reflected by the incidence figures with a constant level of HIV-1 incidence before the outbreak of conflict in 1998, but during and shortly after the war there was a significant increase in HIV-1 incidence to 1.22 per 100 person-years which receded to 0.82 at the end of the study but still remained on a higher level compared to before the conflict (*Figure 8*).

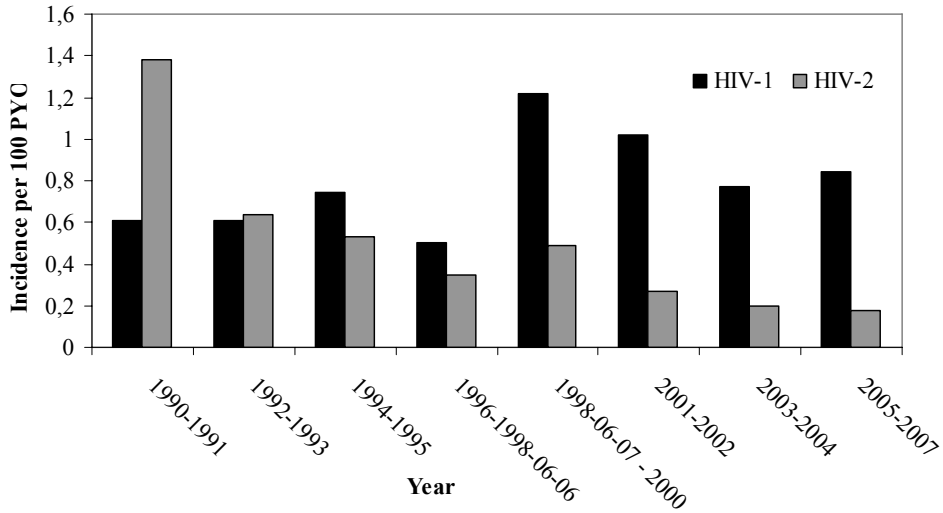



**Figure 8:** HIV-1 and HIV-2 incidence 1990-2007 in police officers. Incidence of HIV-D was not included in the analysis.

The results from the police cohort give support to the results of paper I that decisive events occurred in Guinea-Bissau during and shortly after the war that lead to an increase in HIV-1 incidence in both men and women with a climax of transmission shortly after the conflict. Whether these events consisted of changes in sexual behaviour, mobility or other factors remains to be investigated. Data from conflict situations derive mostly from surveillance of antenatal units while incidence data are scarce. The relationship between HIV and conflict is complex [200] and opposed to earlier beliefs that conflicts in general should aggravate HIV transmission, several publications have reported lower HIV prevalence figures in conflict-affected countries compared to surrounding countries without conflict [38]. This has been shown for example in the Democratic republic of Congo (DRC), Sierra Leone, Uganda, and Angola [38, 201, 202] and the reduction of mobility during a conflict situation is supposed to be the most important factor. There is substantial evidence of mobility as an important factor increasing risk of HIV transmission [203], including one report from Guinea-Bissau and Burkina Faso [36]. Increased mobility during the comparatively smaller conflict in Guinea-Bissau 1998-1999 seems indeed to have enhanced or at lease coincided with HIV transmission. As a comparison, in the Rwandan conflict in the mid-1990s, there have been suggestions of an increase in HIV transmission as a result of the conflict

which included mass population movements and wide-scale rape, but the studies have been inconclusive [38]. Antenatal serosurveys showed a constant decrease in the capital Kigali during the 1990s, in the countryside HIV prevalence was increasing until 1996, thereafter decreasing. There is speculation that if a temporary rise occurred it might just have been part of the natural course of the HIV epidemic in Rwanda. The increase in HIV-1 prevalence and incidence in our observations in Guinea-Bissau could represent a parallel case to the Rwanda situation, though on a much smaller scale, with an increase in HIV prevalence and incidence shortly after a conflict situation and thereafter a decrease to more moderate levels. In Guinea-Bissau there were however no reports of systematic sexual violence.

We did not see any evidence of the civil war in 1998-1999 halting the long-term decline of HIV-2 prevalence or incidence in this cohort, which was also the case for the pregnant women. The results of the studies of pregnant women and police officers are consistent with other studies from Guinea-Bissau where a decline in HIV-2 prevalence and incidence was seen in an urban [30] and in a rural [31] community-based cohort. The decline of HIV-2 prevalence is probably attributed to several factors, for example blood transfusions in Guinea-Bissau were screened for HIV from 1987 [30].

HIV-1 incidence was highest in the youngest age group (17-24 years), similar to figures from a community-based study in Bissau [30] but different from a rural community-based cohort in Guinea-Bissau [31]. Reports from The Gambia have shown similar figures with increasing HIV-1 prevalence and decreasing HIV-2 prevalence although in some cases there were regional differences [29, 204], while rates have been more stable in Senegal [205].

The co-existence of HIV-1 and HIV-2 has been considered in two simulation models, first an early simulation in 1990, where HIV-1 becuase of higher reproductive rates was supposed to gradually outcompete HIV-2 [206]. A more recent mathematical simulation from 2007 suggested that 30% of the decline of HIV-2 prevalence was due to competition from HIV-1 including excess mortality in core groups transmitting both HIV-1 and HIV-2 and the remaining 70% due to behavioural change [207]. HIV-1 was there thought to become the dominant type of HIV by the year 2010 but in reality the shift occurred even faster. This further strengthens the hypothesis of the HIV-2 epidemic having jump-started as a partially parenteral rather than purely sexually transmitted epidemic in the 1960's and 70's [34, 35]. One can speculate whether HIV-2 eventually finally might disappear, transmitting much less efficient via MTCT and sexually, due to the lower viremia levels [131, 208]. It has recently been shown that early infection is associated with onward transmission, just as in HIV-1 infection (Thesis, Carla Van

Tienen 2011). With the HIV-2 epidemic declining it works as an example to what could be achieved in the HIV-1 epidemic with early treatment to prevent further infection, as has recently shown to be a powerful preventive intervention [158].

