Aspects of sexually transmitted infections in Guinea-Bissau

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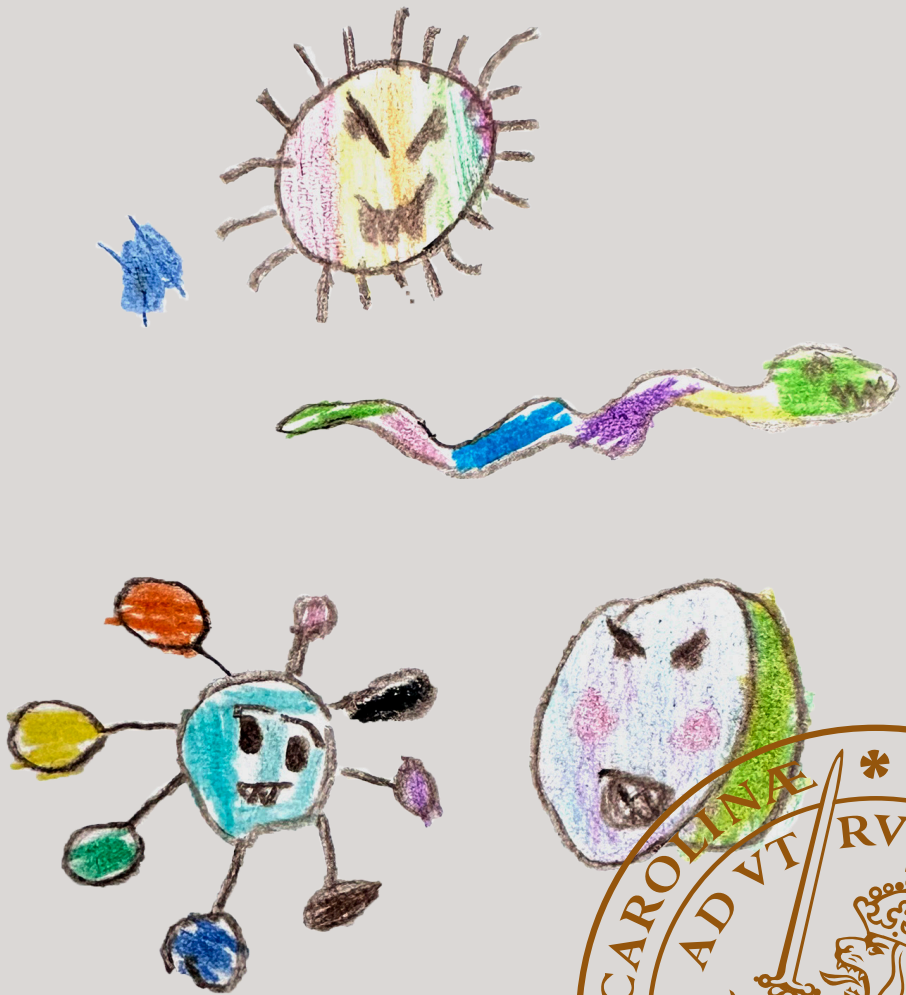

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Jacob Lindman

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DOCTORAL DISSERTATION

Doctoral dissertation for the degree of Doctor of Philosophy (PhD) at the Faculty of Medicine at Lund University to be publicly defended on 05 of September at 09.00 in Belfragesalen, BMC, Sölvegatan 17, Lund.

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Abstract:

Human immunodeficiency virus type 1 (HIV-1) is responsible for the global pandemic, while HIV type 2 (HIV-2) remains largely confined to West Africa. Being able to discriminate between HIV-1, HIV-2 and HIV-1/2 dual infection is important for correct diagnosis and treatment. Female sex workers (FSW) are a key population for transmission of HIV and curable sexually transmitted infections (STIs), however data from this group in Guinea-Bissau are limited. Human T-lymphotropic virus type 1 (HTLV-1) is also endemic in Guinea-Bissau. HTLV-1 has been associated with increased mortality in both HIV negative individuals and people with HIV-1 (PWH-1), although previous findings in the latter group are conflicting.

We investigated three serological assays (Geenius, INNO-LIA and Immunocomb) for HIV type discriminatory capacity. The Geenius Reader showed good concordance with INNO-LIA, while visual reading of Geenius somewhat overestimated HIV-1/2 dual reactivity, though less so than Immunocomb. The study was further strengthened by comparisons with HIV nucleic acid amplification tests (Paper I). We found a high prevalence of both HIV (27%) and curable STIs (47%) among FSW in Guinea-Bissau (Papers II and III). Among all FSW with HIV-1, a third were virally suppressed (viral load ≤1000 copies/ml) and among treatment-naïve FSW a tenth had an HIV drug resistance mutation. Ciprofloxacin resistance was high in Neisseria gonorrhoeae-positive specimens, showing that this drug is no longer appropriate for the treatment of gonorrhoea in Guinea-Bissau, in line with resistance trends reported globally. Finally, we showed that HTLV-1 infection was associated with a threefold increased risk of death among HIV-negative participants with a known HTLV-1 infection date, and with an almost twofold increased risk of death among PWH-1 with a known HIV-1 infection date, despite significantly higher $CD4^+$ T cell counts early in infection.

In this thesis, we explore different aspects of HIV and other STIs in Guinea-Bissau. Our findings highlight persistent gaps in HIV and STI prevention and care among FSW. Future studies should aim to include more representative samples and examine barriers to diagnosis, treatment and viral suppression. Monitoring of HIV drug resistance and antimicrobial resistance in N. gonorrhoeae remains important. The clinical implications of HTLV-1 infection, especially in the context of HIV-1 co-infection, warrant further investigation. Strengthening diagnostic capacity, including HIV confirmatory tests and HTLV-1 testing, is key to improving care and guiding public health interventions.

Key words: HIV-1, HIV-2, FSW, curable STIs, HTLV-1

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Resumo científico de divulgação

O vírus da imunodeficiência humana (HIV) divide-se em dois tipos principais: o HIV-1, causador da pandemia global, e o HIV-2, que se encontra sobretudo na África Ocidental e em países com laços históricos com a região, tal como Portugal e França. A maior incidência de HIV-2 ocorre na Guiné-Bissau, um país da África Ocidental.

Por ano, aproximadamente 1.3 milhões de pessoas são infectadas pelo HIV e, até à data, mais de 88 milhões de pessoas foram infectadas a nível global, resultando em mais de 42 milhões de óbitos. O HIV causa uma infecção crónica que ataca principalmente um tipo de glóbulos brancos chamados células T-auxiliares. Quando o número destas células diminui, o sistema imunitário enfraquece, o que por sua vez aumenta o risco de doenças infecciosas e de certos cancros. Pesquisas anteriores mostraram que a evolução da doença é geralmente mais lenta no caso de HIV -2, quando comparado com o HIV-1.

O diagnóstico do HIV baseia-se em exames laboratoriais que detetam o próprio vírus e os anticorpos específicos produzidos pela pessoa infetada. Por vezes pode ser difícil distinguir entre o HIV-1, HIV-2 e infecção com ambos os vírus (infecção dupla por HIV-1/2), uma vez que os anticorpos podem apresentar reacção cruzada.

O tratamento contra o HIV não cura a própria infecção, mas, quando iniciado a tempo, as complicações podem ser evitadas e a esperança média de vida das pessoas que vivem com HIV pode ser a mesma. Um tratamento considera-se bem-sucedido quando a pessoa afectada deixa de ser transmissível. No entanto, a interrupção do tratamento pode levar à retoma da produção do vírus, e que por sua vez pode levar ao aumento do risco do desenvolvimento de resistência. O HIV-2 é naturalmente resistente a alguns medicamentos frequentemente utilizados para o tratamento contra o HIV-1, o que significa que o tratamento por vezes difere entre as variantes do vírus.

Os estudos desta tese baseiam-se em amostras e informações de diversos grupos da Guiné-Bissau: doentes do programa nacional de tratamento contra o HIV, mulheres que vendem sexo e um grande grupo de polícias. O objectivo foi avaliar o diagnóstico do HIV, descrever a sua prevalência e de outras doenças sexualmente transmissíveis (DSTs), investigar o acesso ao tratamento e mapear a prevalência da resistência aos medicamentos entre mulheres que vendem sexo. Além do acima mencionado, estudámos também a prevalência e consequências da infecção pelo

HTLV-1 (vírus linfotrópico de células T humanas), especialmente em casos de infecção dupla por HIV. O HTLV-1 é um vírus que causa infecção crónica e pode ser transmitido de mãe para filho, por via sanguínea ou por contacto sexual. O HTLV-1 pode causar doenças graves, como leucemia e doenças neurológicas, mas muitas pessoas podem ser portadoras do vírus sem adoecer.

No primeiro trabalho testou-se um novo teste laboratorial, o Geenius, e comparou-se com dois testes já existentes para investigar a sua eficácia na distinção entre HIV-1, HIV-2 e infecção dupla por HIV-1/2. O Geenius não tinha sido previamente testado numa população de doentes com elevada prevalência de HIV-2 e infecção dupla por HIV-1/2. Os resultados mostraram que o Geenius e o INNO-LIA apresentaram resultados equivalentes e foram capazes de distinguir entre os diferentes tipos de infeção por HIV de forma fiável. Uma vantagem do Geenius é que este proporciona um resultado rápido, dentro de uma hora, ao contrário do INNO-LIA, que requer mais tempo e equipamento laboratorial. Isto demonstra que testes mais simples e rápidos, como o Geenius, podem ser de grande importância para o diagnóstico do HIV em países de nível económico mais baixo. Além disso, é importante que os novos testes de HIV continuem a ser avaliados em grupos de doentes onde o HIV-2 é recorrente, uma vez que o diagnóstico correcto é crucial para a escolha do tratamento.

Em ambos os estudos seguintes, examinámos mulheres que vendem sexo, um grupo que apresenta um risco globalmente aumentado para o HIV e outras DST´s. Verificámos uma elevada prevalência de HIV e outras DST´s, como a gonorreia, a clamídia, a tricomoníase e micoplasmose. A maioria das mulheres com DST não apresentavam sintomas. Dessas, uma grande parte das que sabiam estar infectadas pelo HIV teve acesso a tratamento, e verificou-se uma elevada taxa de sucesso. No entanto, muitas desconheciam o facto de estarem infectadas pelo HIV, o que significa que apenas um terço de todas as mulheres infectadas pelo HIV apresentavam uma baixa carga viral no sangue, o que é um sinal de tratamento eficaz. Entre as mulheres que não apresentaram resultados de tratamento satifatórios, metade desenvolveu resistência aos medicamentos antivirais. Mesmo entre as mulheres que nunca tinham sido tratadas anteriormente, aproximadamente 10% já mostravam resistência. Estes resultados indicam que as mulheres que vendem sexo na Guiné-Bissau apresentam um risco muito mais elevado para HIV e outras DST´s. Elas deverias por isso receber tratamento preventivo para HIV (PrEP), testes rápidos de rastreio por DST, mesmo na ausência de sintomas, e tratamento direcionado para as DST´s.

O quarto estudo centrou-se no HTLV-1, onde se analisou a incidência, mortalidade e infecção dupla por HIV entre polícias. Os resultados mostraram que a proporção de pessoas infetadas com HTLV-1 quando foram incluídas no estudo diminuiu entre 1990 e 2008. Mesmo após esse intervalo o número de novas infeções continuou a diminuir, o que pode ser um indício de hábitos sexuais mais seguros. Os indivíduos infetados por HTLV-1 durante o período do estudo tiveram um risco mais elevado

de mortalidade quando comparado com os que permaneceram negativos. Entre os indivíduos infectados com HIV-1 durante o estudo, o risco de mortalidade foi maior se também estivessem infectados com HTLV-1. Este estudo realça a importância de intervenções preventivas e estratégias de acompanhamento de pacientes com infeção dupla por HTLV-1 e HIV-1, especialmente em regiões onde o HTLV-1 é comum.

Concluíndo, esta tese salienta a relevância do diagnóstico correto, de intervenções preventivas e do tratamento individualizado em regiões de incidência de casos de HIV-1, HIV-2 e HTLV-1. Os resultados têm implicações tanto para a prática clínica como para o planeamento de futuras intervenções de saúde pública.

Populärvetenskaplig sammanfattning

Humant immunbristvirus (hiv) förekommer i två varianter: hiv-1, som orsakat den globala pandemin och hiv-2, som framför allt finns i Västafrika samt i länder med historiska band till regionen, såsom Portugal och Frankrike. Den högsta förekomsten av hiv-2 finns i Guinea-Bissau, ett land i Västafrika.

Varje år får cirka 1.3 miljoner människor hiv, och hittills har över 88 miljoner personer globalt levt med viruset. Över 42 miljoner har avlidit till följd av hiv. Hiv orsakar en kronisk infektion som främst angriper en typ av vita blodkroppar kallade T-hjälparceller. När dessa celler minskar i antal försvagas immunförsvaret, vilket ökar risken för infektionssjukdomar och vissa tumörsjukdomar. Tidigare forskning har visat att sjukdomsförloppet vid hiv-2 generellt är långsammare än vid hiv-1.

Diagnos av hiv baseras på laboratorietester som påvisar viruset självt och de antikroppar som en person har bildat som svar på infektionen. Det kan vara svårt att skilja mellan hiv-1, hiv-2 och samtidig infektion med båda virusen (hiv-1/2 dubbelinfektion) eftersom antikroppar kan korsreagera.

Behandlingen mot hiv botar inte infektionen, men om den påbörjas i rätt tid kan komplikationer undvikas och livslängden för personer som lever med hiv förblir normal. Dessutom innebär en välfungerande behandling att man inte längre kan överföra viruset. Däremot kan behandlingsavbrott leda till att virusproduktionen återupptas, vilket ökar risken för resistensutveckling. Hiv-2 är naturligt resistent mot vissa läkemedel som ofta används mot hiv-1, vilket innebär att behandlingen ibland skiljer sig åt mellan virusvarianterna.

Studierna i denna avhandling baseras på prover och information från flera olika grupper i Guinea-Bissau: individer från det nationella hiv-behandlingsprogrammet, kvinnor som säljer sex samt en stor grupp poliser. Syftet har varit att utvärdera hiv-diagnostik, beskriva förekomsten av hiv och andra sexuellt överförbara infektioner (STI:er), undersöka tillgång till behandling och kartlägga förekomst av resistens mot hivläkemedel bland kvinnor som säljer sex. Dessutom har vi studerat förekomst och konsekvenser av infektion med humant T-lymphotropt virus 1 (HTLV-1), särskilt vid samtidig hiv. HTLV-1 är ett virus som orsakar kronisk infektion och kan överföras från mamma till barn, via blod eller sexuella kontakter. HTLV-1 kan orsaka allvarliga sjukdomar som leukemi och nervsjukdomar, men många bär viruset utan att bli sjuka.

I det första arbetet utvärderades ett nytt laboratorietest, Geenius, och jämfördes med två etablerade tester för att undersöka hur väl de kunde särskilja hiv-1, hiv-2 och hiv-1/2 dubbelinfektion. Geenius hade tidigare inte utvärderats med ett större antal prover från personer som lever med hiv-2 eller hiv-1/2 dubbelinfektion. Resultaten visade att Geenius och INNO-LIA gav likvärdiga resultat och kunde särskilja mellan de olika typerna av hiv på ett tillförlitligt sätt. En fördel med Geenius är att det ger ett snabbt resultat, inom en timme, till skillnad mot INNO-LIA som kräver mer tid och laboratorieutrustning. Detta visar att enklare och snabbare tester som Geenius kan ha stor betydelse för hiv-diagnostik i låginkomstländer. Dessutom är det viktigt att nya hiv-tester fortsätter att utvärderas i grupper där hiv-2 är vanligt förekommande, eftersom korrekt diagnos är avgörande för val av behandling.

I de två följande arbetena undersökte vi kvinnor som säljer sex, en grupp som globalt löper ökad risk för både hiv och andra STI:er. Vi fann en hög förekomst av hiv och andra STI:er som gonorré, klamydia, trikomonas och mykoplasma. De flesta kvinnor som testade positivt för en STI hade inga symtom. Majoriteten av kvinnorna som visste om att de levde med hiv hade tillgång till behandling, och de flesta av dessa var framgångsrikt behandlade. Däremot saknade en stor del av kvinnorna kännedom om att de levde med hiv, vilket innebar att endast en tredjedel av alla kvinnor med hiv hade en låg virusnivå i blodet, som är ett tecken på effektiv behandling. Bland kvinnor där behandlingen inte fungerade som avsett hade hälften utvecklat resistens mot antivirala läkemedel. Även bland kvinnor som aldrig tidigare behandlats hade cirka ~10% redan resistens. Dessa resultat visar att kvinnor som säljer sex i Guinea-Bissau har en kraftigt ökad risk för hiv och andra STI:er. De bör därför erbjudas förebyggande hivbehandling (PrEP), STI-testning även vid avsaknad av symtom, samt riktad behandling mot STI:er.

Det fjärde arbetet fokuserade på HTLV-1 där vi studerade förekomst, dödlighet och dubbelinfektion med hiv bland poliser. Resultaten visade att andelen personer som hade HTLV-1 när de inkluderades i studien minskade under perioden 1990 till 2008. Även antalet nya fall av HTLV-1 under uppföljningen sjönk, vilket kan tyda på säkrare sexualvanor. Personer som fick HTLV-1 under studietiden hade en högre risk att avlida jämfört med dem som förblev negativa. Bland personer som fick hiv-1 under studiens gång var risken att avlida högre om de samtidigt levde med HTLV-1. Denna studie understryker behovet av förebyggande insatser och strategier för uppföljning av personer som lever med både HTLV-1 och hiv-1, särskilt i regioner där HTLV-1 är vanligt förekommande.

Sammanfattningsvis belyser denna avhandling vikten av korrekt diagnostik, förebyggande insatser och anpassad behandling i regioner där både hiv-1, hiv-2 och HTLV-1 förekommer. Resultaten har betydelse både för klinisk praxis och för utformning av framtida folkhälsoinsatser.

List of papers

Papers included in this thesis

This thesis is based on the following papers, which will be referred to in the text by their roman numerals (I-IV).

Paper 1

Lindman J, Hønge BL, Kjerulff B, Medina C, da Silva ZJ, Erikstrup C, Norrgren H, Månsson F. Performance of Bio-Rad HIV-1/2 Confirmatory Assay in HIV-1, HIV-2 and HIV-1/2 dually reactive patients - comparison with INNO-LIA and Immunocomb discriminatory assays. *Journal of Virological Methods*. 2019;268:42-7

Paper II

Lindman J, Djalo MA, Biai A, Månsson F, Golparian D, Esbjörnsson J, Jansson M, Medstrand P, Unemo M, Norrgren H, Sweden Guinea-Bissau Cohort Research (SWEGUB CORE) group. Prevalence of sexually transmitted infections and associated risk factors among female sex workers in Guinea-Bissau. *Sexually Transmitted Infections* 2024;100:411-417

Paper III

Lindman, J, Djalo, M.A, Biai, A, Månsson F, Esbjörnsson J, Jansson M, Medstrand P, Norrgren H, Sweden Guinea-Bissau Cohort Research (SWEGUB CORE) group. The HIV care continuum and HIV-1 drug resistance among female sex workers: a key population in Guinea-Bissau. *AIDS Res Ther* 17, 33 (2020)

Paper IV

Lindman J, Månsson F, da Silva ZJ, Biai A, Malm K, Esbjörnsson J, Jansson M, Medstrand P, Unemo M, Andersson S, Norrgren H, Sweden Guinea-Bissau Cohort Research (SWEGUB CORE) group. HTLV-1 mortality and co-infection with HIV-1 or HIV-2 in Guinea-Bissau: findings from a prospective open cohort study. *Manuscript*

Papers not included in the thesis

Johansson E, Nazziwa J, Freyhult E, Hong M-G, **Lindman J**, Neptin M, Karlson S, Rezeli M, Biague AJ, Medstrand P, Månsson F, Norrgren H, Esbjörnsson J, Jansson M. HIV-2 mediated effects on target and bystander cells induce plasma proteome remodeling. *iScience*. 2024;27(4):109344

Johansson E, Kerkman PF, Scharf L, **Lindman J**, Szojka ZI, Månsson F, Biague A, Medstrand P, Norrgren H, Buggert M, Karlsson AC, Forsell MNE, Esbjörnsson J, Jansson M, the SCG. Hierarchical Clustering and Trajectory Analyses Reveal Viremia-Independent B-Cell Perturbations in HIV-2 Infection. *Cells*. 2022;11(19)

Palm AA, Veerla S, **Lindman J**, Isberg P-E, Johansson E, Biague A, Månsson F, Norrgren H, Esbjörnsson J, Medstrand P, Jansson M. Interferon Alpha-Inducible Protein 27 Expression Is Linked to Disease Severity in Chronic Infection of Both HIV-1 and HIV-2. *Frontiers in Virology*. 2022;2

Scharf L, Pedersen CB, Johansson E, **Lindman J**, Olsen Lars R, Buggert M, Wilhelmson S, Månsson F, Esbjörnsson J, Biague A, Medstrand P, Norrgren H, Karlsson AC, Jansson M, Group tSC. Inverted CD8 T-Cell Exhaustion and Co-Stimulation Marker Balance Differentiate Aviremic HIV-2-Infected From Seronegative Individuals. *Front Immunol*. 2021;12

Jespersen S, Mansson F, **Lindman J**, Wejse C, Medina C, da Silva ZJ, Te D, Medstrand P, Esbjornsson J, Honge BL. HIV treatment in Guinea-Bissau: room for improvement and time for new treatment options. *AIDS Res Ther*. 2020;17(1):3

Esbjörnsson J, Månsson F, **Lindman J**, Rowland-Jones SL, Jansson M, Medstrand P, Norrgren H. New insights are game-changers in HIV-2 disease management – Authors' reply. *The Lancet HIV* 2019;6(4):e214-e215

Esbjörnsson J, Jansson M, Jespersen S, Månsson F, Hønge BL, **Lindman J**, Medina C, da Silva ZJ, Norrgren H, Medstrand P, Rowland-Jones SL, Wejse C. HIV-2 as a model to identify a functional HIV cure. *AIDS Res Ther*. 2019;16(1):24

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Lindman JL, Månsson F, Biague A, Da Silva ZJ, Andersson S, Norrgren H. Declining prevalence rates of syphilis among police officers in Guinea-Bissau, West Africa, 1990-2010. *Sexually transmitted diseases*. 2013;40(10):794-796.

Dunér S, **Lindman JL**, Ansari D, Gundewar C, Andersson R. Pancreatic Cancer: The Role of Pancreatic Stellate Cells in Tumor Progression. *Pancreatology*. 2011;10(6):673-681.

Abbreviations

AIDS	Acquired immunodeficiency syndrome
ART	Antiretroviral treatment
ATL	Adult T-cell leukemia/lymphoma
CCR	CC chemokine receptor
CD	Cluster of differentiation
CDC	United States Center for Disease Control
CRF	Circulating recombinant forms
CTA	Centro de tratamento ambulatório, the HIV clinic at Hospital National Simão Mendes
CXCR	CXC chemokine receptor
DNA	Deoxyribonucleic acid
DRM	Drug resistance mutations
DTG	Dolutegravir
FSW	Female sex workers
FTC	Emtricitabine
GALT	Gut-associated lymphoid tissue
gp	Glycoprotein
HAM/TSP	HTLV-1-associated myelopathy/tropical spastic paraparesis
HIV-1	Human immunodeficiency virus type 1
HIV-2	Human immunodeficiency virus type 2
HIVDR	HIV drug resistance
HR	Hazard ratio
HSNM	Hospital National Simão Mendes
HTLV-1	Human T-lymphotropic virus type 1

HTLV-2	Human T-lymphotropic virus type 2
INH	Isoniazid
INSTI	Integrase strand transfer inhibitor
IQR	Interquartile range
MSM	Men who have sex with men
NAAT	Nucleic acid amplification test
NK	Natural killer
NNRTI	Non-nucleoside reverse transcriptase inhibitor
OR	Odds ratio
PI	Protease inhibitors
PID	Pelvic inflammatory disease
POC	Point-of-care
PrEP	Pre-exposure prophylaxis
PRR	Pattern recognition receptor
PTMCT	Prevention of mother-to-child transmission
PVL	Proviral load
PWH	People with HIV
PY	Person years
RNA	Ribonucleic acid
RR	Relative risk
SIV	Simian immunodeficiency virus
STI	Sexually transmitted infections
TDF	Tenofovir disoproxil fumarate
TMA	Transcription-mediated amplification
UNAIDS	Joint United Nations Programme on HIV and AIDS
VL	Viral load
WHO	World Health Organization

Introduction

Although the studies presented in this thesis cover a broad range of topics, they are all centred on a common theme: human immunodeficiency virus (HIV). HIV remains one of the most significant global health challenges of the 21st century. Since its emergence in the early 1980s, an estimated 88 million people worldwide have acquired HIV, and over 40 million have died as a result of AIDS-related illnesses. By the end of 2023, an estimated 40 million people with HIV (PWH) were living globally, representing approximately 0.5% of the global population. Most of these individuals resided in sub-Saharan Africa (Figure 1) (1). Key populations, such as men who have sex with men (MSM), people who inject drugs, female sex workers (FSW), and transgender people, represent a significant share of new infections, particularly in regions where stigma, discrimination, and legal barriers limit access to prevention and treatment services.

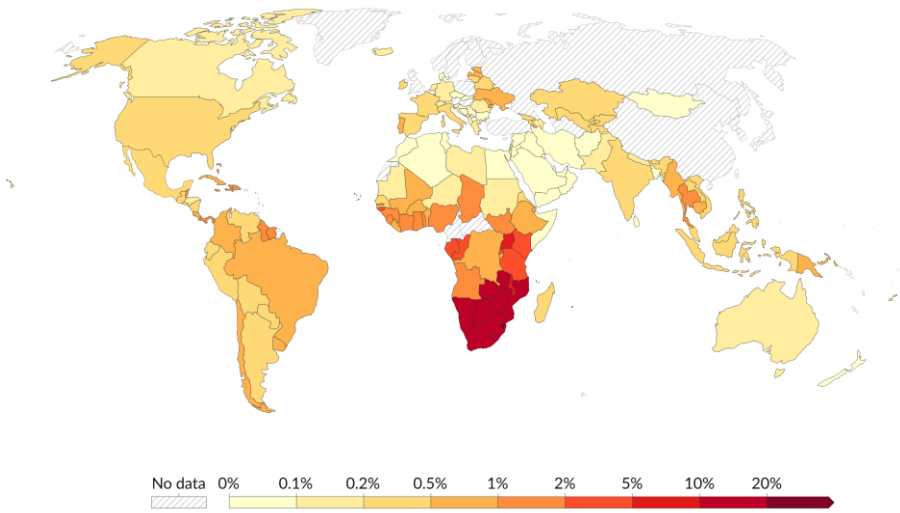

Figure 1. Global prevalence of HIV. Source: Joint United Nations Programme on HIV/AIDS (2024) – with minor processing by Our World in Data (2).

The global rollout of antiretroviral treatment (ART) over the past two decades has led to substantial public health gains. New HIV infections have decreased by 59%

since the peak in 1995, and AIDS-related deaths have declined by 69% since the peak in 2004 (3).

In 2014, the Joint United Nations Programme on HIV and AIDS (UNAIDS) launched the 90-90-90 targets, which aimed that by 2020: (i) 90% of all PWH would be diagnosed, (ii) 90% of those diagnosed would receive ART, and (iii) 90% of those on ART would achieve viral suppression (4). In 2021, UNAIDS updated these goals to the more ambitious 95-95-95 targets, to be achieved by 2025 (5).

Of the approximately 40 million PWH currently living worldwide, 86% are diagnosed, 89% of those diagnosed are on ART, and 93% of those on ART are virally suppressed (3). However, significant regional differences persist. In Guinea-Bissau for instance, between 2014 and 2016, only 19% of PWH were diagnosed, of these 49% received ART and 75% of those on ART achieved viral suppression (6). Sweden, on the other hand, reached the UNAIDS 90-90-90 targets by 2015 and achieved the 95-95-95 targets by 2022 (7, 8).

Thus, despite remarkable advances in medical research, ART and public health interventions, the virus persists as a major public health concern, particularly in low- and middle-income countries.

Discovery and global emergence of HIV

The first reports of acquired immunodeficiency syndrome (AIDS) appeared in 1981, when several cases of *Pneumocystis* pneumonia and Kaposi´s sarcoma were observed in previously healthy men in the United States (9-12). Both conditions represent rare opportunistic infections that are usually linked to profound immunosuppression. Although the first identified cases occurred among MSM, later findings revealed that other groups were also affected (13-15). By the end of 1981, the first case in Europe had been identified (16), and in 1982, the syndrome was named AIDS (17), although its underlying cause was still unknown at that time. The virus responsible for AIDS, later known as HIV, was discovered in 1983 by Luc Montagnier and Françoise Barré-Sinoussi at the Pasteur Institute in Paris (18), and by Robert Gallo's research group in the United States (19). Initially referred to as Lymphadenopathy-Associated Virus or Human T-lymphotropic Virus type III, the virus was officially renamed HIV type 1 (HIV-1) in 1986 (20), and established as the virus causing AIDS. In 1986, a second retrovirus was identified in individuals from West Africa and was named HIV type 2 (HIV-2) (21). Unlike HIV-1, which is responsible for the global pandemic, HIV-2 remains largely confined to West Africa and to countries with historical colonial ties to the region, such as Portugal and France (22, 23). HIV-2 is considered less transmissible and less pathogenic than HIV-1 (24, 25). Yet an estimated 1 to 2 million PWH-2 are currently living globally (26).

Reports of AIDS cases in Africa first appeared in 1984 and 1985, mainly from the Democratic Republic of Congo and Uganda (27, 28). However, retrospective analysis of samples collected in 1959 and 1960 in the Democratic Republic of Congo revealed that HIV had been circulating much earlier (29, 30). Eastern and Southern Africa have been the most affected regions, reporting the highest prevalence rates of HIV-1 infection. In contrast, West African countries have consistently reported significantly lower HIV-1 prevalence rates (31). For HIV-2, the first study conducted between 1985 and 1987 reported high prevalence levels in West African countries (32). Evidence from serological studies suggests that HIV-2 may have been present in human populations as early as 1966 (33), while phylogenetic analyses estimate its introduction into humans around 1935 (34, 35).

The origin of HIV

HIV-1 and HIV-2 originated from cross-species transmission of simian immunodeficiency viruses (SIVs) from primates to humans in West and Central Africa (36).

HIV-1 strains are divided into four different genetic groups: M (Main), N (Non-M/Non-O), O (Outlier), and P (Pending) (37, 38). Groups M and N are thought to have emerged from cross-species transmission of SIV from chimpanzees to humans, whereas groups O and P are believed to have originated from gorilla SIV (38). HIV-2, on the other hand, originates from multiple transmissions of SIV from sooty mangabeys to humans, resulting in HIV-2 groups A-I (39). Among these, only HIV-1 groups M and O, and HIV-2 groups A and B, have established wider transmission among humans (38).

HIV-1 group M likely originated from chimpanzees in south-eastern Cameroon and thereafter spread to Kinshasa in the Democratic Republic of Congo during the 1920s (30), where the virus began to circulate more widely by the 1960s (30, 40). Through extensive viral evolution, group M has diversified into nine major subtypes (A-D, F-H, J, and K) as well as numerous circulating recombinant forms (CRFs) (41). Subtype C is currently the most prevalent globally (46%), followed by subtype B (12%), subtype A (10%), CRF02_AG (8%), CRF01_AE (5%), subtype G (5%), and subtype D (3%), while subtypes F, H, J, and K each represent less than 1% (42).

HIV-2 groups A and B, the only groups known to have become endemic, likely originated from sooty mangabeys in the Taï Forest region of Côte d'Ivoire during the 1940s (34, 35, 43). HIV-2 group A accounts for over 90% of all HIV-2 infections. Other HIV-2 groups, have been identified only in isolated cases (43). As with HIV-1 group M, HIV-2 prevalence appears to have grown rapidly in the 1960s (34). Phylogenetic studies have indicated that Guinea-Bissau may have played a central role in the regional spread of HIV-2 across West Africa (43, 44).

HIV epidemiology among female sex workers in Africa

FSWs play a key role in the transmission of HIV in sub-Saharan Africa and are at a high risk of acquiring HIV infection (45). Several systematic reviews and meta-analyses have reported a substantially higher HIV prevalence among FSWs in the region compared to the general population (45-47). It has been estimated that FSWs represent approximately 1% of women aged 15-49 years in sub-Saharan Africa, but account for approximately 6% of women with HIV in the region (47). The increased risk of HIV acquisitions is due to a combination of biological and sociostructural factors, including biological vulnerability, stigma and criminalisation (48).