In addition to the epidemiological trends of HIV-1 and HIV-2, there was no evidence of a protective effect exerted by HIV-2 against HIV-1 infection, but while a previous report from the same cohort showed HIV-2 as a risk factor with Incidence Rate Ratios (IRR) of 1.65 - 1.98 (depending on adjustment), the IRR had now fallen to 1.02 – 1.18 (the latter figure for men only). Interestingly, in the HIV-negative persons the risk of HIV-1 infection decreased by age, while it increased among HIV-2-positives.

**In conclusion, we found that the previously noted trend of decreasing HIV-2 prevalence and incidence continued, while HIV-1 prevalence increased and surpassed HIV-2 prevalence before stabilising after the civil war in 1998-1999. We found that during and shortly after the conflict, the HIV-1 incidence increased significantly among the police officers, indicating an effect on HIV-1 transmission exerted by the war which was also seen as a significant rise in HIV-1 prevalence among the pregnant women. However, in the years after the conflict HIV-1 prevalence stabilized in both groups, not giving evidence of any long-term effect of the war on HIV-1 prevalence. As in previous studies from this and most other cohorts, we found no evidence of a protective effect of HIV-2 against HIV-1 infection.**

# Natural course of HIV-2 infection (III)

While the natural course of HIV-1 infection has been studied thoroughly, most reports of the more benign disease course of HIV-2 were derived from seroprevalent individuals, without knowledge of the duration of infection. Thus, it was not clear whether the seroprevalent positive individuals were representative for all HIV-2-positive cases or were partially a selection of LTNP.

After 21 years of surveillance in the police cohort, the outcomes of the seroincident participants were examined to investigate the natural course of HIV-2 87 HIV-2 seroincident individuals were followed for a median follow-up time of 6.5 years (mean follow-up time of 7.4 years). Comparisons were made with 225 HIV-1 seroincident (median follow-up time of 4.8 years), 295 HIV-2 seroprevalent (median follow-up time 5.9 years) and 126 HIV-1 seroprevalent individuals (median follow-up time 3.8 years).

We performed the calculations on total non-accidental mortality and disease progression to WHO clinical stage 4-qualifying events.

The mortality rate was 3.0/100 person-years (PYO), significantly lower compared to 6.9/100 PYO in HIV-1 seroincident study participants. The incidence rate of disease progression to WHO stage 4 was 2.6/100 PYO, significantly lower than 6.1/100 PYO in HIV-1 seroincident study participants (*Table 5*).

**Table 5**: Mortality rates and Progression to WHO stage 4 rates per 100 person-years, with 95% CI (the full table is shown in table 2, paper III.

| | HIV-2 | | | HIV-1 | | |
|---|---|---|---|---|---|---|
| **Rates** | **incident** | **prevalent** | **Total** | **incident** | **prevalent** | **Total** |
| **Mortality** | 3.0 (1.9-4.6) | 4.3 (3.6-5.3) | 4.0 (3.4-4.8) | 6.9 (5.5-8.6) | 6.9 (5.0-9.6) | 6.9 (5.7-8.3) |
| **WHO 4** | 2.6 (1.6-4.1) | 3.1 (2.4-3.9) | 2.9 (2.4-3.6) | 6.1 (4.8-7.7) | 6.9 (5.0-9.6) | 6.4 (5.3-7.7) |

There are no previous reports of mortality rates in HIV-2 seroincident individuals but mortality rates of seroprevalent HIV-2-positive individuals have been described from other cohorts – 4.5/100 PYO in HIV-2 prevalent persons in rural Guinea-Bissau [209] and 2.6/100 PYO in urban Guinea-Bissau [101]. In a study of older age in urban Bissau, the total HIV-2 mortality was 6.2/100 PYO, although mortality rate ratios remained unchanged, approximately double compared to HIV-negative individuals [103].

For the HIV-1 seroincident individuals, the mortality rate was very similar to two other studies HIV-1 seroincident individuals in Africa before the introduction of ART, both community-based studies in rural Uganda, with mortality rates of 7.0/100 PYO among 240 HIV-1 seroincident persons [98] and 6.7/100PYO among 168 HIV-1 seroincident individuals [97]. In a study of Hotel employees in Tanzania, the mortality rate was 5.0/100 PYO in 120 seroincident individuals [210].

In the survival analysis, the median survival time in HIV-2 seroincident individuals was 15.0 years, significantly longer than the 8.6 years in HIV-1 seroincident participants and the median disease progression time to WHO clinical stage 4 was 15.6 years, also significantly longer than the 8.7 years in HIV-1 seroincident participants (*Table 6, Figure 9*).