A recent meta-analysis found that HIV incidence among FSWs was nearly eight times higher than in the general female population in sub-Saharan Africa. The difference was even greater in Western and Central Africa, where incidence among FSWs compared to the general female population was approximately 20 times higher, compared to around five times higher in Eastern and Southern Africa. Interestingly, the same study reported that the median incidence was lower in Western and Central Africa compared to Eastern and Southern Africa (49). This pattern is likely explained by the concentration of HIV transmission within key populations, such as FSWs, in Western and Central Africa, resulting in much higher incidence rates among these groups compared to the general population (50). Between 1985 and 2020, HIV incidence among FSWs declined at a rate similar to that observed among matched women in the general population (49). Supporting these findings, other studies from Western and Central Africa have also reported that the proportion of new HIV infections attributable to commercial sex has decreased over time (51, 52). It has been suggested that initial reductions in incidence among FSWs were driven by the implementation of prevention programmes and health services specifically targeting this group (53), whereas more recent declines likely reflect the impact of HIV treatment as prevention strategies (54).

In sub-Saharan Africa, HIV prevention programmes targeting FSWs were a key part of the early HIV response. However, from the early 2000s, reduced funding and a strategic shift toward broader population-level interventions led to a decline in programmes specifically addressing the needs of FSWs (53). In recent years, renewed focus on key population-specific strategies has contributed to the expansion of targeted services for FSWs in sub-Saharan Africa (55).

In summary, while HIV incidence and prevalence among FSWs in sub-Saharan Africa have declined over time, they remain substantially higher than in the general female population. Continued efforts to expand targeted prevention and treatment

programmes are essential to further reduce the disproportionate burden of HIV within this key population.

HIV in Guinea-Bissau

The first study investigating HIV-2 prevalence in Guinea-Bissau was performed using blood samples collected between 1985 and 1987 across various groups in six West African countries. In Guinea-Bissau, the HIV-2 prevalence among the control population (including healthy individuals, pregnant women, prisoners and hospital patients) was 9%, while among FSWs it was 64%. HIV-1 prevalence at that time was 0% (32). Later studies from the late 1980s and early 1990s confirmed these findings, reporting HIV-2 prevalence rates of 8-10%, the highest documented worldwide, and reporting no or only a few cases of HIV-1 infection (56-61). Following these initial reports, HIV-2 prevalence steadily declined, whereas HIV-1 prevalence increased and surpassed HIV-2 prevalence after the civil war in 1998-1999, before eventually reaching a plateau (62-69). The most recent study, conducted between 2014 and 2016, confirmed this trend, reporting an HIV-1 prevalence of 4%, an HIV-2 prevalence of 3% and a prevalence of HIV-1/2 dual infection of 0.1% (69). The HIV-1 epidemic in Guinea-Bissau is dominated by the recombinant form CRF02_AG, sub-subtype A3 and an A3/CFR02_AG recombinant form (70, 71).

Modes of HIV transmission and key population in Guinea-Bissau

HIV-1 and HIV-2 are primarily transmitted through sexual contact, exposure to infected blood, or vertically (during pregnancy, delivery or breast-feeding). In sub-Saharan Africa, heterosexual transmission is the most common mode of HIV spread, followed by vertical (mother-to-child) transmission (72). In comparison to HIV-1, HIV-2 has consistently been demonstrated to have lower rates of transmission both through sexual contact and vertically (73, 74).

Control measures for blood transfusions have been in place in Guinea-Bissau since 1987 (63). In 2002, the first programme for the prevention of mother-to-child transmission (PTMCT) was introduced in Bissau, using a nevirapine-based regimen and implemented by a non-governmental organization (75). Access to PTMCT services was further strengthened when the national ART programme was initiated in 2006, particularly in the capital, Bissau. However, the coverage has remained incomplete; a cross-sectional study conducted between 2008 and 2013 at the maternity ward of Hospital National Simão Mendes (HNSM) reported that 15% of mothers with HIV and 22% of infants born to mothers with HIV did not receive ART as part of PTMCT (76).

The spread of HIV-2 in Guinea-Bissau is believed to have coincided with the prolonged war of liberation against Portuguese colonial rule between 1963 and 1974 (34, 77). Evidence suggests that during this period, parenteral transmission rather than sexual transmission may have been the primary mode of spread, potentially linked to medical procedures or female genital cutting (34, 58, 77, 78). Previous studies suggest that the subsequent decline in HIV-2 prevalence observed in Guinea-Bissau over the past few decades has largely been driven by changes in risk behaviours (43, 79, 80). However, during this same period, HIV-1 began to spread within the population and is thought to compete with HIV-2, primarily as a result of excess mortality among key populations transmitting both viruses (79, 80). Because of the lower efficiency of HIV-2 transmission through sexual contact and mother-to-child transmission, mainly attributed to its lower viral load (VL) (73, 81, 82), it is believed that HIV-2 will continue to decline and may eventually disappear (83). HIV-1 is thought to have been introduced into Guinea-Bissau during the late 1970s, likely as a result of migration patterns that followed the conclusion of the war of independence in 1974 (71). Phylogeographic analyses suggest that the initial introduction of HIV-1 occurred in the capital, Bissau, before spreading to rural regions (71).

As mentioned above, FSWs play a key role in the transmission of HIV in sub-Saharan Africa and are at a heightened risk of acquiring HIV infection. In Guinea-Bissau, sex work is not legally specified, meaning it is neither criminalized nor legalized (84). In 2020, the number of FSW in four of the five largest cities in Guinea-Bissau (Bissau, Gabú, Bafatá and Bissorã) was estimated at 7,900 (85). A recent study explored the relationship between mobility and HIV-related vulnerabilities among FSWs in the country (86). Mobility has been identified as a potential risk factor for HIV infection, particularly due to its potential association with intermittent access to prevention and care services (87). Findings indicated that mobility was associated with both condomless sex with clients and non-paying partners, as well as increased likelihood of receiving HIV prevention information and engaging with HIV-related organizations, suggesting that mobility may have complex and heterogeneous effects within this population (86). Another study found that early initiation into sex work was common, with 26% of adult FSWs in Guinea-Bissau having started selling sex before the age of 18 (85). However, prior to our investigations, little information was available regarding HIV prevalence and access to ART among this population, providing the rationale for the study presented in Paper II.

In addition to FSWs, MSM represent another key population in Africa with a higher risk of acquiring HIV compared to the general population (88). As with sex work, homosexuality is also not legally specified in Guinea-Bissau although high levels of stigma contribute to widespread secrecy. According to personal communication with Kátia Ribeiro Barreto, Program Director of ENDA Guinea-Bissau, a 2023 survey found an HIV prevalence of 3% among 542 MSMs in Guinea-Bissau. These

findings warrant further studies to investigate the reasons behind the observed low HIV prevalence among MSM in this setting.

Although often overlooked people who use drugs represent another emerging key population in Guinea-Bissau. West Africa and Guinea-Bissau serves as a major transit point for drug trafficking. Significant amounts of drugs, primarily cocaine, are trafficked through Guinea-Bissau towards Europe. In addition but to a lesser extent, heroin and methamphetamine are trafficked through West Africa towards other regions in Africa and the United States (89). A recent study investigating the prevalence of blood borne infections and drug use practises among 750 participants reported an HIV-1 prevalence ranging between 2% and 5%, and a prevalence of ever injecting between 6% to 44% (90).

Virology

HIV structure and genome

HIV-1 and HIV-2 are part of the *Retroviridae* family and the *Lentivirinae* genus (91). The mature HIV virion particle is approximately 100 nm in diameter (92). The viral envelope is derived from the host cell membrane during the budding of new viral particles (93). The viral envelope also contains viral envelope glycoproteins which consist of gp120/gp125 (surface subunits) and gp41/gp36 (transmembrane subunits) in HIV-1 and HIV-2, respectively (94). Beneath the envelope lies the matrix protein, which surrounds the conical capsid composed of p24 protein in HIV-1 and p26 protein in HIV-2. Enclosed in the capsid are two copies of single-stranded positive-sense RNA along with viral enzymes (Reverse Transcriptase, Integrase and Protease) (95).

The HIV-1 genome comprises approximately 9.700 base pairs while the HIV-2 genome comprises approximately 10.500 base pairs (94). The HIV genome encodes three structural genes (gag, pol and env) as well as six accessory genes: tat, rev, vpr, neg, nif, and vpu in HIV-1 or vpx in HIV-2 (96). The *gag* gene encodes the capsid proteins (p24 for HIV-1 and p26 for HIV-2); the *pol* gene encodes the viral enzymes protease, reverse transcriptase, and integrase, and the *env* gene encodes the envelope glycoproteins (gp120 and gp41 in HIV-1, and gp125 and gp36 in HIV-2) (97).

HIV replication cycle

HIV primarily targets immune cells expressing the CD4 receptor, mainly T helper lymphocytes, macrophages and dendritic cells (98, 99). The replication cycle is initiated by the binding of viral envelope glycoproteins and the CD4 receptor, and

a co-receptor, most commonly CCR5 or CXCR4 in HIV-1. In contrast to HIV-1, HIV-2 appears to use a broader range of co-receptors (100).

Binding to the CD4 receptor is facilitated by gp120, whereas membrane fusion is driven by the fusion peptide within gp41 (101). Following receptor binding, the viral envelope fuses with the host cell membrane, allowing the viral capsid to enter the cytoplasm. Once inside, the capsid dissembles, traditionally the disassembly has been believed to occur in the cytoplasm, but recent evidence suggests that this process might occur in the nucleus or at nuclear pores (102). Then the viral RNA is reverse-transcribed into complementary DNA by the viral reverse transcriptase enzyme. The high mutation rate of HIV and frequent recombination during reverse transcription, when the viral reverse transcriptase switches between two non-identical RNA strands packaged in the same virion, are major contributors of the genetic diversity and the high evolutionary rate of HIV (103, 104). These mechanisms also promote the emergence of drug resistance mutations (DRMs) as well as immune evasion. After reverse transcription complementary DNA is transported to the nucleus and integrated into the host genome by the viral integrase enzyme, forming the provirus (105).

Although HIV can enter both resting and activated cells, transcription of the proviral genome depends on the host´s cellular machinery and generally occurs only after the infected cell is activated. Once transcription is initiated, newly produced viral proteins and RNA genomes accumulate in the cytoplasm and are assembled at the cell membrane, where new viral particles bud from the host cell. Maturation of the viral particle, mediated by the viral protease, results in the mature viral particle (106).

HIV-1 and HIV-2 pathogenesis

HIV infection leads to a progressive loss of $CD4^+$ T cells, resulting in gradual deterioration of the immune system and increased susceptibility to opportunistic infections (107). The clinical course of HIV infection is typically divided into three different phases: the acute phase, the asymptomatic or chronic phase and the AIDS phase. Figure 2 presents each phase and their associated immunological and virological characteristics.

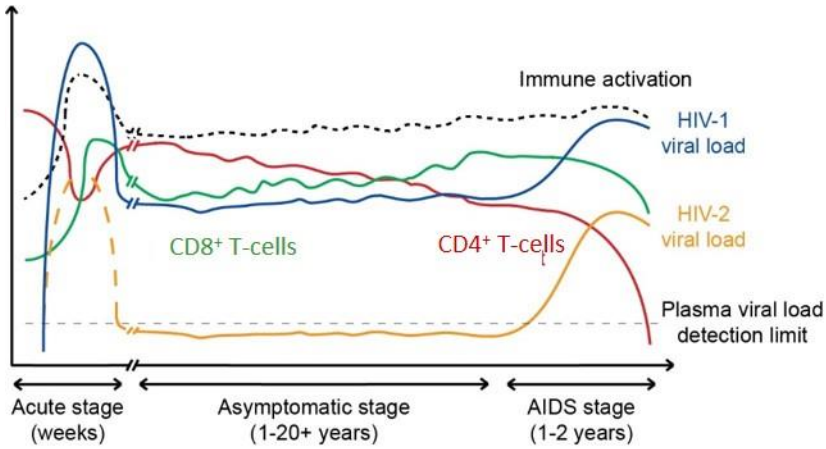

Figure 2. Illustration of general HIV disease progression. Due to the lack of studies on plasma viral load dynamics during acute HIV-2 infection, the trajectory is represented hypothetically using a dashed line (108). Reprinted with permission.

The acute phase

Following sexual transmission which is the predominant mode of HIV transmission globally (109), the first cells to be infected are believed to be $CD4^+$ T cells at the site of HIV entry or neighbouring lymphoid tissue.

Initial HIV replication occurs locally, during a period known as the eclipse phase, when plasma VL remains undetectable. This phase typically lasts 6-10 days (110, 111). The virus then disseminates systemically, with replication becoming established in secondary lymphoid tissues, particularly the gut-associated lymphoid tissue (GALT), which contains a high number of activated $CD4^+$ T cells. Extensive viral replication in the GALT leads to a rapid and permanent depletion of up to 80% of the $CD4^+$ T cells in the GALT (112-114). Following the eclipse phase, plasma VL increases sharply, reaching peak levels before declining as the adaptive immune response develops. This decline results in a steady-state level of viraemia, referred to as the viral set point, which coincides with a partial recovery of $CD4^+$ T cell counts (113, 114). The level of the viral set point has been shown to correlate with the rate of disease progression (115-118).

The acute phase, sometimes referred to as primary HIV infection, can be associated with a mononucleosis-like illness featuring self-limiting symptoms such as fever, rash, myalgias, and headache (119).

The asymptomatic phase

The duration of the asymptomatic phase of HIV infection varies considerably between individuals, ranging from less than one year to over 20 years. The rate of disease progression during the asymptomatic phase is affected by a combination of host and viral factors (120). Although individuals typically remain free of clinical symptoms, this phase is characterized by a gradual decline in CD4+ T cell counts, driven by ongoing viral replication and chronic immune activation (121). Plasma VL remains relatively stable, although a slow increase is generally observed to occur over time (118). Persistent HIV replication results in exhaustion of HIV-specific immune cells, while chronic inflammation contributes to a generalized dysfunction and exhaustion of both B cells and T cells (122, 123). The combined effect of immune cell exhaustion and progressive $CD4^+$ T cell loss leads to immune system collapse, resulting in increased susceptibility to opportunistic infections, marking the transition to the AIDS phase of the disease.

We have shown that the asymptomatic phase is approximately twice as long among PWH-2 compared to PWH-1 (124). In HIV-2 infection, the viral set point has been reported to be associated with disease progression (82), but others have found that general immune activation were associated with disease progression rather than plasma VL (125, 126). Moreover, progression to immunodeficiency has been observed in the absence of detectable viraemia (127), suggesting that factors beyond viral replication contribute to disease progression in HIV-2 infection.

Additionally, HIV-1 disease progression appears to be slower in individuals living with both HIV-1 and HIV-2, particularly when HIV-2 acquisition precedes HIV-1. Progression to AIDS and HIV-related death has been reported to occur approximately 50% more slowly in those with prior HIV-2 acquisition compared to PWH-1 alone (128, 129). Subsequent studies have proposed that the attenuation of HIV-1 disease progression by prior HIV-2 infection may be mediated by cross-reactive T cell responses (130, 131), cross-reactive antibodies (132, 133), the HIV-2-mediated release of β-chemokines that block CCR5 receptor binding (134, 135), along with interference in intracellular HIV-1 replication processes within co-infected cells (136, 137).

The AIDS phase

The progressive loss of $CD4^+$ T cells, together with chronic immune activation, leads to immune system exhaustion and a reduced capacity to control the HIV infection (138). This is accompanied by an increase in plasma VL, which further accelerates CD4+ T cell depletion and leads to increased susceptibility to opportunistic infections (139). The clinical manifestations in HIV-1 and HIV-2 infection are largely similar in individuals reaching AIDS, although Kaposi's sarcoma seem to be less common in PWH-2 (140, 141). We and others have also

reported that the onset of AIDS tends to occur at higher $CD4^+$ T cell counts in PWH-2 compared to PWH-1 (124, 140).

Both the United States Center for Disease Control and Prevention (CDC) and the World Health Organization (WHO) define AIDS using clinical and immunological criteria, although their frameworks differ slightly. According to the CDC, PWH is diagnosed with AIDS if they have a $CD4^+$ T cell count below 200 cells/ μl or present with one or more AIDS-defining illnesses (142). WHO initially developed a clinical staging system in the early 1990s that was revised in 2007, which classified HIV infection into four stages based only on clinical signs and symptoms (143). The WHO clinical stages reflect disease progression from stage 1 (asymptomatic/post-acute), to stage 2 (chronic with mild symptoms), stage 3 (symptomatic with advanced disease) and stage 4 (AIDS defining condition, Table 1). However, in more recent guidelines, the WHO has incorporated $CD4^+$ T cell counts into the definition of advanced HIV disease (144). Advanced HIV disease is defined as either a $CD4^+$ T cell count below 200 cells/μl or the presence of WHO stage 3 or 4.

Table 1. The World Health Organization classification of AIDS defining symptoms, WHO stage 4.

HIV wasting syndrome unexplained involuntary weight loss >10% of baseline (or BMI <18.5) plus either chronic diarrhoea (three or more loose stools per day for >1 month) or persistent fever/night sweats (>1 month) unresponsive to antibiotics or antimalarials (malaria must be excluded in endemic areas).
Pneumocystis pneumonia
Recurrent bacterial pneumonia
Chronic herpes simplex infetion (orolabial, genital or anorectal for >1 month or visceral at any site
Esophageal candidiasis (or candidiasis of trachea, bronchi or lungs)
Extrapulmonary tuberculosis
Kaposis sarcoma
Cytomegalovirus infection (retinitis or infection of other organs)
Central nervous system toxoplasmosis
HIV encephalopathy
Extrapulmonary cryptococcosis including menigitis
Disseminated non-tuberculous mycobacterial infection
Progressive multifocal leukoencephalopathy
Chronic cryptosporidiosis (with diarrhea)
Chronic isosporiasis
Disseminated mycosis (histoplasmosis, coccidiomycosis, penicilliosis)
Recurrent non-typhoidal Salmonella bacteraemia
Lymphoma (cerebral or B-cell non-Hodgkin) or other sold HIV-associated tumours
Invasive cervical carcinoma
Atypical disseminated leishmaniasis
Symptomatic HIV-associated nephropathy
Symptomatic HIV-associated cardiomyopathy
Reactivation of American trypanosomiasis (meningoencephalitis and/or myocarditis)

HIV and the immune system

The immune system is typically divided into the innate and adaptive parts. The innate response relies on germline-encoded pattern recognition receptors (PRRs) that detect pathogen-associated molecular patterns for example HIV DNA and initiate inflammation, while in the adaptive immune system, B and T cell receptor diversity is generated through somatic recombination (145). Upon HIV infection, both parts of the immune system are activated but eventually dysregulated due to persistent viral replication and antigen exposure (107).

Innate immunity

The innate immune system includes physical barriers, antimicrobial peptides and restriction factors which inhibit viral replication (146, 147). Following PRR engagement, cytokine and chemokine release promotes inflammation (148). Cells such as dendritic cells, macrophages, neutrophils, and NK cells play important roles in early viral control (149). NK cells, important for killing infected cells via cytotoxic molecules or antibody-dependent cellular cytotoxicity, are modulated by MHC class I interactions (150). Enhanced antibody-dependent cellular cytotoxicity, and specific HLA-C alleles have been linked to protection from HIV acquisition (151, 152). NK cells may also exhibit memory-like features similar to the adaptive immune response (153), although their contribution to HIV control in vivo is unknown.

Adaptive immunity

Adaptive immunity is driven by B and T cells in HIV infection (145). $CD8^+$ T cells target infected cells but become progressively exhausted during chronic infection, characterized by upregulation of inhibitory receptors and impaired capacity for differentiation into long-lived memory cells (154). HIV-2 tends to induce a broader, more polyfunctional $CD8^+$ T cell response, which potentially contributes to slower disease progression seen in HIV-2 (155). $CD4^+$ T cells, the main targets of HIV, play a central role in immune coordination. Like $CD8^+$ T cells they also become exhausted during HIV infection and the progressive depletion leads to immune collapse (122).

B cell responses are also impaired in chronic HIV infection. During the acute phase, B cells primarily differentiate into short-lived plasmablasts within secondary lymphoid tissues, producing low-affinity antibodies. High-affinity antibody production develop later through germinal center maturation, a process that depends on the support of follicular dendritic cells and follicular helper T cells derived from $CD4^+$ T cells (156). In HIV infection, follicular helper T cells become functionally

exhausted and provide inadequate help to B cells during affinity maturation, thereby hindering the development of neutralizing antibodies (157). Persistent antigenic stimulation and proinflammatory cytokines releases drives B-cell exhaustion and dysfunction (123). Compared to HIV-1, the antibody response in HIV-2 infection has been reported to be broader and more potent (132, 158-160).

Laboratory diagnosis of HIV

HIV diagnosis relies primarily on immunoassays that target either the p24 antigen, anti-HIV antibodies or both. Traditionally, these assays have been classified by "generation," but a more recent framework categorizes them according to their analytic targets (IgG-sensitive, IgM-sensitive, or combined antigen/antibody detection) and testing environment, distinguishing between laboratory-based and point-of-care (POC) tests (161). A major innovation in HIV testing has been the decentralization of diagnostics through the development of HIV rapid tests, which can now be used either at the POC by healthcare providers or by individuals in home-based settings through self-testing. These tests provide results within 30 minutes and are compatible with various sample types, including whole blood and oral fluid (162, 163).

Modern rapid HIV tests demonstrate diagnostic performance comparable with third-generation laboratory assays (164). However, despite including components for p24 antigen detection, their sensitivity is typically lower than that of fourth-generation laboratory-based assays, especially during acute infection (165). All reactive results should also be confirmed with supplemental diagnostic testing. Rapid diagnostic testing has played an important role in increasing access to HIV screening, especially in low-resource settings. Here, testing algorithms involving three sequential rapid tests, are widely implemented and recommended by the WHO. Additionally, the WHO has recommended discontinuing the use of Western blot and line immunoassays in favor of HIV rapid tests (166). CDC recommends an algorithm that begins with an antigen/antibody combination screening test, followed by a confirmatory immunoassay that differentiates HIV-1 from HIV-2 antibodies (e.g., Geenius HIV-1/2 assay). If results are non-reactive or indeterminate, HIV-1 nucleic acid amplification tests (NAATs) is used as a final step (167). Dried blood spots and self-testing have also further improved accessibility, especially in remote regions and settings with limited laboratory infrastructure (168).

NAATs, such as RT-PCR and transcription-mediated amplification (TMA), are used to detect HIV RNA or proviral DNA and are considered the gold standard for measuring HIV VL and are important tools for confirming acute infection (161). The diagnostic window includes the initial “eclipse” phase, lasting approximately 7 to 21 days, during which the virus establishes infection at the site of exposure and

remains undetectable in systemic circulation. The "window period" refers to the interval between HIV exposure and the first reliable detection of viral or immunologic markers (161). Fiebig et al. defined six laboratory stages of HIV seroconversion, from RNA detection alone (Stage I) to the appearance of p24 antigen (Stage II), followed by various phases of antibody development and Western blot reactivity (Stages III-VI) (169).

While these staging criteria are primarily applicable to HIV-1, HIV-2 diagnosis presents additional challenges, due to serologic cross-reactivity. Many screening assays fail to discriminate HIV-1 from HIV-2 antibodies, and Western blot bands can yield false-positive or indeterminate HIV-1 results in HIV-2-positive individuals (170). As previously described, Guinea-Bissau has had the highest HIV-2 prevalence rates globally, but over the last 30 years, HIV-2 prevalence has steadily declined while HIV-1 prevalence has increased. Consequently, both viruses co-circulate in the population, resulting in a proportion of individuals acquiring HIV-1/2 dual infection.

In individuals with suspected HIV-1/2 dual infection, HIV-1 can often be confirmed by NAAT, whereas HIV-2 confirmation is more difficult due to low or undetectable plasma VLs (171) and, in some cases, undetectable proviral HIV-2 DNA (172). Despite these limitation NAAT remains the gold standard for confirming HIV-1/2 dual infection. However, HIV-2 NAATs remain largely unavailable in many low-resource settings, in contrast to HIV-1 NAATs, which have become increasingly more available. As a result HIV type discrimination still often relies on rapid diagnostic tests (173). In Guinea-Bissau, quantitative HIV-1 RNA testing for VL measurement was implemented in January 2017 at the National Public Health Laboratory (LNSP) in Bissau, but HIV-2 NAATs have yet to be implemented.

Commercially available HIV-2 RNA assays for VL measurements have historically been unavailable, leading many reference laboratories to use in-house methods for measuring HIV-2 RNA levels (174). However, a recent evaluation has demonstrated that certain commercially available assays now offer reliable options for HIV-2 RNA quantification thereby improving diagnostic availability (175). Additionally, the Alere q HIV-1/2 Detect qualitative test, which was recently renamed to m-PIMA q HIV-1/2 Detect qualitative test has demonstrated promise as a POC test for detecting HIV-1 RNA and HIV-2 RNA in low-resource settings (176).

Misclassification of HIV-2 as HIV-1 can have implications for the clinical management of HIV, as standard HIV-1 VL assays do not detect HIV-2 (168). This type of misclassification could also result in suboptimal treatment and can limit second-line options in the event of treatment failure (177). This is especially relevant in resource-limited settings like Guinea-Bissau, where the availability of second-line treatment options is limited. Fortunately misclassification of HIV-2 as HIV-1 is rare. A more common error is the misdiagnosis of HIV-2 as HIV-1/2 dual infection. While this do not result in suboptimal treatment, it can still have clinical

implications, as individuals with HIV-1/2 dual infection progress to AIDS more rapidly than those with HIV-2 mono-infection (124, 128).

In Guinea-Bissau, Determine HIV-1/2 assay (Abbott Laboratories, Tokyo, Japan) has been the primary test for HIV screening. For confirmation and differentiation between HIV-1, HIV-2 and HIV-1/2 dual infection, several different HIV rapid tests have been utilized, including SD Bioline HIV-1/2 3.0 (Standard diagnostics Inc, Kyonggi-do, South Korea), First Response HIV Card 1-2.0 (Premier Medical Corporation Private Limited, Kachigam, India), Genie III HIV-1/HIV-2 (Bio-Rad, Steenvorde, France) and MULTISURE HIV Rapid Test (MULTISURE HIV, MP Diagnostics, CA, USA). While these rapid HIV tests generally demonstrate high sensitivity and specificity for detecting HIV, their ability to accurately discriminate between HIV types has been suboptimal (178-182).

ImmunoComb HIV-1/2 Bispot (Orgenics, Yavne, Israel) (62, 66, 183, 184) and INNO-LIA HIV I/II Score (Fujirebio, Ghent, Belgium) (185, 186) have also been widely used as serological assays for confirming HIV infection and distinguishing between HIV-1 and HIV-2. However, following the discontinuation of ImmunoComb production, there was a need to assess an alternative strategy for HIV confirmation and HIV-type discrimination. The Geenius HIV-1/2 Confirmatory Assay (Bio-Rad, Marnes-al-Coquette, France) was launched in 2013. Reported sensitivities for detecting HIV-1 infection range from 92% to 100% (185, 187-194), while for HIV-2, sensitivity varied between 85% and 100% (185, 187-189, 191-195). The Geenius assay can be interpreted either by visual reading or with the use of a dedicated Geenius Reader. Visual reading of the Geenius assay has been associated with an overestimation of HIV-1/2 dual infections due to cross-reactivity between HIV-2 antibodies and HIV-1 antigens. In contrast, the use of the Geenius Reader has shown better accuracy in discriminating between HIV-1 and HIV-2 infections (185, 188, 193, 196). However, previous evaluations have included none or only a limited number of samples from individuals with HIV-1/2 dual infection, which provided the rationale for the study presented in Paper I.

HIV prevention and treatment

Pre-exposure prophylaxis

Oral pre-exposure prophylaxis (PrEP) using the nucleoside reverse transcriptase inhibitor (NRTI) tenofovir disoproxil fumarate (TDF), has demonstrated high efficacy in preventing HIV infection, particularly among MSM. In randomised clinical trials, TDF-based PrEP has reduced the risk of HIV infection in MSMs by up to 92% in individuals with detectable drug levels (197-199), though effectiveness in real-world settings is typically somewhat lower (200). Among cisgender women,

PrEP has shown inconsistent effectiveness, largely due to challenges with adherence and acceptability (201, 202). Nevertheless, a pooled analysis of 11 studies involving emtricitabine (FTC) and TDF PrEP found very low HIV incidence among women with high adherence (defined as four to six doses per week) (203).

Other delivery methods for PrEP have also been explored with mixed results. Daily application of tenofovir-based topical gels showed limited success (204) and monthly vaginal rings containing the non-nucleoside reverse transcriptase inhibitor (NNRTI) dapivirine demonstrated only modest efficacy in a placebo-controlled trial (205). Despite this, WHO has recommended dapivirine rings as a potential PrEP option for use in low-resource settings (206). An alternative approach, known as "on-demand" or event-driven PrEP, where TDF-FTC is taken as two pills 2-24h before sex, followed by one pill 24h later and another 48h after the initial dose, has also shown efficacy in the MSM population (198).

The development of long-acting injectable PrEP represents a significant advancement. The integrase strand transfer inhibitor (INSTI) cabotegravir, administered every 8 weeks, has demonstrated superior efficacy compared to daily TDF-FTC, reducing HIV-incidence by 66% among MSM and by 89% among cisgender women (207, 208). A potential drawback is that cases of HIV infection while on cabotegravir PrEP have often involved resistance to integrase (209), and the high costs of the drug may limit its accessibility. Nonetheless, WHO has included long-acting cabotegravir as an option for PrEP in its updated PrEP recommendations for low-resource settings (206). The capsid inhibitor lenacapavir is an even longer-acting PrEP option, administered twice-yearly. In a recent trial conducted in South Africa and Uganda, lenacapavir demonstrated superior efficacy compared to daily TDF-FTC, with no incident HIV infections reported among participants receiving lencapavir. In contrast, HIV incidence among participants receiving TDF-FTC was comparable to background rates (210).

Several additional PrEP strategies are currently under clinical evaluation, including subdermal implants of cabotegravir or tenofovir alafenamide, a rectal douche formulation of tenofovir, and a dual-compound insert (tenofovir alafenamide and elvitegravir) designed for vaginal or rectal use (211).

Preventative vaccines

Despite significant investments in research and clinical trials, no effective HIV vaccine is currently available. An adenovirus vector-based candidate not only failed to offer protection but was associated with an increased risk of HIV acquisition (212). Another strategy using a recombinant canarypox vector boosted with envelope protein demonstrated 26% efficacy, though the protective effect was short-lived (213). A modified version of this vaccine specific for HIV subtype C induced immunogenicity (214) but did not prevent HIV infection (215). Due to the limited

success of earlier vaccine targeting T cell responses or non-neutralizing antibodies, current strategies are focused on generating broadly neutralizing antibodies (211).

Antiretroviral therapy

The first antiretroviral drug effective against HIV was introduced in 1987. However, it was the introduction of combination ART in 1996 that marked a turning point in HIV treatment, enabling sustained viral suppression and offering a high genetic barrier to resistance (216, 217). Initially, ART initiation was recommended for PWH with $CD4^+$ T cell counts ≤200 cells/μL. Between 2006 and 2010, this threshold was raised to ≤350 cells/μL, and subsequently to ≤500 cells/μL between 2009 and 2013 (218). The final shift occurred between 2012 and 2015, when universal treatment was recommended for all PWH regardless of $CD4^+$ T cell count, a change supported by evidence from three major randomized clinical trials (219-221). Several classes of ART have been developed, including NRTIs, NNRTIs, protease inhibitors (PIs), INSTIs, entry inhibitors and capsid inhibitors. Since 2019, WHO has recommended a dolutegravir (DTG)-based regimen as the preferred first-line treatment for all adult PWH (222), based on clinical trials demonstrating greater efficacy, improved tolerability and a higher barrier to resistance compared to NNRTI-based regimens (223, 224). This has led to a more simplified approach to HIV treatment, especially beneficial in settings like Guinea-Bissau where both HIV-1 and HIV-2 can be managed with the same first-line regimen. Due to the difficulties associated with maintaining daily medication adherence, ongoing research is increasingly directed toward the development of long-acting and extended-release treatment strategies. Recent advances include a two-drug combination of cabotegravir and the NNRTI rilpivirine given intramuscular every 1-2 months that has been approved for treatment in PWH-1 with viral suppression (225).

HIV-2 treatment depends entirely on the use of antiretroviral drugs originally developed for HIV-1, as no therapies have been specifically designed for HIV-2 (177). However, HIV-2 is intrinsically resistant to NNRTIs (43), the fusion inhibitor enfuvirtide (226), some PIs (nelfinavir and atazanavir) (227) and the attachment inhibitor fostemsavir (228, 229). Emerging data also demonstrated lower efficacy of the capsid inhibitor lenacapavir against HIV-2 in comparison to HIV-1 (230). Moreover, HIV-2 appears to develop antiretroviral drug resistance more rapidly than HIV-1, further complicating treatment efforts (231). PWH-2 have also been shown to experience slower $CD4^+$ T cell recovery following antiretroviral therapy compared to PWH-1, particularly among individuals who initiate treatment with $CD4^+$ T cell counts below 200 cells/μL (232, 233). Until recently, few studies had evaluated ART specifically for HIV-2, and no randomized trial data were available to guide clinical decision-making. As a result, existing treatment guidelines have been based largely on laboratory data and small clinical studies (177). The first randomized trial of HIV-2 treatment, conducted in four West African countries,

evaluated three ART regimens in treatment-naive adults with $CD4^+$ T cell counts >200 cells/μL (234). All participants received two NRTIs (TDF plus lamivudine or FTC), combined with either zidovudine, lopinavir/ritonavir, or raltegravir. The triple NRTI arm was discontinued early due to adverse events and virological failure. At 96 weeks, treatment success (defined as viral suppression and $CD4^+$ T cell count increase) was achieved in 57% and 59% of participants in the protease inhibitor and integrase inhibitor arms, respectively. However, assessment was complicated by the high proportion of participants with undetectable VL at baseline and by the failure of many to reach the prespecified increase in $CD4^+$ T cell count. Additional clinical studies are needed to ensure that PWH-2 have equal access to evidence-based treatment options comparable to those available for HIV-1. For instance, current long-acting therapies, as the one described above, cannot be used in HIV-2 treatment.