**Table 6:** Median survival time and disease progression time to WHO stage 4, with 95% CI (the full table is shown in table 3, paper III).

| | HIV-2 | | | HIV-1 | | |
|---|---|---|---|---|---|---|
| **Median time** | **Incident** | **Prevalent** | **Total** | **Incident** | **Prevalent** | **Total** |
| **Survival** | 15.0 (11.4-.) | 15.9 (13.2-17.3) | 15.9 (13.1-17.3) | 8,6 (7.9-9.1) | 6,9 (6.1-9.8) | 8.2 (7.2-9.0) |
| **WHO 4** | 15.6 (14.3-.) | 17.8 (15.7-.) | 17.8 (15.6-.) | 8.7 (8.0-9.7) | 6.9 (6.1-11.3) | 8.6 (7.4-9.8) |

The differences with shorter median survival times than disease progression times to WHO stage 4 rather than the other way around, is quite representative for the imperfect diagnostic measures in Guinea-Bissau. Several WHO stage 4-classifying diseases, for example extrapulmonary tuberculosis, will certainly in many cases have been underdiagnosed, leading to death without the recognition of the diagnosis of an AIDS-defining symptom.

The median survival time in HIV-1 seroincident individuals in the two Ugandan studies was 9.0 years, and 9.8 years respectively, which was also quite similar to the 8.6 years in our study. Thus we believe our figures were not too biased by eventual loss to follow-up.

Adjusted for gender and age in a Cox regression model, the HIV-2 seroincident study participants progressed to clinical AIDS at a rate of 0.26 and to death at a rate of 0.27 compared to the HIV-1 seroincident participants.

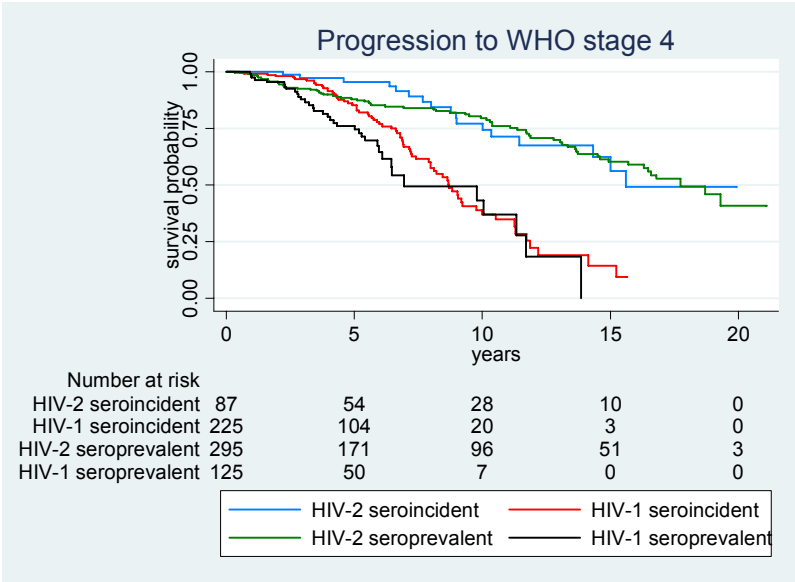



**Figure 9:** Kaplan-Meier curve displaying disease progression to AIDS according to WHO stage 4 definition, with median progression to AIDS being 15.6 years in HIV-2 seroincident compared with 8.7 years in HIV-1 seroincident ($p<0.001$log-rank test) and 17.8 years in HIV-2 seroprevalent individuals ($p<0.69$).

The results of HIV-2 seroincident data mainly confirm previous reports of seroprevalent HIV-2 infection being less aggressive than HIV-1 infection, and we did not see significant differences between HIV-2 seroincident and seroprevalent infection.

Our second aim was to determine how the first recorded $CD4^+$ T cell counts after estimated date of seroconversion were correlated to outcome. The first recorded $CD4^+$ T cell of each patient were of the same levels in HIV-2 seroincident and seroprevalent individuals, just as they were in HIV-1 infection (*Figure 10*).

We stratified the first recorded $CD4^+$ T cell absolute count and $CD4^+$ T cell percentage sample into categories and counted survival time from the date of first $CD4^+$ T cell count. The median survival times of HIV-2 seroincident participants were significantly shorter in the lower ranges of $CD4^+$ T cell counts (absolute counts below 350 cells/µl and percentages below 28%). At levels below 350 cells/µl and 21%, no significant differences with HIV-1 seroincident individuals were found, indicating the early loss of $CD4^+$ T cells is associated with mortality in both HIV-2 and HIV-1 infection (*Figure 11*).

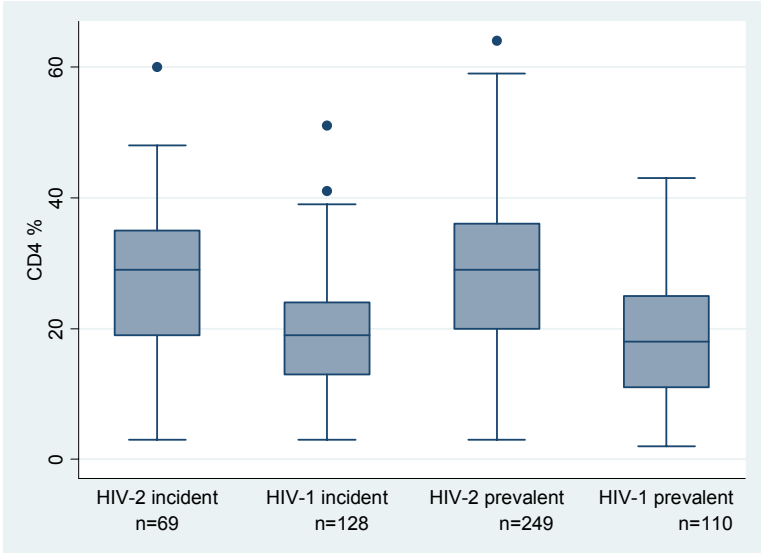



**Figure 10:** CD4$^+$ T cell % levels at first measurement after diagnosis of HIV of estimated seroincident or registered seroprevalent infection. Box plots with inter-quartile ranges inside boxes. P<0.001 between HIV-2 and HIV-1-seroincident participants (Wilcoxon rank-sum test).