Guinea-Bissau launched its national ART programme in 2005. By the end of 2022 the Bissau HIV Cohort, which includes individuals from the country´s nine largest HIV clinics and covering approximately 90% of all registered cases, had enrolled 34929 PWH (235). However, ongoing political instability continues to affect the entire health system (236). The national ART programme has historically faced continuous challenges, including poor treatment adherence, recurrent stock-outs of ART and laboratory supplies, and high rates of loss to follow-up (237-240). Moreover, many PWH are diagnosed late, with around half presenting with advanced HIV disease at diagnosis (241).

Global targets for ending the HIV epidemic

The 90-90-90 targets were introduced by UNAIDS in 2014 as a global strategy to end the AIDS epidemic. The goal was that by 2020, 90% of all PWH would know their HIV status, 90% of those diagnosed would receive sustained ART, and 90% of those on ART would achieve viral suppression. This translates to 73% of all PWH achieving viral suppression. In 2020, UNAIDS updated these goals to the more ambitious 95-95-95 targets for 2025, aiming for 95% diagnosed, 95% on treatment, and 95% virally suppressed, corresponding to approximately 86% of all PWH being virally suppressed (5).

In 2018, regional progress in West and Central Africa was reported as 64%-51%-39% (242). However, significant data gaps persist due to missing information from several countries. Accurate assessments of the HIV care continuum across a country´s entire population of PWH is possible in only a few countries, such as Sweden, due to a National Quality Registry (InfCareHIV) (7). In many countries, estimates are based on population-based surveys that use diverse methodologies, which can affect the accuracy and comparability of the data. As mentioned in the introduction, a study conducted in Bissau between 2014 and 2016 reported that only

27% of all PWH-1 were virologically suppressed (6). This estimate highlights the challenges of achieving the 95-95-95 targets in some low-resource settings.

HIV drug resistance

HIV is highly prone to developing DRMs, due to it high replication rate, error-prone reverse transcriptase, and frequent recombination events (103, 104, 243). DRMs may emerge under selective pressure during suboptimal treatment (treatment-emergent resistance) or be acquired at the time of infection (transmitted resistance) (244). Resistance mechanisms are typically specific to individual drugs (drug-specific) or drug classes (class-specific) and often involve mutations that reduce the binding efficiency of antiretroviral drugs to their intended molecular targets (243). While some DRMs reduce viral replication capacity and can lead to reversion to the wild-type virus in the absence of ART (245), these mutations persist within latently infected cells due to the integration of HIV DNA into the host genome (246). As a result, resistant variants can re-emerge if the same antiretroviral pressure is reintroduced (246).

A systematic review from 2018, estimated that pretreatment NNRTI resistance in Western and Central Africa was 7% (247). Another recent review found low levels of INSTI resistance across sub-Saharan Africa (248). A systematic review among FSW in Africa reported a 33% prevalence of pretreatment drug resistance, with resistance to NNRTIs and PIs being the most frequently observed (249). These findings highlight the need for targeted drug resistance surveillance in key populations disproportionately affected by HIV. In Bissau, a study conducted between 2017 and 2018 found that approximately 10% of pregnant women with HIV-1 had NNRTI resistance before initiating treatment. Furthermore, among 15 PWH-1 on ART with virological failure 53% had NNRTI resistance (250). However, the lack of DRMs estimates among FSW prior to Paper II provided further rational for the study.

Curable Sexually Transmitted Infections

In 2020, WHO estimated that more than one million sexually transmitted infections (STIs) are acquired globally each day, with approximately 374 million new annual infections caused by *Treponema pallidum*, *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis* (251). *T. vaginalis* is the most common pathogen, with an estimated 156 million new annual cases (251). While *Mycoplasma genitalium* is acknowledged as a significant pathogen (252), WHO has not yet provided global prevalence estimates.

The treatment of gonorrhoea is becoming increasingly challenging due to the development and spread of antimicrobial resistance in *N. gonorrhoeae* (253, 254). Over the past decade, resistance to ceftriaxone and azithromycin which are among the last remaining treatment options have been reported (255). Furthermore, *M. genitalium* has shown increasing resistance to macrolides and fluoroquinolones, the preferred first- and second-line treatment options (256, 257). However, two novel drugs, gepotidacin and zoliflodacin, have recently demonstrated non-inferiority to the standard first-line treatment ceftriaxone-plus-azithromycin for the treatment of urogenital *N. gonorrhoeae* (254, 258).

The following section focuses on symptoms of curable STIs in women. Most urogenital infection caused by *N. gonorrhoeae* (~50%) and *C. trachomatis* (more than 70%) are asymptomatic (254, 259). However, untreated *N. gonorrhoeae* infections can lead to pelvic inflammatory disease (PID), ectopic pregnancy and infertility (260). *C. trachomatis* can cause cervicitis and urethritis, with ascending infections potentially resulting in PID, peritonitis and sequelae such as infertility and ectopic pregnancy (254). *M. genitalium* has been associated with urethritis, cervicitis and PID, as well as with infertility and adverse pregnancy outcomes including preterm delivery and spontaneous abortion (261). *T. vaginalis* is asymptomatic in approximately 85% of women and affects the vagina, urethra and endocervix. Symptomatic infections generally present with dysuria, vaginal discharge and vulvovaginal irritation (262).

Curable STIs have been shown to be associated with an increased risk of HIV transmission and acquisition (263). STIs increase the risk of HIV transmission primarily by increased HIV shedding in the genital tract (264-266). Bacterial vaginosis has also been implicated in increasing the HIV transmission risk (267). The risk of HIV acquisition is elevated through mucosal damage caused by inflammation or genital ulcers, and recruitment of HIV-susceptible cells with an increased expression of CCR5 and CD4 receptors (263). Alterations in the vaginal microbiota, particularly bacterial vaginosis has also been associated with increased risk of HIV acquisition (268, 269). Despite these associations, several randomized trials, including three community-based studies (270-272) and two studies among FSW (273, 274), did not demonstrate a reduction in HIV incidence after different STI interventions. An exception, was a community trial conducted in Tanzania reporting a 40% reduction in HIV incidence (275). Poor adherence to STI treatment and difficulties in treatment timing have been suggested as key reasons why studies have failed to demonstrate an effect of STI interventions on HIV prevention (276).

Diagnosis and management

NAATs is the gold standard for diagnosing *C. trachomatis*, *N. gonorrhoeae*, *T. vaginalis* and *M. genitalium* (277). However, these molecular tests are generally unavailable in low-resource settings. Unfortunately, POC antigen-based lateral flow

assays for the detection of *C. trachomatis* and *N. gonorrhoeae* have low sensitivities (277, 278). In contrast, several POC tests for *T. vaginalis* and *T. Pallidum* are available and perform well (279).

Near-POC NAAT platforms such as GeneXpert offer assays for *C. trachomatis*, *N. gonorrhoeae*, *T. vaginalis* and *M. genitalium* but their use in low-resource settings is limited by costs, reliance of electricity and long turn-around time (~90 minutes). In addition, a recent study reported suboptimal sensitivity for detecting of *C. trachomatis*, *N. gonorrhoeae* and *T. vaginalis* (280). GeneXpert Edge, which uses an external battery pack, eliminates the need for direct electricity but still shares the other limitations (281). Other POC options include the Visby Medical Sexual Health Test (Visby Medical, San Jose, CA, USA) (282) and the binx health ioCT/NG assay (binx health, Boston, MA, USA) (283) which both produce results within 30 minutes. However, affordability is still an issue for use in low-resource settings.

Other diagnostic options for *N. gonorrhoeae* include light microscopy of Gram-stained or methylene blue-stained samples though this is primarily an option in symptomatic males. In women, endocervical and urethral samples show low sensitivity. Culture is recommended for test-of-cure and complete antimicrobial resistance testing (260). *T. vaginalis* can be detected by wet-mount microscopy and culture, while *T. Pallidum* diagnosis relies on non-treponemal and treponemal serological tests (284).

In most low-resource settings, including Guinea-Bissau, management of STIs still relies on syndromic approaches, where treatment is based on reported symptoms and clinical findings rather than laboratory confirmation. However, as many STIs are asymptomatic, these infections are often undetected and untreated. The limitations of syndromic management have long been recognized and supported by more recent studies as well (285, 286). New affordable and rapid POC tests for detection of both pathogens and antimicrobial resistance mutations are needed for reducing the spread of STIs and improve surveillance.

Previous studies on the prevalence of curable STIs

A 2018 systematic review summarising the genital prevalence of *N. gonorrhoeae* and *C. trachomatis* among FSW in sub-Saharan Africa reported estimates ranging from 4% to 7% for *C. trachomatis* and 5% to 11% for *N. gonorrhoeae* (287). The prevalence of *T. vaginalis* among FSW in Africa was estimated to range between 7% and 46% in a 2021 systematic review (288). For *M. genitalium*, a global systematic review published in 2018 reported prevalence estimates between 14% and 19% (289).

In Guinea-Bissau, a 1997 study investigating the prevalence of *N. gonorrhoeae* and *C. trachomatis* among women presenting with vaginal discharge reported prevalences of 4% and 17% respectively (290). A study conducted between 1997

and 1998 among pregnant women found a prevalence for *M. genitalium* of 6% and 4% for *T. Pallidum* (291, 292). Another study from 2006-2008 assessing STI prevalence among women with urogenital symptoms attending sexual health clinics, reported prevalences of 13% for *C. trachomatis*, 1% for *N. gonorrhoeae*, 8% for *M. genitalium*, 20% for *T. vaginalis* and 1% for *T. Pallidum* (293). A study conducted by our group on *T. pallidum* prevalence among police officers reported a decline from 4.5% in 1990 to 0.4% in 2010 (294). The most recent community-based study, conducted in 2016, reported prevalences of 6% for *C. trachomatis*, 4% for *N. gonorrhoeae*, 3% for *M. genitalium*, 10% for *T. vaginalis* and 1% for *T. Pallidum* (295).

However, prior to the study in paper III, no data existed on the prevalence of curable STIs among FSW in Guinea-Bissau, providing the rationale for the investigation. Older studies from the neighbouring Senegal have reported high prevalences of *C. trachomatis*, *N. gonorrhoeae*, *T. vaginalis* and *T. Pallidum* among FSW (296, 297). A study conducted in 2000 among FSW reported prevalences of 22% for *C. trachomatis*, 24% for *N. gonorrhoeae*, 22% for *T. vaginalis* and 24% for *T. Pallidum* (296).

Human T-lymphotropic virus type 1

"HTLV-1 is the neglected of neglected diseases…" (298)

-Lucy B M Cook 2020

Worldwide, an estimated 5 to 10 million people are living with Human T-lymphotropic virus type 1 (HTLV-1) (299). However, the true global burden is likely underestimated due to insufficient data from several regions.

HTLV-1 is a single-stranded RNA virus that is part of the *Retroviridae* family and *Deltaretrovirdae* genus (91). The origin of HTLV-1 stems from zoonotic animal-to-human transmission of the closely related simian T-lymphotropic virus type 1, which is present in several African and Asian Monkeys (299). The HTLV-1 virion is around 100 nm in diameter and contains two copies of single-stranded RNA containing nine genes which are encapsulated in a capsid and surrounded by a lipid envelope (300).

HTLV-1 is mainly transmitted vertically from mother-to-child (mainly through breastfeeding, with limited evidence of intrauterine or peripartum transmission), sexually, or through exposure to infected blood (301). As HTLV-1 is poorly infectious in the absence of cell-to-cell contact, HTLV-1 transmission between individuals is thought to occur mostly through direct cell-to-cell contact through formation of viral synapses and/or viral biofilm (302). HTLV-1 has mainly tropism for $CD4^+$ T cells but can also infect $CD8^+$ T cells, B lymphocytes, dendritic cells,

monocytes and endothelial cells (303). Entry of HTLV-1 into target cells is thought to involve interactions between the surface subunit of the envelope glycoproteins with three cellular surface receptors: Glucose Transporter, Heparin Sulfate Proteoglycane and Neuropilin-1. Attachment of HTLV-1 to the host cell triggers fusion of the viral membrane and the host cell membrane. The viral genome is then released into the cytoplasma and reverse transcription is thought to occur. The double-stranded DNA is transported to the cell nucleus and integrated into the host genome, forming provirus (304).

Unlike HIV-1, HTLV-1 does not lead to progressive loss of $CD4^+$ T cells. Instead HTLV-1 is thought to induce cell-cycle arrest initially and later during chronic infection induce further modifications that promote cell proliferation which can potentially lead to malignant proliferation (305). The acute phase of HTLV-1 infection is generally considered to span the first three months post-infection but research on this early phase of HTLV-1 infection is limited. During the acute phase, the virus spreads rapidly between $CD4^+$ T cells (306). Additionally the HTLV-1 nuclear transcriptional transactivator protein, Tax, enhances the expression of viral structural proteins which facilitates the cell-to-cell spread (307). A study of three individuals who acquired HTLV-1 through organ transplantation found that the HTLV-1 proviral load (PVL) doubled approximately every 1.4 days during the early weeks of infection, before stabilizing around six weeks post-infection (308). However, it remains uncertain whether the immunosuppressive therapy used during transplantation enhanced or restricted viral spread. Additionally, the administration of antiretroviral therapy may have influenced the results. The acute phase is halted due to activation of the adaptive immune system, targeting viral structural proteins, including Tax, leading to the elimination of many infected cells (307). The activation of the adaptive immune system marks the beginning of the chronic phase of HTLV-1 infection, which is characterized by reduced Tax expression and lower levels of viral replication due to immune-mediated transcriptional suppression. During the chronic phase, HTLV-1 persists in the peripheral blood primarily through clonal expansion of infected cells (309). HTLV-1 PVL has been shown to decline over time in the chronic phase. In one study investigating sequential samples from asymptomatic carriers over a median follow-up of 6.5 years, the PVL decreased from approximately 2% to 1%, with PVL (%) representing the proportion of infected peripheral blood mononuclear cells (310). Tax reactivation has been reported to occur during the chronic phase of HTLV-1 infection, leading to occasional infective spread between $CD4^+$ T cells (311). Tax contributes to DNA damage, genomic instability and inflammation (312), and stimulates the production of inflammatory cytokines such as IL-2, IL-6 and tumor necrosis factor as well as the immunosuppressive cytokine IL-10 (313, 314). Tax induces HLA class II expression in HTLV-1-infected T cells, which may allow them to function as tolerogenic antigen presenting cells and trigger antigen-specific T cell anergy (315). Prolonged low-level expression of Tax throughout the chronic phase of HTLV-1 infection may explain the oncogenic and inflammatory effects associated with

HTLV-1 (301). In addition, the HTLV-1 protein p30 suppresses the transcription factor PU.1, which is important for the development of granulocytes, monocytes, macrophages and lymphoid cells (316).

The most well-established complications of HTLV-1 infection are adult T-cell leukemia/lymphoma (ATL) (317) and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) (318), both of which occur exclusively in people with HTLV-1. The estimated lifetime risk of developing HAM/TSP and ATL among people with HTLV-1- is approximately 2% (319) and 5% (320), respectively. Additionally, HTLV-1-associated uveitis (321) and infective dermatitis (322) are also conditions seen only in those with HTLV-1 infection. HTLV-1 has also been associated with tuberculosis (323-328), eczema (329), seborrheic dermatitis (329-331), lung disease (bronchiectasis, bronchitis, bronchiolitis) (332, 333), urinary tract infections (327), lymphoma other than ATL (334), Strongyloides stercoralis infection (335) and hyperinfection syndrome (336).