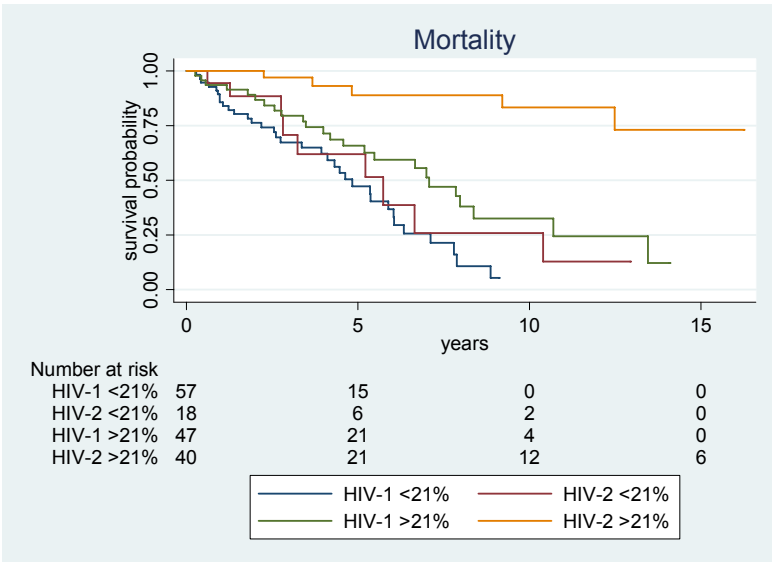



**Figure 11:** Kaplan-Meier curve of mortality of HIV-2 and HIV-1 seroincident individuals depending on the first recorded CD4$^+$ T cell % level (</≥ 21%). P<0.001 between HIV-2 seroincident participants </≥ 21%. P<0.001 between HIV-1 and HIV-2 ≥21%, p=0.13 between HIV-1 and HIV-2 <21%.

VL has not been a routine diagnostic tool at the LNSP, but there was a limited amount of VL measurement results available (performed for research purposes with two different tests in the periods 1993-1995 and 2004-2006). Due to few samples we had to group all HIV-2-positive individuals together in the survival analysis. VL>4 $\log_{10}$ was independently associated with mortality and disease progression to WHO stage 4.

When both VL and $CD4^+$ T cell counts were introduced in the Cox regression model, the $CD4^+$ T cell absolute and percentage counts lost significance while VL remained correlated both to mortality and disease progression to WHO stage 4. VL >4 $\log_{10}$ copies/ml was significantly correlated to mortality while $CD4^+$ T cell levels were not, VL thus being the most accurate variable for prediction of mortality, though it must be remembered the low number of subjects with both VL and $CD4^+$ T cell counts that could be included in the analysis. The results are, however, in agreement with the observations from a community-based study in rural Guinea-Bissau of HIV-2 seroprevalent individuals where undetectable plasma VL was a strong predictor of survival as was high $CD4^+$ T cell % counts, but in multivariate analysis $CD4^+$ T cell % lost significance [209]. Our results are also in line with previous studies where high HIV-2 plasma VL predicted faster $CD4^+$ T cell decline and clinical disease progression in the minority of HIV-2-positive individuals that progressed rather rapidly to disease and death [211], with mortality rates similar to HIV-1-positive individuals [113]. In a study from the HIV-2 cohort in France, some HIV-2 seroprevalent controllers with low VL still experienced disease progression and some were long-term non-progressors despite higher VL [212]. This was also the case in our data, reflecting a more complex relationship between virus and host. In practice, there is a lack of commercially available high-sensitivity HIV-2 plasma VL assays, which, just as well as for HIV-1, would be desirable for prognostic purposes as well as surveillance of treatment efficacy, also in resource-limited settings such as Guinea-Bissau.

The third aim was to investigate the rates of $CD4^+$ T cell depletion during the course of infection. With lest-square linear regression of all individuals with 2 or more recorded $CD4^+$ T cell % counts, we found that the median decline rates were 0.22 per year in HIV-2 seroincident and 1.10 in HIV-1 seroincident study participants. Thereafter we divided the decline rates into three categories (more than 1.5, 1.5 to 0.1 and less than 0.1 per year). In the HIV-2 seroincident group 44% belonged to the group with no or very low decline rate (<0.1 per year), and 27% in the group with steepest decline (>1.5 per year) whereas in HIV-1 seroincident individuals the situation was the opposite with 43% of participants in the group with steep decline and 25% in the group with slow decline. We also performed an analysis with a mixed linear model with the time since

seroconversion as a covariate, giving the results of mean $CD4^+$ T cell % decline rates in HIV-2 seroincident individuals of 0.53 and 1.00 in HIV-1 seroincident participants. In a follow-up of study of 49 HIV-2 seroincident individuals within the French HIV-2 cohort, the $CD4^+$ T cell % decline rate was only 0.04 per year, although the decline rate in HIV-2 seroprevalent individuals, 0.58, was similar to our results. [115] A reason for the difference compared with our result could be a lower immune activation as presumably fewer co-infections were encountered by the patients in France compared to Africa, especially as parasitic infections known to contribute to immune activation [213].

Between 32 and 44 % of the HIV-2-positive participants in our study belonged to the group with no or very low decline rate of $CD4^+$ T cell % over the study period. This is again in accordance with the same community-based study from rural Guinea-Bissau where 37% of HIV-2-prevalent individuals had an undetectable VL at study entry and similar survival rate compared to HIV-negative controls [209]. It may imply that approximately one third of HIV-2-positive subjects are long-term non-progressors. The corresponding proportion of HIV-1-positive individuals with no or very low decline rate of $CD4^+$ T cell % was 25%, but this may be an overestimation due to the fact that the HIV-1 epidemic has quite recently entered Guinea-Bissau and many of the HIV-1-positive subjects would still be in the early asymptomatic phase of the infection.

**In conclusion, we found that the observations of HIV-2 seroincident disease course affirmed results of previous studies of seroprevalent HIV-2 infection, regarding the less severe clinical course of HIV-2 infection compared with HIV-1 infection. We showed that median survival time in HIV-2 seroincident individuals was 15.0 years compared with 8.9 years in HIV-1 seroincident individuals and that the risk of mortality and disease progression was between one third and one fourth the risk in HIV-1 seroincident participants. The first recorded $CD4^+$ T cell levels correlated to survival, suggesting that in HIV-2, like in HIV-1, early events are decisive for the clinical outcome. Initial $CD4^+$ T cell levels and HIV-2 VL levels correlated to mortality in univariate analysis but in multivariate analysis only VL remained significantly associated (however in small groups due to limited availability of samples). We did not see any differences in HIV-2-seroincident and HIV-2-seroprevalent individuals, indicating that previous studies of HIV-2-prevalent individuals were quite representative for description of the natural course of HIV-2 infection.**

# Influence of HIV-2 on HIV-1 infection (IV)

Experimental studies using the macaque model have shown inhibition against both immunosuppression and SIV-induced disease as a result of concomitant HIV-2 infection [146, 214], raising the question of a possible mitigating effect exerted by HIV-2. Previous reports of disease progression and mortality among dually infected HIV-1 and HIV-2-individuals concerned seroprevalent infections where mortality of HIV-D had been higher or on the same level as HIV-1 single infected individuals [80]. We wanted to investigate whether this was the case also in HIV-1-seroincident study participants and to correlate it with molecular and immunological investigations.

We analysed data of 223 HIV-1-seroincident individuals - 191 HIV-1- single-infected and 32 HIV-D-infected, whereof 12 had simultaneously recorded dual HIV reactivity and 20 had HIV-2 infection preceeding the HIV-1 seroconversion. Of the latter 20 participants, 4 were initially seronegative and 16 were HIV-2 seroprevalent at study inclusion. To measure the progression time to AIDS we used the definition of CDC stage C (including $CD4^+$ T cell absolute count $\leq$200 or $CD4^+$ T cell % $\leq$14).

The median progression-time to AIDS was 104 months (8.7 years) in dual-infected individuals, significantly higher than 68 months (5.7 years) in single-infected individuals. Adjusting for gender and age, there was a significant 2.8 times higher risk of progression to AIDS in HIV-1 single-infected compared to HIV-D-infected individuals.

To evaluate the effect of infection order, we stratified the dual-group into those with HIV-2 seroreactivity preceding the dual seroreactivity (HIV-$D_{2\rightarrow D}$, n=20) and those with a simultaneous recorded dual seroreactivity (HIV-$D_{0\rightarrow D}$, n=12). The HIV-$D_{2\rightarrow D}$-infected individuals had a progression-time to AIDS of 129 months (10.8 years), significantly longer compared than the single-group (*Figure 12*). Even though the progression-time to AIDS for the HIV-$D_{0\rightarrow D}$ infected individuals was intermediate, 88 months (7.3 years), it did not differ significantly from either the HIV-$D_{2\rightarrow D}$-group or the single-group The adjusted hazard ratio (aHR) of progression to AIDS in single versus HIV-$D_{2\rightarrow D}$-infected individuals was significantly higher, 3.1 times. There was a strong trend of longer progression time in single vs. HIV-$D_{0\rightarrow D}$-infected individuals (aHR 2.3) while there was no difference between HIV-$D_{0\rightarrow D}$ and HIV-$D_{2\rightarrow D}$-infected individuals (aHR 1.4).

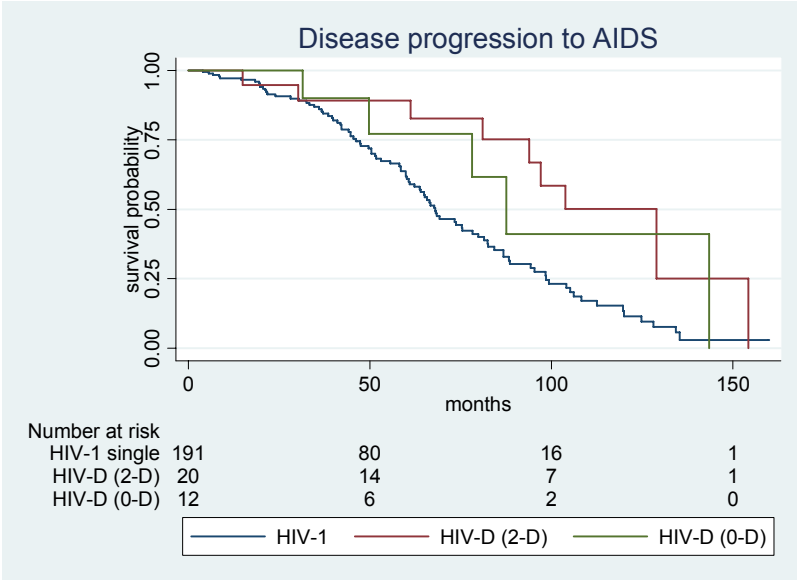



**Figure 12:** Progression time to AIDS in HIV-1-single-infected and HIV-D-infected. HIV-1 vs HIV-D (total) p=0.003. HIV-1 vs HIV-$D_{0\text{-}3}$ p=0.13, HIV-1 vs HIV-$D_{2\text{-}3}$, p=0.007, log-rank test.