In addition to specific disease associations, HTLV-1 infection has been linked to increased overall mortality. A recent meta-analysis of eight studies reported a pooled relative risk (RR) of 1.57 (95% CI 1.37-1.80) for the association between HTLV-1 infection and all-cause mortality (337). The included studies were conducted across various geographical regions, including Japan (338-340), Guinea-Bissau (341-344), and the USA (345). Two studies that examined all-cause mortality excluding ATL reported slightly lower RRs: 1.3 (95% CI 1.0-1.7) (339) and 1.87 (95% CI 1.12-3.12) for women (340), and 1.77 (95% CI 0.93-3.38) for men (340). One study stratified mortality risk by HTLV-1 PVL and found that higher PVL was associated with an increased risk of all-cause mortality (342). Additionally, another study reported that higher HTLV-1 antibody titres were associated with increased all-cause mortality (338), and higher antibody titres have previously been correlated with higher HTLV-1 PVL (346). A high HTLV-1 PVL has also been shown to precede and predict the development of ATL and HAM/TSP (347). Furthermore, elevated HTLV-1 PVL has been associated with an increased risk of bloodstream infections (348), bronchiectasis and the severity of pulmonary disease (332), urinary tract infections (349), and several chronic non-communicable conditions, including kidney disease (350), diabetes (350), and diffuse coronary artery disease (351).

HTLV-1 epidemiology in Guinea-Bissau

To our knowledge, the first study reporting on HTLV-1 prevalence in Guinea-Bissau was conducted in 1989 in the capital Bissau and found a prevalence of 9% (344). A pronounced difference in HTLV-1 prevalence was observed between women (12%) and men (5%), though the study included only individuals over 50 years of age. A later nationwide study among police officers conducted from 1990 to 1992 reported a lower HTLV-1/2 prevalence of 4% and an annual HTLV-1/2

incidence of 0.4 per 100 person years (PY) (352). In Bissau, community surveys reported HTLV-prevalence estimates of 4% in 1996 (353), 2% in 2006 (354) and 3% in the most recent survey conducted between 2014 and 2016 (355). The incidence of HTLV-1 in Bissau was estimated at 0.09 per 100 PY between 1996 and 2006 (354) and remained stable at 0.09 per 100 PY between 2006 and 2016 (355). Among women attending sexual health clinics in Bissau, the prevalence of HTLV-1 was 3% between 2006 and 2008 (293).

Older age, female sex, HIV-2 infection and a history of blood transfusions have been identified as risk factors for HTLV-1 infection in Guinea-Bissau (353-356)

In contrast to the capital, higher HTLV-1 prevalence has been reported in the rural region of Caió, situated in the northwestern Guinea-Bissau approximately 100 km from Bissau near the border with Senegal. Community-based studies in Caió reported HTLV-1 prevalence rates of 6% in 1990 (357), 6% in 1997 (356) and 5% in 2007 (356). The incidence of HTLV-1 in the Caió region was estimated at 0.18 per 100 PY between 1990 and 1997 and remained stable at 0.16 per 100 PY between 1997 and 2007 (356). The reasons for the marked difference in HTLV-1 prevalence rates between rural and urban areas in Guinea-Bissau is not clear, though cultural and/or ethnic differences have been suggested as possible explanations (356, 358).

As noted above, the spread of HIV-2 is believed to have coincided with the war of liberation against Portuguese colonial rule and that parenteral rather than sexual transmission was playing a major role (34, 77, 78). These events may account for the elevated HTLV-1 prevalence reported during the 1990s as well. HTLV-1 prevalence decreased in both urban and rural areas of Guinea-Bissau between the 1990s and 2000s (355, 356), similar to the decrease observed in HIV-2 prevalence during the same period, but contrasting the increasing prevalence of HIV-1 (62-69, 355, 356). However, the most recent community survey reported a slight increase of HTLV-1 prevalence, particularly among men aged 15-24 years, suggesting ongoing transmission, most likely through vertical transmission (355).

HTLV-1 and HIV co-infection

Previous studies suggest that HTLV-1 and HIV-1 co-infection may accelerate progression to AIDS and increase the risk of death (359-361). However, existing evidence is inconsistent, with studies reporting contradictory findings (362, 363). A case-control study by Schechter et al. in 1994 involving 27 individuals with co-infection, observed that although individuals with co-infection had higher $CD4^+$ T cell counts compared to individuals with HIV-1 mono-infection, they also exhibited a more advanced HIV disease (364). Similarly, a study from South Africa reported that individuals with co-infection maintained elevated $CD4^+$ T cell count despite virological failure on ART, compared to those with HIV-1 mono-infection (365). Among ART naïve individuals, another study found that individuals with co-

infection had higher initial $CD4^+$ T cell count than those with HIV-1 mono-infection, with no correlation between $CD4^+$ T cell count and HIV-1 VL (366). Although $CD4^+$ T cell counts may be preserved or even elevated in individuals with co-infection, these cells may exhibit functional alterations, with a study reporting a reduction in naïve subsets and elevated levels of immune activation (367).

Table 2 summarizes the key studies investigating mortality among individuals with HTLV-1 and HIV-1 co-infection. A common limitation in these studies is the inability to determine the infection order, whether HTLV-1 preceded or followed HIV-1 infection. Moreover, the studies either lacked data on ART adherence, included individuals both on and without ART or did not report ART status at all. An additional study, not included in Table 2, found a significantly higher proportion of deaths among 35 children with HTLV-1 and HIV-1 co-infection (34.3%) compared to 39 children with HIV-1 mono-infection (7.7%, p=0.01). The study also reported that survival was shorter among children with co-infection compared to children with HIV-1 mono-infection. However, it was not clearly stated whether the children with co-infection had HTLV-1, Human T-lymphotropic virus type 2 (HTLV-2), or both viruses (368).

Regarding HIV-2, one study reported a higher risk of tuberculosis among individuals with HTLV-1 and HIV-2 co-infection compared to those with HIV-2 mono-infection (369). Additionally among tuberculosis patients, individuals with HTLV-1 and HIV-2 co-infection experienced higher mortality than those HIV-2 mono-infection (370).

The limited and partly conflicting data on HTLV-1 and HIV co-infection, combined with the lack of studies from Africa, provided the rationale for the study presented in Paper IV.

Table 2. Summary of studies comparing mortality among individuals with HTLV-1 and HIV-1 co-infection and HIV-1 mono-infection

Study	Country and year	Study design	Data source	Participants	All-cause mortality
Brites (360)[1]	Brazil 1989-1999	Retrospective Case-control	Medical charts. Mortality source not reported.	63 HTLV-1/ HIV-1 vs 135 HIV-1. 75% men, Median age 33.	Median survival was shorter in HTLV-1/HIV-1 vs HIV-1: 2378 days vs 3000 days, p=0.0001.
Collins (363)[2]	Peru 1990-2004	Retrospective Case-control	Medical charts. Mortality source not reported.	50 HTLV-1/HIV-1 vs 100 HIV-1. 87% men. Mean age 41 (SD 11).	aHR 1.2 (95% CI 0.4-3.6) for HTLV-1/HIV-1 vs HIV-1.
Brites (359)[3]	Brazil	Retrospecitve Case-control	Medical charts. Mortality data from National Mortality Registry.	149 HTLV-1/HIV-1 vs 149 HIV-1. 40% men. Mean Age 39 (SD 9).	Mean survival was shorter in HTLV-1/HIV-1 vs HIV-1: 16.7 vs 18.1 years, p=0.001. Among those with pVL >50 copies/mL: 8.4 vs 12.9 years, p=0.02; with pVL <50 copies/mL: 19.0 vs 20.2 years, p=0.5.
Beilke (362)[2]	USA 1993-2002	Case-control	Medical charts. Mortality source not reported.	62 HTLV-1/HIV-1 vs. 824 HIV-1. HTLV-1/HIV-1: 76% males, 79% ≥36 years. HIV-1: 74% males, 45% ≥36 years.	aHR 0.84 (95% CI 0.52-1.36 for HTLV-1/HIV-1 vs HIV-1.
Amanzo-Vargas (361)[2]	Peru 1989-2022	Retrospective cohort	Medical charts. Mortality data from National Mortality Registry.	54 HTLV-1/HIV-1 vs 108 HIV-1. 61% men. Median age 42 (IQR 34-51).	aHR 11.75 (95% CI1.55-89.00, p=0.017) for HTLV-1/HIV-1 vs HIV-1.

aHR= Adjusted Hazard Ratio. CI= Confidence interval. SD= Standard Deviation. pVL=plasma viral load. IQR= Interquartile range. HTLV-1/HIV-1= Individuals with HTLV-1 and HIV-1 co-infection. HIV-1= Individuals with HIV-1 mono-infection.

[1]No information on ART use.

[2]Adjusted for ART in the multivariate analysis.

[3]All participants on ART.

Setting

Guinea-Bissau

The republic of Guinea-Bissau is a country in West Africa that covers 36,125 square kilometres. Guinea-Bissau shares borders with Senegal to the north, Guinea-Conakry to the southeast and the Atlantic Ocean to the west (Figure 3). As of 2023, the population was estimated at 2.1 million inhabitants (371). The capital Bissau is the largest city, however population estimates are uncertain due to limited census data.

Guinea-Bissau has a tropical climate, characterized by two distinct seasons: a rainy season, from May to November, and a dry season from December to April. The economy is primarily based on traditional agriculture, with cashew nuts being the main export product, and fishing.

A former Portuguese colony, Guinea-Bissau gained independence in 1974 after a protracted war of liberation that lasted over a decade. The aftermath of the conflict left the country with underdeveloped economic, administrative, educational and healthcare systems. In 1998-1999, a civil war took place, with intense fighting in the capital Bissau, which significantly disrupted infrastructure and the national economy. Since then, the country has experienced two major coup d'états and several other politically destabilising events.

The population of Guinea-Bissau is ethnically diverse, with major ethnic groups including Balanta, Fula, Mandjaco, Mandinga and Papel. While each ethnic group has its own language, Portuguese remains the official language due to the colonial legacy. However, the most widely spoken language in everyday life is Kriol, a Portuguese-based creole language. Religion practices are diverse, and includes traditional animist beliefs, Islam and various Christian denominations.

Guinea-Bissau is one of the least developed countries in the world, ranking as country number 179 of 193 in the latest United Nations Development Programme report of 2024. The country faces significant public health challenges, with a mean life expectancy at birth of 58,6 years, a maternal mortality rate of 603 per 100.000 births and an under-five mortality of 72 per 1000 live births (372).

Figure 3. Map of Guinea-Bissau, No 4063 R5, February 2018, United Nations, Department of Field Support, Geospatial Information Section. Reprinted with permission.

National Public Health Laboratory

Following Guinea-Bissau's independence, Sweden became a key bilateral aid partner, with substantial support continuing until 1997. In 1987, LNSP in Bissau was established with funding from the Swedish International Development Cooperation Agency and technical input from the Swedish Institute for Communicable Disease Control. The initiative included the introduction of routine HIV diagnostics and foundational systems for epidemiological surveillance. Early research activities were also launched with support from the Swedish Agency for Research Cooperation. Until the broader implementation of rapid HIV tests in 2005, most HIV testing in the country was conducted at the LNSP.

ENDA SANTÉ

ENDA Santé is a non-governmental organization based in Dakar, Senegal, involved in public health initiatives across West and Central Africa. Active in Guinea-Bissau since 2008 as part of the ENDA Tiers Monde network, the organization has

contributed to strengthening the health system and improving access to care. ENDA Santé runs community-based programmes focused on HIV prevention and treatment, sexual and reproductive health, and harm reduction. These activities target key populations often underserved by traditional care systems, including FSWs, people who use drugs, and MSMs. In addition to these activities, ENDA Santé is involved in research and work to promote policies aimed at ensuring fair and sustainable health care in the region. ENDAs work with FSW has focused on HIV prevention through behaviour change communication, condom distribution (mostly male condoms but occasionally female condoms), HIV-testing and facilitating access to treatment. STI management has included both syndromic treatment and periodic presumptive treatment, depending on access to antimicrobial drugs.

Aims

The papers in this thesis aimed to investigate:

I. three serological HIV tests (Geenius, INNO-LIA and Immunocomb) with an emphasis towards the ability to discriminate between HIV-1, HIV-2 and HIV-1/2 dual infection.

II. the HIV care continuum and HIV drug resistance (HIVDR) among FSW in Guinea-Bissau.

III. the prevalence of curable STIs, identify associated risk factors and assess ciprofloxacin resistance in *N. gonorrhoeae* among FSW in Guinea-Bissau.

IV. the prevalence and incidence of HTLV-1, assess all-cause mortality in individuals with HTLV-1, and examine time to death and time to AIDS in individuals with HTLV-1 and either HIV-1 or HIV-2 co-infection.

Material and Method

Study design at a glance

	Paper I	Paper II	Paper III	Paper IV
Study design	Retrospective diagnostic evaluation nested in a cohort study	Cross-sectional	Cross-sectional	Prospective open cohort study
Participants	ART naïve PWH from the Bissau HIV cohort (n=131)	FSW in seven cities in Guinea-Bissau (n=440)	FSW in eight cities in Guinea-Bissau (n=467).	Police offiers in Guinea-Bissau (n=4607)
Data sources	Microbiology diagnostics and case report forms	Microbiology diagnostics and case report forms	Microbiology diagnostics and case report forms	Microbiology diagnostics and case report forms
Main Outcomes	Measurement of agreement between the three serological tests (INNO-LIA, Geenius and Immunocomb) and concordance with HIV RNA/ DNA results	HIV prevalence, HIV care continuum outcomes and prevalence of HIV-1 DRMs	Prevalence of curable STIs and associated risk factors	HTLV-1 incidence and prevalence; all-cause mortality in participants with HTLV-1 vs HTLV-1-negative participants, and in participants with HTLV-1 and HIV-1 co-infection vs those with HIV-1 mono-infection

Study population and study design

The Bissau HIV cohort (Paper I)

The Bissau HIV cohort was established in 2007 as a single-centre cohort based at the HIV clinic (CTA) at HNSM, the national reference hospital in Bissau (373). The cohort was implemented through a collaboration between Aarhus University Hospital in Denmark, the Bandim Health Project in Guinea-Bissau and local healthcare personnel, including researchers, physicians and nurses. PWH in Bissau can be referred to CTA HSNM from hospital wards at HSNM, the blood bank or transferred from other HIV clinics, hospitals and health centres. All patients at CTA HNSM are invited to participate in the Bissau HIV cohort. At enrolment, participants complete a study questionnaire and as part of routine care a blood sample is collected for $CD4^+$ T cell count, biochemistry and haematology analyses. Surplus plasma and cell suspension are frozen and stored in Guinea-Bissau prior to shipment to a biobank in Aarhus, Denmark. In 2017, the cohort expanded to include eight additional major HIV clinics across the country. By 31 December 2021 the cohort comprised 34,929 PWH (235).

For paper I, we used a subset of samples from a previous study comparing the performance of INNO-LIA and Immunocomb assays, with an emphasis on the ability to discriminate between HIV-1, HIV-2 and HIV-1/2 dual infection (179). In that study, 239 samples were retrospectively selected from the biobank and stratified by HIV-type to ensure equal representation of HIV-1, HIV-2 and HIV-1/2 dually reactive samples. Stratification was based on the initial HIV-type discrimination performed in Bissau. All samples were originally collected at CTA HNSM between September 2007 and May 2012 (179).

Female sex workers (Paper II-III)

This cross-sectional study was designed and initiated through a collaboration between Lund University in Sweden, LNSP and ENDA Santè—Bissau in Guinea-Bissau.

The study was conducted from a mobile clinic staffed by a trained nurse, a social worker and a driver. FSW in Bissau were recruited using venue-based convenience sampling at nine predefined sites across Bissau. In cities outside Bissau, FSW were recruited through peer-based chain referral at one to four predefined sites per city, depending on city size, totalling 14 sites. Inclusion of participants took place between October 2014 and September 2017 for paper II and between October 2014 and May 2019 for paper III. The following criteria were applied for inclusion in the study; being biologically female, age ≥16 years old, self-reported engagement in selling sex in the last 12 months and capacity to consent. At inclusion, all

participants were interviewed following a study questionnaire to obtain information on demographics, HIV testing history, HIV care and treatment engagement, sexual history and symptoms related to STIs. Venous blood samples were collected and screened on site using a rapid HIV antibody test, with results provided immediately. Blood samples were subsequently transported to LNSP for $CD4^+$ T cell count, confirmatory discriminatory HIV test and *T. pallidum* serology. Until March 2016 HIV-1 VL measurements were conducted at Lund University, Sweden and subsequently at LNSP. Plasma and cell suspension were stored at -20°C before being shipped on dry ice to Lund University, Sweden for HIV-1 drug resistance genotyping. High vaginal swabs were collected under nurse supervision and stored in Aptima vaginal swab specimen collection tubes. As with plasma and cell suspension, swabs were stored at -20°C at LNSP before being shipped on dry ice to Örebro University, Sweden.

Throughout the study, pre-test and post-test counselling was provided. Condoms were distributed, and FSW with HIV were referred to HIV clinics for treatment per national guidelines. Women reporting symptoms of STI received syndromic treatment, while asymptomatic women with curable STI were offered directed treatment as per national guidelines.