Decline rates of $CD4^+$ T cells and $CD8^+$ T cells were analysed with a mixed linear model with time since seroconversion as covariate. The rate of decline in $CD4^+$ T cell % was similar in single and dual-infected individuals, with an average decline of 1.22 per year. However, the level of $CD4^+$ T cell % was significantly higher in dual (31.26%) than in single-infected individuals (23.34%). A stratified analysis showed similar decline rates in $CD^+$ T cell 4% in HIV-$D_{0\rightarrow D}$ and HIV-$D_{2\rightarrow D}$-infected individuals. The HIV-$D_{2\rightarrow D}$-group had, however, a significantly higher level of $CD^+$ T cell 4% (32.30%) than HIV-1-single-infected individuals. The HIV-$D_{0\rightarrow D}$-group showed an intermediate level (28.13%), with no statistical difference from either the single-group or the HIV-$D_{2\rightarrow D}$-group.

Next, we examined differences in $CD8^+$ T cell % over time, and found a slower increase in $CD8^+$ T cell % levels in dual (1.54 per year) than in single-infected individuals (2.95 per year). This difference was evident also between the single-group and the HIV-$D_{2\rightarrow D}$-group, but not between the single-group and the HIV-$D_{0\rightarrow D}$-group which showed steeper increase than the other groups, though not significant.

As we observed a difference in the rate of $CD8^+$ T cell % elevation between single and dual-infected individuals, we investigated possible differences in the immune activation markers of beta-2-microglobulin (b2m) or neopterin but the results were

inconclusive with considerable inter-patient variation. However, the slower increase in $CD8^+$ T cell % over time after HIV-1 infection among dual-infected individuals suggests that alteration in cellular immune activation may contribute to the disease outcome. It has been suggested that levels of soluble immune activation markers could be influenced by different endemic parasite and bacterial diseases present in African populations [215]. This could at least partly explain the results of levels of b2m and neopterin. There have been several reports of a positive correlation between $CD8^+$ T-cell activation and HIV-1 disease progression rate [216, 217]. There are reports of HIV-2 *env* and *nef* proteins having higher inhibitory effect against T-cell proliferation than their HIV-1 counterparts [218, 219]. *In vitro* studies have shown that HIV-2 infection generates higher levels of β-chemokines (the natural ligands of the HIV coreceptor CCR5) in peripheral blood mononuclear cells, and that this can inhibit HIV-1 infection and replication [220-223]. There is evidence of high frequencies of cross-reactivity between samples of HIV-1 and HIV-2 regarding heterologous T-cell responses, and that higher HIV-1 plasma VL is exhibited in those without this cross-reactivity [224], which is also the case in dual-infected individuals [225]. Antibodies elicited by HIV-2 infections that cross-neutralize HIV-1 have been described [226]. Hence, humoral HIV-2 immune responses could also play a role in controlling HIV-1 in dual-infected individuals.

As mentioned before, since VL measurements have not been included as a standard procedure in Guinea-Bissau, and in the VL data used in study III there were too few dually infected samples for usage in study IV. As many of the stored plasma samples were freeze-thawed several times which causes degradation of the genetic material, retrospective VL measurements was not an option. However, it was possible to detect and clone RNA from plasma samples enabling molecular analyses of diversity and divergence. Studies have suggested that there may be a positive correlation between HIV-1 diversity and VL [61, 227]. Diversity has also been positively correlated with HIV-1 replication efficiency and rate of progression to AIDS [62, 228]. The “diversity threshold theory” assumes that AIDS develops when diversity exceeds a critical threshold that varies individually [60]. The concept of the “diversity threshold theory” has been debated, and the high level of diversity seen close to AIDS may as well be a consequence of the disease progression rather than a cause of disease. The observation that HIV-1 diversity was significantly lower in dual than in single-infected individuals at comparable time-points after HIV-1 infection, while the rates of increase in diversity was similar supports the hypothesis that the slower disease progression rate may be related to inhibitory effects early in the HIV-1 infection. We wanted to test if we could find any correlate with such threshold and the difference in

diversity level found between single and dual-infected individuals. The average progression-time to AIDS was 68 months in single-infected individuals. At this time-point the estimated mean diversity was $13.52x10^{-3}$ substitutions/site for single-infected individuals. Dual-infected individuals were estimated to reach the same mean diversity after 105 months, close to the average observed progression-time to AIDS of 104 months. The correlation between diversity and disease progression time was seen with different phylogenetic approaches. Thus, the mean diversity threshold was almost identical for dual and single-infected individuals, indicating that diversity evolution could be used as a clinical marker for disease progression rate. The average increase in HIV-1 sequence diversity over time, was similar in single and dual-infected individuals with an average of $1.75x10^{-3}$ substitutions per site per year. The diversity was significantly lower in dual than in single-infected individuals, after adjusting for differences in sampling time between individuals.