The Police cohort (paper IV)

An open, prospective cohort of police officers was established in Guinea-Bissau on February 6, 1990. Police officers were selected due to their permanent employment status, which was anticipated to increase the likelihood of sustained follow-up. In June 1998, a civil war broke out in Guinea-Bissau, leading to a temporary suspension of recruitment and follow-up visits. Follow-up resumed in 2000, and new enrolments restarted in 2003, continuing until September 28, 2009. All participants were followed until February 25, 2011. However, follow-up for participants with HIV-1 or HIV-2 continued until September 28, 2013. By that time 4820 police officers had enrolled, of whom 4817 had a recorded HIV test result. 76% of all study participants returned for at least one follow-up visit.

The study was conducted at a health post located within the main police station (2° Esquadra) in the capital, Bissau, and was managed by a team comprising one physician, two to three nurses, and three to four auxiliary staff. Follow-up visits were scheduled every 12-18 months at police stations in both urban and rural regions. All police officers with regular employment were invited to participate, and over 98% consented to participate in the study.

At enrolment and each follow-up visit, participants completed a questionnaire collecting demographic data and symptoms related to HIV and STIs. Blood samples were collected for HIV and *T. pallidum* serology at inclusion and follow-up visits, and baseline serology at enrolment for HTLV was performed until 25 April 2008.

For the study presented in paper IV, we also retrospectively tested stored samples from 10 of 14 participants with HIV-1 and 10 of 11 participants with HIV-2 enrolled after 25 April 2008. Up to and including 1992, all follow-up samples were tested for HTLV. Between 1992 and 2006, HTLV testing of follow-up samples was conducted intermittently and in batches, dependent on reagent availability. After 2006 no HTLV testing was carried out on follow-up samples. From 1993, $CD4^+$ T cell counts were measured for participants with HIV and for a subset of HIV-negative participants as controls. Bodyweight was measured, and clinical symptoms were classified according to the WHO clinical staging system.

Throughout the study, participants received both pre-test and post-test counselling, as well as health education on HIV and STIs. Condoms were distributed, and basic medical care, including free medications, was provided. ART was introduced in Guinea-Bissau through the national treatment programme in 2005 and became available to the police cohort in January 2006. In addition, participants with HIV were offered trimethoprim-sulfamethoxazole prophylaxis and, from 2005, isoniazid (INH) prophylaxis in accordance with WHO guidelines at the time, which recommended INH for PWH with a positive tuberculin skin test (374).

Participants who initiated ART were censored at the time of treatment initiation. Participants receiving trimethoprim-sulfamethoxazole were not censored due to uncertain adherence and the likelihood of unrecorded or irregular usage both within and outside the study. Similarly, participants who received INH were not censored, given the high incidence of tuberculosis in Guinea-Bissau and the continued risk of reinfection (375). Moreover, INH prophylaxis was introduced in 2005 and, as noted, was offered only to participants with a positive tuberculin skin test.

Mortality reports obtained from the Ministry of the Interior were cross-checked by health post staff at the main police station in Bissau. Reported deaths were clinically classified based on symptoms documented prior to death.

Due to frequent transfers of police officers between units across the country, maintaining consistent follow-up was more challenging than in community-based cohorts. Consequently, the date of the last recorded visit was used as the endpoint for follow-up.

Laboratory methods

Serological determination of HIV-1 and HIV-2 infection

In paper I, the initial screening was performed at CTA HNSM using Determine HIV-1/2 assay and HIV type discrimination was performed in Bissau using the SD Bioline HIV 1/2 3.0 (179). In Aarhus University Hospital, Denmark, samples were

further evaluated with INNO-LIA HIV I/II Score and ImmunoComb HIV-1/2 Bispot. Samples were subsequently further analysed at Lund University, Sweden, using the Geenius HIV-1/2 Confirmatory Assay. The Geenius assay, is a single-use immunochromatographic test designed to discriminate between HIV-1 and HIV-2 antibodies. The assay includes antigens attached on a membrane strip on a cartridge: HIV-1 (p31, gp160, p24, gp41) and HIV-2 (gp36, gp140). Cartridges were analysed both automatically and visually. Automated interpretation was conducted using the Geenius Reader (S/N DP3B002818) connected to a Lenovo Mini PC (Geenius Reader software). For visual reading, two independent observers assessed the cartridges in accordance with the manufacturer's guidelines, which specify that even faint bands must be considered reactive. According to the manufacturer's interpretive criteria, a result was considered HIV-1 reactive if at least two HIV-1 bands were visible, including at least one envelope antigen (gp160 or gp41). HIV-2 reactivity required both HIV-2 bands (gp36 and gp140) to be present. Results with both HIV-2 bands and HIV-1 envelope bands (gp160 and gp41) and/or additional HIV-1 bands (p24 and/or p31) were classified as HIV-1/2 dual reactive (labelled as 'untypable' in the manufacturer's instructions. It should be noted that the Geenius Reader uses an algorithm that incorporates band intensity into its interpretation of results, whereas visual interpretation, as previously described, follows simplified instructions in which a strict binary assessment of band reactivity is recommended.

In Papers II-III, initial HIV screening was conducted in the mobile clinic using Determine. Confirmatory and discriminatory testing was performed LNSP. Up to September 2016, ImmunoComb was used. From October 2016 onwards, the Geenius assay was used.

In paper IV, from 1990 to 1994, screening for HIV-1 and HIV-2 antibodies was performed using the Behring Enzygnost HIV-1+2 ELISA (Behring, Marburg, Germany), the Wellcozyme recombinant anti-HIV-1 (Wellcome, Dartford, UK) and an in-house HIV-2 (SBL6669) ELISA assay (376). From 1995 onwards, screening was conducted with the Behring Enzygnost HIV-1/HIV-2 Plus ELISA (Behring). Until 1997, reactive samples were confirmed by Western blot analysis using either the Diagnostic Biotechnology anti-HIV-1 Blot 2.2 (Diagnostic Biotechnology, Science Park, Singapore) or an in-house HIV-2-specific Western blot (377). Samples reactive to both HIV-1 and HIV-2 were confirmed by Pepti-lav (Sanofi Diagnostic Pasteur, Marnes-la-Coquette, France. In 1999, the confirmation algorithm was revised to Capillus HIV-1/HIV-2 assay (Cambridge Biotech Limited, Galway, Ireland) and ImmunoComb (378).

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

In paper III, samples were screened for evidence of past or present T.pallidum infection with *T.pallidum* particle agglutination assay (TPPA; Fujirebio, Tokyo,

Japan) and if positive further analysed with Venereal Disease Research Laboratory test (VDRL; Sypal, Diagast, Lille Cedex, France) to identify active cases.

Screening of samples for HTLV was performed using enzyme immunoassays, mainly Murex HTLV 1+2 (Murex Biotech Ltd, Dartford, UK). Reactive samples were confirmed in Guinea-Bissau using Western blot (HTLV-blot 2.3, Genelabs Diagnostics, Singapore) or retrospectively at the Department of Laboratory Medicine, Örebro University using INNO-LIA HTLV I/II Score (Innogenetics, Gent, Belgium). The inclusion samples from the 20 participants with HIV enrolled after 25 April 2008 were screened using LIAISON XL Murex recHTLV-I/II assay (DiaSorin, Saluggia, Italy).

Laboratory methods for $CD4^+$ T cell measurement

In paper I, $CD4^+$ T cell counts were performed by flow cytometry at LNSP, using a Cyflow instrument (Partec, Münster, Germany) (241).

In paper II, $CD4^+$ T cell counts were performed at LNSP, using the FACSPresto-Near Patient CD4 counter (Becton-Dickinson, NYSE:BDX, USA) (379).

In paper IV, $CD4^+$ T cell counts were determined by flow cytometry. From 1993 to 2005, analyses were performed using the FACStrak instrument (Becton Dickinson, San José, USA), and from 2006 onward, the Cyflow instrument (Partec, Münster, Germany) was used. A comparative evaluation of the two testing methods was conducted in parallel, which showed a strong correlation for $CD4^+$ T cell percentages and acceptable correlation for absolute $CD4^+$ T cell counts (380).

Quantification of HIV-1 and HIV-2 viral load

In Paper I, HIV-1 and HIV-2 VL measurement was done at Aarhus University Hospital, Denmark. Quantification of HIV-1 VL was performed using Abbott m2000 system (Abbott RealTime HIV-1, version 9.00; Abbott Molecular Inc, Abbot Park, II, USA) and HIV-2 VL was quantified using an in-house method (179). The limit of detection for the HIV-2 assay was approximately 50 copies/ml.

In Paper II, HIV-1 VL was quantified at Lund, University, Sweden, using an in-house Taqman quantitative PCR performed in the ABI StepOnePlus System (Applied Biosystems) and the Superscript III Platinum One-Step qRT-PCR Kit (Invitrogen), until March 2016 (381). The qPCR assay had a limit of quantification of 5 RNA copies/qPCR reaction. From March 2016 onwards, plasma HIV-1 VL was quantified at LNSP, using the GeneXpert HIV-1 Quant Assay (Cepheid Innovations Pvt. Ltd., USA), with a detection limit of 40 copies/ml.

HIV-1 drug resistance genotyping

HIV-1 drug resistance genotyping was performed by extracting viral RNA from plasma, followed by amplification of the HIV-1 pol sequence using RT-PCR and nested PCR, as previously described (382). After removal of regions corresponding to the primers, editing and sequence alignment, the final analysed fragment was 1035 bases in length, corresponding to nucleotide positions 2268-3302 of the HXB2 reference strain (GenBank accession K03455), spanning position 6-99 of the protease regions and 1-251 of the reverse transcriptase region. DRMs were assessed using the Stanford Genotypic Resistance Interpretation Algorithm (383). For FSW who reported no prior ART, DRMs were evaluated using the Calibrated Population Resistance tool (v6.0 beta), based on the 2009 WHO surveillance transmitted drug resistance list (384, 385). Among FSW currently on ART or reporting treatment interruption, DRMs was assessed using the Stanford HIVdb program (386).

HIV-1 and HIV-2 DNA measurement

DNA extraction was performed on cell suspensions using the EZ1 DNA Blood Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. The extracted DNA was then subjected to PCR amplification using specific primers and probes as described by Gueudin et al. (387). No plasmid standards were included in the analysis and therefore our results were qualitative. Each PCR reaction was carried out in a total volume of 40 μL.

HIV DNA analyses were performed on all available samples that were typed as HIV-1/2 dually reactive, as well as on samples that yielded discordant results between Geenius, INNO-LIA, and ImmunoComb.

Molecular detection of *Neisseria gonorrhoeae, Chlamydia trachomatis, Mycoplasma genitalium* and *Trichomonas vaginalis*

Vaginal swabs were tested at the WHO Collaborating Centre for Gonorrhoea and Other STIs, Örebro University Hospital, Sweden, in accordance with the manufacturer's instructions, using the Aptima Combo 2 assay for *C. trachomatis* and *N. gonorrhoeae*, Aptima *T. vaginalis* assay and Aptima *M. genitalium* assay (only for samples collected from July, 2015 onwards) on a Panther instrument (Hologic, San Diego, CA, USA). Previous evaluations have reported sensitivities and specificities of 99% and 100%, respectively, for these assays (388). In brief, the Aptima platform uses a three-step process: capture of target (*C. trachomatis* 23S rRNA, *N. gonorrhoeae* 16S rRNA, *T. vaginalis* 16S rRNA, *M. genitalium* 16S rRNA), amplification through TMA and detection via hybridisation with chemiluminescent-labelled probes. The TMA process is an isothermal technique

that amplifies RNA from the target acid using the combined activity of reverse transcriptase and RNA polymerase.

Ciprofloxacin resistance in *Neisseria gonorrhoeae*

Ciprofloxacin resistance was assessed in *N. gonorrhoeae*-positive Aptima samples (collected before September 2017) using the ResistancePlus GC assay (SpeeDx, Sydney, Australia), which detects the GyrA S91F mutation. This assay has demonstrated 100% sensitivity and specificity for detection of the GyrA S91F mutation (389). In brief, the assay detects five targets across separate channels: (i) *N. gonorrhoeae* opa gene; (ii) *N. gonorrhoeae* porA pseudogene; (iii) N. gonorrhoeae wild-type GyrA S91; (iv) *N. gonorrhoeae* GyrA S91F; and (v) an internal control for extraction efficiency and detection of PCR inhibition. Results were analysed using ResistancePlus GC (7500) software. The assay reports (i) presence or absence of *N. gonorrhoeae*, and (ii) if detected, whether GyrA is wild type, harbours the GyrA S91F mutation, or is indeterminate.

Statistical analysis

In papers I, II and IV, no a priori power calculations were performed. In paper I, the sample size was determined by the number of available samples with sufficient remaining material from a previous study (179). For paper II, the sample size was determined by the number of FSW who visited the mobile clinic during the study period. In paper IV the sample size was determined by the number of included police officers diagnosed with HIV and HTLV-1 during the study period. In paper III, to ensure adequate statistical power, the study aimed to recruit 400 participants. This sample size was determined based on an anticipated 40% prevalence of any of the following infections among FSW: *C. trachomatis*, *N. gonorrhoeae*, *M. genitalium*, *T. pallidum*, or *T. vaginalis* (296, 297). The calculation assumed a 95% confidence level ($Z = 1.96$), a significance level of 5% ($\alpha = 0.05$), and a degree of absolute precision of 5% ($d = 0.05$).

In paper I, kappa statistic was used to assess the level of agreement between Geenius and INNO-LIA, as well as between Geenius and ImmunoComb. Additionally, agreement between the Geenius Reader and visual reading of Geenius was evaluated using the same measure.

In paper II, FSW were classified as virally suppressed if HIV-1 VL was <1000 copies/ml (166, 390). The Cochran-Armitage test for trend adjusted for age, was used to assess linear trends in HIV prevalence over the study period. HIV care continuum outcomes were analysed using two approaches: (1) all FSW with HIV-1 and HIV1/2 dual infection as the denominator, and (2) only those who had reached the previous step in the continuum. Logistic regression was used to examine

predictors of diagnosed versus undiagnosed HIV infection. Covariates assessed in univariable and multivariable models included age, region, education, condom use, number of clients on the last day of sex work, alcohol use, and number of children. The final model was derived using backward stepwise selection, with model fit assessed using likelihood ratio tests and Akaike's information criterion. Age, region, education, and condom use were retained in the final model.

In paper III, univariable logistic regression, adjusted for age, was used to explore associations between STI status and potential risk factors. Variables with p-values <0.10 in univariable analysis were included in the multivariable model. In the final model, factors with p-values <0.05 were considered independently associated with curable STIs.

In paper IV the HTLV-1 prevalence and incidence were analysed using six and eight time strata, which were chosen based on a previous study (66). Date of estimated seroconversion of HTLV and HIV was set to the midpoint between last negative and first positive sample. Prevalence was evaluated using logistic regression and incidence using Poisson regression. Univariable analyses were adjusted for age and sex (for HTLV-1 infection at enrolment) and time period (for incident HTLV-1 infections). Variables with p <0.10 were included in multivariable models, those with p <0.05 in the final models were considered independently associated with HTLV-1 infection. To examine the association between HTLV-1 and HIV-1 co-infection and $CD4^+$ T cell count, we performed multivariable linear regression on log-transformed $CD4^+$ values, adjusting for age, sex, and time since estimated HIV-1 infection. Time to death was analyzed among HIV-negative participants with prevalent or incident HTLV-1 infection and those who remained HTLV-1-negative. Among participants with HTLV-1 and HIV co-infection, we assessed time to death and AIDS, defined as WHO stage IV, $CD4^+$ T cell count ≤200 cells/μL or $CD4^+$ T cell percentage ≤14%, Survival and AIDS-free time were estimated using Kaplan-Meier curves, with group comparisons by log-rank test and Cox proportional hazard model adjusted for age and sex. For incident HTLV-1 infection a final multivariable Cox proportional hazards model additionally adjusted for education, calendar period of cohort entry, and reported urethral discharge. Participants who did not reach AIDS or death during follow-up were right-censored at the date of their last clinical examination. Participants receiving ART were censored from the analysis at the date of ART initiation. Proportional hazards assumptions were assessed using Schoenfeld residuals and log-minus-log plots.

In general, 95% confidence intervals were reported, parametric and non-parametric tests were applied as appropriate. Two-sided p-values were used, with statistical significance set at p<0.05. Data management was performed using Excel for paper I and EpiInfo for papers II-IV. Statistical analyses were conducted using Stata.

Ethical considerations

Participants in all studies received study information prior to enrolment: written and oral information in Papers I-III, while in Paper IV, participants received oral information up to 2011, in subsequent visits written information was also provided. For Papers I-III, informed consent was obtained in writing. In paper IV, up to 2011 only oral consent was required. Thereafter written informed consent was required from all participants. For illiterate participants, thumbprints were used in place of signatures. For Papers II-IV, pre-test and post-test counselling was provided, whereas participants in Paper I had already been diagnosed with HIV.