In order to study the impact of potential confounders in our analyses, we performed several complementary analyses. We did not find any significant impact of censoring on the the difference in disease progression between the two groups. We performed simulations and did not find evidence of misclassified dual-infected individuals, selection for long-term disease controllers in the dual-group or any effect of HIV-1 subtype. The study of paper IV was performed and analysed before study III, hence the lower number of participants in the HIV-1 single infected group, and as we could see, the seroconversion of additionally 34 additional participants and prolonged follow-up time for 191 individuals in the study gave an increase in median progression time to AIDS from 68 moths (5.7 years) - to 6.2 years in study III. Also in the $CD4^+$ T cell % decline rate over time (measured with a mixed linear model using time since seroconversion as a covariate) there was a difference in study IV compared with study III - as more samples were included in study III the mean decline rate decreased from 1.20 to 1.00 per year. For the simulations to investigate whether a possible selection bias towards HIV-1 LTNPs we used the figure of 20-30% disease progressors, while we later in the analysis of study III saw indications that a higher proportion could be disease progressors. However, as most other studies reported following of the participants for less than 10 years, this was a too short time span for the majority HIV-2-positive individuals to start progressing to clinical disease. When we measured the disease progression in the HIV-2 seroincident individuals with the same definition of AIDS as in study IV, the progression rate at the mean time of AIDS in the HIV-D group (104 months = 8.9 years) was 34% - close to the stipulated upper limit of 30% HIV-2-positive disease progressors. Thus there should be a low risk of selection bias towards HIV-2 long-term-non-progressors

that should eventually also be HIV-1 LTNPs. However, even selection bias should have occurred, the lower disease progression rate in the HIV-D group would still have been an interesting finding.

**In conclusion, we saw that dually-infected individuals with a previous HIV-2 infection preceding the HIV-1 infection had a longer progression-time to AIDS compared with HIV-1 single-infected individuals. The slower disease progression was reflected by higher levels of CD4+ T-cells at comparable time-points and different kinetics in levels of CD8+ T-cells. Phylogenetic analysis revealed differences in HIV-1 molecular evolution with lower viral diversity at comparable time-points after infection in dual-infected individuals. Further investigations of the interactions between HIV-1 and HIV-2 and the immunologic effects of a contemporaneous HIV-2 infection preceeding HIV-1 seroconversion are needed to find new entry points for therapeutic HIV-1 interventions.**

# Sexually Transmitted Infections and relation to HIV (V)

As there was a lack of prevalence data of common STI pathogens in Guinea-Bissau, we wanted to investigate the prevalence of what we expected to be the most common non-ulcerative (*Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Mycoplasma genitalium, Trichomonas vaginalis*) and ulcerative (HSV-1 and HSV-2, *Haemophilus ducreyi*, *Treponema pallidum*) STIs. We did not include HBV and HCV, as we were interested in genital infection. We did however include HTLV in the report, as we already had available HTLV diagnostics and were interested in comparisons with previous studies from Guinea-Bissau, were HTLV had been diagnosed.

A total of 711 women were examined. 89.7% presented with vaginal discharge and 21.8% with genital ulcers. We found that HIV-1 and HIV-2 prevalence was higher than in the pregnant women of paper I. The difference was more marked in HIV-1 where the total HIV prevalence of 10.6% (including HIV-D) was approximately double compared to 4.9% of pregnant women in the survey in 2006. HIV-2 prevalence was 3.0 (including HIV-D), compared with 2.0% of pregnant women in 2006. This were quite expected findings considering the nature of the study population with symptomatic urogenital disease. Surprisingly, we did not see any statistical differences in HIV prevalence between women with or without genital ulcers or vaginal discharge. The most prevalent pathogen encountered was *Trichomonas vaginalis*, with a prevalence of 20.4%. The finding of a correlation between HSV-2 and HIV was expected as genital HSV-2 infection is a well known risk factor for HIV acquisition [170]. *Mycoplasma genitalium* is an STI that is attracting more and more attention. We found a correlation with HIV which was not surprising since it has been associated with HIV shedding in the female genital tract [229] and has recently been shown to increase HIV acquisition in Zimbabwe and Uganda where it was also more prevalent than other non-viral STIs [230].

The unexpectedly low prevalence found of *Neisseria gonorrhoeae* was probably at least partly the result of problems with culture, especially incubator time in correct temperature. There is definitely a need for functioning culture for better diagnostics of *Neisseria gonorrhoeae*, both for diagnostics of individual patients and for vigilance for antibiotic resistance. The syphilis prevalence was surprisingly low, 1.0%. However, this is consistent with a finding of decreasing syphilis prevalence in The Gambia, which has been observed in spite of any directly targeted treatment programme [231]. For comparison, 12 % of the participants in

the police cohort showed evidence of earlier syphilis serology (with positive TPHA reactivity at inclusion in the cohort).

HTLV-1 has been stable or slightly decreasing in Guinea-Bissau [177, 232]. We found that HTLV-1 was not associated with HIV, contrasting recent findings from rural Guinea-Bissau where there was an association. [232]. However, there HIV was a risk factor for HTLV-1 and not vice versa. As the women in our study were quite young (mean age 24.5 years), a longitudinal follow-up study could reveal later HTLV-1 acquisition.

One would have expected that also STIs other than *Mycoplasma genitalium* and HSV-2 should have been associated with HIV infection, and we haven't got any good explanation for that finding, apart from the fact that diagnostics of *Neisseria gonorrhoeae* were suboptimal.

At the time, there were no standardized treatment guidelines for STIs in Guinea-Bissau. We have later shown that there was severely reduced susceptibility of *Neisseria gonorrhoeae* for several different antibiotics (B Olsen et al, submitted manuscript), a pattern following earlier reports from The Gambia where it was shown increasing antibacterial resistance in *Neisseria gonorrhoeae* isolates already in 1992 and 1997 [233, 234]. Guinea-Bissau will need STI management guidelines based on evidence and ongoing antibiotic susceptibility testing to treat STIs correctly. Further studies regarding resistance will certainly be needed.

Further studies are also needed to explore sexual behaviour - there is actually very limited knowledge even of heterosexual sex patterns in Guinea-Bissau. Heterosexual sex is understood as purely vaginal intercourse although several reports have emerged of different heterosexual practices in Africa [235] There is certainly a need for more studies on STI prevalence in other populations in Guinea-Bissau – including sexually transmitted pathogens that were not covered here such as Hepatitis B Virus and Hepatitis C Virus. Another interesting survey to perform could be that of genital schistosomiasis which is emerging as a possible cause of genital ulcers and a risk for HIV acquisition [236], as *Schistosoma haematobium* is present in Guinea-Bissau and there is known high prevalence in other West African countries [237].

**In conclusion, we found that both HIV-1 and HIV-2 prevalence was higher among symptomatic women than in the pregnant women in Bissau. We found high prevalence levels of other STIs, though we could only see statistical correlations to HIV in the HSV-2 and the *Mycoplasma genitalium* infections. Better diagnostic setups are necessary and evidence-based guidelines of treatment of STIs are needed in order to improve sexual and reproductive health as well as to limit HIV transmission.**

# Concluding remarks

The fight against AIDS is multifaceted, needing a comprehensive approach with many different efforts from different actors. While the epidemic has passed it's peak and the numbers of newly infected individuals become fewer, it is necessary to bear in mind that still in 2012, for every person put on antiretroviral treatment in Africa two new individuals get infected. Many different kinds of interventions are needed, from prevention, education and destigmatisation, to surveillance, treatment and basic scientific research. The results presented in this thesis add more evidence to different areas of action.

The surveillance of HIV-1 and HIV-2 in Guinea-Bissau has shown a steady decline of HIV-2 while HIV-1 has come to a steady-state level. The stabilisation of the HIV-1 epidemic may be due to information and education, sexual behaviour change, the national ART programme launched in 2005 but most probably a combination of factors. Further surveillance is needed to give possibilities of direct reaction if the trends suddenly diverge from expected scenarios.

The evidence of a further decline of the HIV-2 epidemic could act as a role model for the HIV-1 epidemic. The knowledge of the lower transmission rates of HIV-2 and the correlation to lower plasma VL levels can be seen as a call for expansion of early treatment of HIV-1. The pharmaceutical treatment of HIV-1 and HIV-2 is outside the scope of this thesis but it is still important to point out that the most important thing in HIV care in Guinea-Bissau will be to retain people initiated on treatment. Early treatment of HIV has now shown to be an efficient way of preventing new transmissions.

The effect of armed conflict has most often shown to be limiting to HIV spread, but in the case of the civil war in Guinea-Bissau 1998-1999 there was significant increase in new HIV-1 cases during and shortly after the war. We interpret this as the conflict being very much different to other wars fought in Sub-Saharan Africa, with the example of Guinea-Bissau creating a more mobile situation compared to other countries where populations were rather obstructed to move for prolonged times. Prevention efforts are important to sustain even during conflict situations.

There was previously a lack of data on the natural course of HIV-2 infection based on observations of individuals with known seroconversion date. In our study of

HIV-2 seroincident individuals, we confirmed results from earlier studies of a much less severe clinical course in HIV-2 infection. The clinical course of HIV-2 did not significantly differ in the seroincident compared with the seroprevalent cases, indicating that previous studies of HIV-2 were representative of the whole HIV-2-positive community. There was a clear correlation between the first recorded $CD4^+$ T cell counts after seroconversion, both with mortality and disease progression, indicating that early events are impportant for the clinical outcome, just as in HIV-1 infection. Plasma VL measurements for prognostic and diagnostic purposes should ideally be implemented, also for HIV-2, and if possible to a greater extent in low-income countries such as Guinea-Bissau, where actually the need is the greatest.

The interactions of concomitant HIV-1 and HIV-2 infection are most interesting as they can give clues for therapeutic interventions against HIV-1 infection. We ovserved longer disease progression time in HIV-1-seroincident individuals with simultaneous HIV-2 infection and the findings were more marked in the participants that had a documented HIV-2 infection preceeding the seroincident HIV-1 infection. We tried to control for the risk of selection bias towards HIV-1 long-term non-progressors and our conclusion was that we saw a true mitigating effect against disease progression of HIV-1, emanating from HIV-2 infection. It stresses even more the importance to correctly identify and map all possible mechanisms of HIV-2-elicited immune responses and other actions for partial control of HIV-1 infection.

The high prevalence of STIs is a call for better and more available diagnostics. There is also a need for further studies in other locations in Guinea-Bissau to map the prevalence of STIs in order to gain knowledge for constructing correct guidelines of treatment and prevention. These guidelines will need to be consistent with studies in order to avoid antibiotic resistance. Treatment of all STIs are essential, but especially treatment of *Mycoplasma genitalium* and HSV-2 is needed for the HIV prevention. New studies to recognise present and changing patterns of sexual behaviour could give insight in areas where traditional prevention methods presently are not effective.

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