For the Bissau HIV cohort (paper I), a subset of samples was used from a previous study comparing INNO-LIA and Immunocomb assays for HIV type discrimination (179). Results from that study were sent to Bissau immediately after testing as HIV type had implications for the effectiveness of the ART regimens at the time.

For the FSW (paper II-III), blood samples were screened in the mobile clinic using a rapid HIV antibody test. $CD4^+$ T cell count and VL results were provided to participants at their next visit, with the study nurse assisting with interpretation of the results. All women who received an HIV diagnosis were referred to an HIV treatment clinic of their choice for follow-up and treatment in accordance with national guidelines. HIVDR results were sent to Bissau immediately after testing, and the responsible medical consultant was notified. Women reporting STI symptoms received syndromic treatment, while asymptomatic women with curable STI received directed treatment according to national guidelines.

For participants in the police cohort (paper IV), receipt of HIV test results was voluntary. ART was introduced in Guinea-Bissau in 2005 through a national treatment programme initiated by The Ministry of Health. Following this, interest among participants in receiving HIV test results increased, and by the final years of the study, nearly all participants received their test results. Follow-up and treatment were provided at a health post located at the main police station in Bissau.

Ethical Approval was granted by the National Health Ethics Committee in Guinea-Bissau (Parecer NCP/No. 15/2007, 010/CNES/INASA/2014 and 039/CNES/INASA/2016) (Paper I-IV), by the Regional Ethical Review Board, Lund Univerity (Dnr 2014/424) (Paper II-III), by the Interior Ministry of Guinea-Bissau (Paper IV) and by the Research Ethical Committee at the Karolinska Institute, Stockholm (Paper IV).

Author contributions

For papers II-III, the ethical application process was led by me and Hans Norrgren. Papers I and IV had an existing ethical approval. For papers II-III, I was involved in the design of the studies in a shared lead role with Hans Norrgren. For papers II-III, I had a lead role concerning data collection. For Papers I-IV I had either a lead or shared lead role regarding data curation, methodology, statistical analysis, drafting the manuscript, reviewing and editing.

Results

Paper I

Participants

A total of 131 samples from ART-naïve patients were included in this study. Median age was 37 years (Interquartile range (IQR) 30-46), 72.5% were women and median $CD4^+$ T cell count was 245 cells/µl (IQR 126-433 cells/µl). HIV RNA analyses were performed on all samples while HIV-DNA analyses were performed in available samples typed HIV-1/2 dually reactive and in samples with divergent results by Geenius, INNO-LIA and Immunocomb. Two samples identified as HIV-1/2 dually reactive by Immunocomb, but not confirmed by INNO-LIA or the Geenius Reader, lacked sufficient cell suspension for DNA analysis; therefore, DNA testing was performed on 59 samples.

Key findings

According to the Geenius Reader 62 (47.3%) samples were HIV-1 positive, 37 (28.2%) were HIV-2 positive and 32 (24.4%) were HIV-1/2 dually reactive. Visual reading of the Geenius assay, following the manufacturer's instructions, classified 10% more samples as HIV-1/2 dually reactive (n=35) compared with the Geenius Reader. There was accordance between the Geenius Reader and NAAT in all samples except one in which HIV RNA and DNA were measured; none of the HIV-1-positive samples had detectable HIV-2 RNA or DNA, one sample typed as HIV-2-positive by the Geenius Reader had detectable HIV-1 RNA but not DNA (Table 3). Of the 32 HIV-1/2 dually reactive samples, 15 (46.9%) could not be confirmed by nucleic acid detection.

The agreement between the Geenius Reader and INNO-LIA was 92.4%. Results from NAAT were fully consistent with those from INNO-LIA; no HIV-1-positive samples showed detectable HIV-2 RNA or DNA, and no HIV-2-positive samples showed detectable HIV-1 RNA or DNA.

The agreement between the Geenius Reader and Immunocomb was 84.0%, and thus lower than for the Geenius Reader and INNO-LIA (92.4%). The observed discrepancy was primarily due to challenges in distinguishing HIV-2-positive

samples from HIV-1/2 dually reactive samples. Of the 43 samples classified as HIV-1/2 dually reactive by Immunocomb (N=45) in which both HIV-RNA and DNA were measured, 25 (58.1%) could not be confirmed by NAAT.

Table 3. HIV RNA and DNA detection by INNO-LIA, Geenius Reader and Immunocomb results

	INNO-LIA			Geenius Reader			Immunocomb		
	HIV-1	HIV-2	HIV-1/2	HIV-1	HIV-2	HIV-1/2	HIV-1	HIV-2	HIV-1/2
	N=63	N=36	N=32	N=62	N=37	N=32	N=63	N=23	N=45
HIV-1 RNA detected n(%)	62 (98.4)	0 (0.0)	30 (93.8)	61 (98.4)	1 (2.7)	30 (93.8)	62 (98.4)	0 (0.0)	30 (66.7)
HIV-2 RNA detected n(%)	0 (0.0)	21 (58.3)	13 (40.6)	0 (0.0)	23 (62.2)	11 (34.4)	0 (0.0)	14 (60.9)	20 (44.4)
HIV-1 DNA detected n(%)	8 (100.0)	0 (0.0)	28 (87.5)	7 (100.0)	0 (0.0)	29 (90.6)	8 (100.0)	0 (0.0)	28 (65.1)
HIV-2 DNA detected n(%)	0 (0.0)	19 (100.0)	18 (56.3)	0 (0.0)	20 (100.0)	17 (53.1)	0 (0.0)	8 (100.0)	29 (67.4)
Samples not confirmed HIV-dually reactive by HIV-1/2 RNA/DNA detection			14 (43.8)			15 (45.5)			25 (58.1)
HIV-1/2 dually reactive samples only positive for HIV-1 DNA and/or RNA			12 (37.5)			13 (40.6)			12 (27.9)
HIV-1/2 dually reactive samples only positive for HIV-2 DNA and/or RNA			2 (6.3)			2 (6.3)			13 (30.2)

Paper II

Participants and HIV prevalence

Between October 2014 and September 2017, 847 women visited the mobile clinic and were screened for eligibility. Of these, 490 provided written informed consent to participate. 50 women did not meet the study definition of sex work and were excluded. The final analytic sample comprised 440 women who met the inclusion criteria. Median age was 28 (IQR 22-35). At inclusion, the overall HIV-prevalence was 26.8%. HIV-1 prevalence was 23.6% (including FSW with HIV-1/2 dual infection), HIV-2 prevalence was 6.4% (also including HIV-1/2 dual infections) and

the prevalence of HIV-1/2 dual infection was 3.2%. To assess temporal trends in HIV prevalence, the study period was divided into three intervals: October 2014-September 2015 (32.6%), October 2015-September 2016 (26.0%), and October 2016-September 2017 (21.4%). Although HIV prevalence appeared higher during the first two intervals compared to the final period, these differences were not statistically significant after adjustment for age (p=0.580).

HIV care continuum

Among FSW with HIV-1 (including those with HIV-1/2 dual infection), 58.7% were aware of their HIV status. Of those aware, 70.5% reported current use of ART. When using all FSW with HIV-1 as the denominator, 41.4% reported current ART use, and among them, 55.8% were virally suppressed, corresponding to 23.1% of all women with HIV-1. An additional 6.7% of FSW with HIV-1 were virally suppressed despite not reporting ART use, bringing the total proportion of virally suppressed FSW to 29.8%.

Among FSW with HIV-2 only (not including FSW with HIV-1/2 dual infection), 64.3% were aware of their HIV status, and 28.6% reported current ART use. HIV-2 VL was not measured.

We investigated factors associated with undiagnosed HIV infection and found that younger FSW (<30 years) were more likely to be unaware of their HIV status (odds ratio (OR) 2.7, 95% CI 1.2-6.5). Undiagnosed HIV infection was also associated with inconsistent condom use with clients (OR 2.7 95% CI 1.04-8.9).

HIV-1 resistance mutations

HIVDR was measured in 53 treatment-naïve FSW with HIV-1, including three with seroincident HIV-1, and in 22 ART-experienced FSW with HIV-1 (reporting current ART use or treatment interruption) with VLs ≥1000 copies/mL. One sample from a treatment-naïve participant could not be analysed due to insufficient plasma volume. HIVDR was also investigated in nine FSW with HIV-1 and VLs <1000 copies/mL: no resistance mutations were detected in this subgroup.

Among treatment-naïve FSW with HIV-1, HIVDR was detected in 9.4%, with NNRTI-associated mutations in 5.7% and NRTI-associated mutations in 3.8%. Among ART-experienced FSW with HIV-1, HIVDR was identified in 50.0%: 18.2% had NNRTI mutations only, 9.1% had NRTI mutations only, and 18.1% had both NNRTI and NRTI mutations. The most frequently observed NNRTI mutation was K103N/S, found in 43.8% of those with HIVDR, conferring reduced susceptibility to efavirenz and nevirapine (391-393). M184V was the most common NRTI mutation, detected in 37.5% of those with HIVDR (exclusively among ART-

experienced FSW), conferring reduced susceptibility to lamivudine and FTC (394, 395).

Paper III

Participants

Between October 2014 and May 2019, 1082 women visited the mobile clinic and were screened for eligibility. Of these, 639 provided written informed consent to participate. 52 women did not meet the study definition of sex work and were excluded. 120 women declined to provide vaginal sample. The final analytic sample comprised 467 women who met the inclusion criteria and had a complete biological sample (blood sample and vaginal sample).

The median age of the women was 27 years (IQR 22-33), and most were Bissau-Guinean nationals (90.6%). The study also included women from The Gambia, Ghana, Guinea-Conakry, Liberia, Nigeria, Senegal, and Sierra Leone. Approximately one-third (30.8%) were recruited in the capital, Bissau. The median duration of formal education was 6 years (IQR 0-9), and the median number of live children was 1 (IQR 0-3). Nearly half (43.4%) reported additional income from informal sector activities, predominantly street vending of food items. Most women were single (56.8%), while 17.5% were divorced or separated, 14.0% married, and 11.6% widowed. The median age at initiation of sex work was 16 years (IQR 15-18). Participants reported a median of 1 sex partner in the previous week (IQR 1-12) and 1 client on the most recent day of sex work (IQR 1-2). Median income from the last client was US$4.1 (IQR 2.5-8.2).

Curable STIs: Prevalence, HIV co-infection, and associated risk factors

The prevalence of current infection with any curable STI was high, affecting nearly half of the women (46.7%). A similar proportion (46.5%) reported experiencing current STI symptoms. However, there was no significant association between reported symptoms and laboratory-confirmed curable STIs (OR 0.97 95% CI 0.67-1.41). The distribution of curable STIs were similar between among women with or without symptoms (Figure 4). *T. vaginalis* was the most frequently detected pathogen (26.3%), followed by *M. genitalium* (21.9%), *C. trachomatis* (11.8%), *N. gonorrhoeae* (10.3%), and *T. pallidum* (2.8%). Co-infection with two or more pathogens was identified in 15.2% of women, with the most common co-infection being *C. trachomatis* and *N. gonorrhoeae* (N=23). Among the *N. gonorrhoeae*-positive samples collected before September 2017, a *gyrA* result was successfully

generated for 31 of 42 samples, with the majority (83.9%) showing the GyrA S91F mutation, which confers resistance to ciprofloxacin.

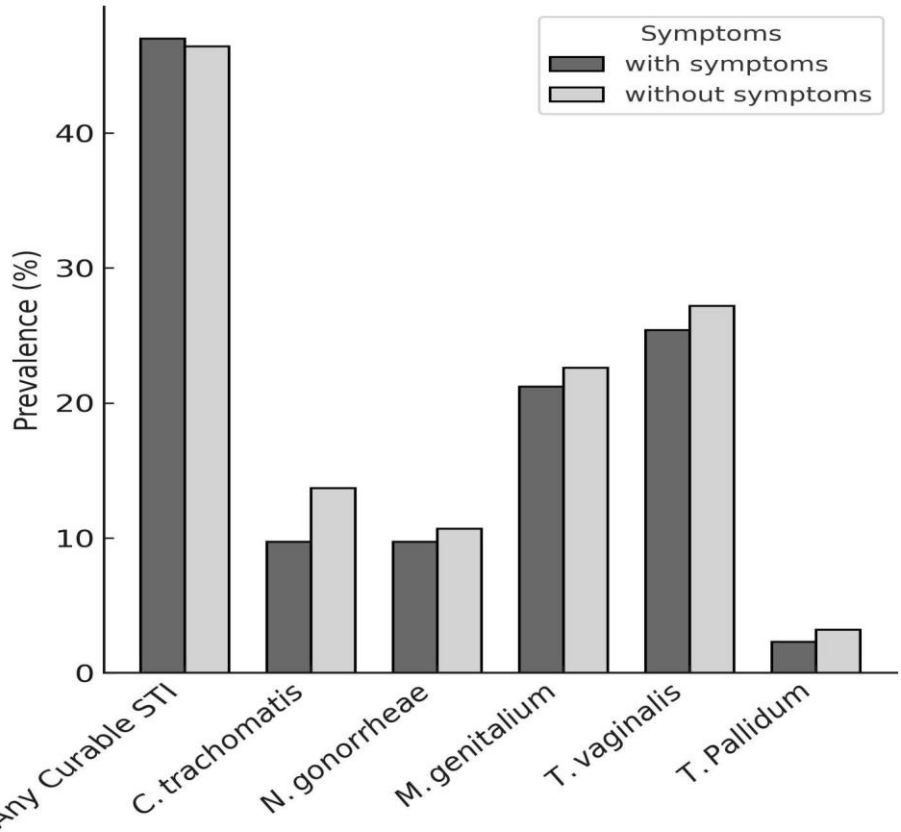

Figure 4. STI prevalence by current symptoms.

HIV-1 prevalence was 27.0% (including FSW with HIV-1/2 dual infection), HIV-2 prevalence 6.2% (including FSW with HIV-1/2 dual infection), and the prevalence of HIV-1/2 dual infection was 3.0%. Curable STIs were more prevalent among FSW with HIV-1 (56.3%) than among those without HIV-1 (43.1%), with a significant association after age adjustment (OR 1.91, 95% CI 1.25-2.91). Among FSW with HIV-1, those with viraemia (VL ≥1000 copies/mL) had higher STI prevalence than those with suppressed VL (63.2% vs 37.9%, OR 2.89, 95% CI 1.19-7.10). No significant difference in STI prevalence was observed between FSW with HIV-2 (48.3%) and HIV-2-negative women (47.7%) (OR 1.32, 95% CI 0.59-2.96). *M. genitalium* was significantly more common among FSW with HIV-1 (30.2%) compared with HIV-1-negative women (18.8%) (OR 2.05, 95% CI 1.14-3.70), as

was *T. Pallidum* seropositivity (TPPA positive: 17.6% vs 6.5%, OR 2.56, 95% CI 1.34-4.90).

In univariable analysis, risk factors for curable STIs included older age, HIV-1 infection, and having multiple clients on the most recent day of sex work. Residence in the capital Bissau was also borderline associated with increased risk. Prior use of female condoms was associated with a reduced risk of infection. In multivariable analysis, women aged 26-35 years (OR 1.86, 95% CI 1.05-3.27) and those with HIV-1 (OR 1.90, 95% CI 1.18-3.03) had significantly higher odds of having a curable STI, while previous use of female condoms remained a protective factor (OR 0.41, 95% CI 0.17-0.95).

Paper IV

Participants

Of the 4607 individuals enrolled between 1990 and 25 April 2008, 4590 were screened for HTLV at enrolment. A total of 185 participants were diagnosed with HTLV-1 at enrolment, and an additional 41 acquired HTLV-1 during follow-up. Among those with HTLV-1, 28 had HIV-1 co-infection and 48 had HIV-2 co-infection, either at enrolment or during follow-up. The timing of co-infection varied: some participants acquired HTLV-1 before HIV, others acquired HIV before HTLV-1, while some had co-infection at enrolment or aquired both viruses concurrently during follow-up. Additionally, four participants were diagnosed with HTLV-2 at enrolment and one individual acquired HTLV-2 during follow-up.

Women represented 12.8% of the cohort. Median age among women was 30 years (IQR 25-38), significantly lower than that of men (36 years, IQR 28-45, $p < 0.001$). The majority of participants were married (74.1%), and among these, nearly one-third (27.6%) reported practising polygamy. Condom use was infrequent, with 58.7% reporting never having used condoms. Among male participants, 8.0% reported ever having visited a FSW. Self-reported urethral or vaginal discharge was common, reported by 41.2% of participants

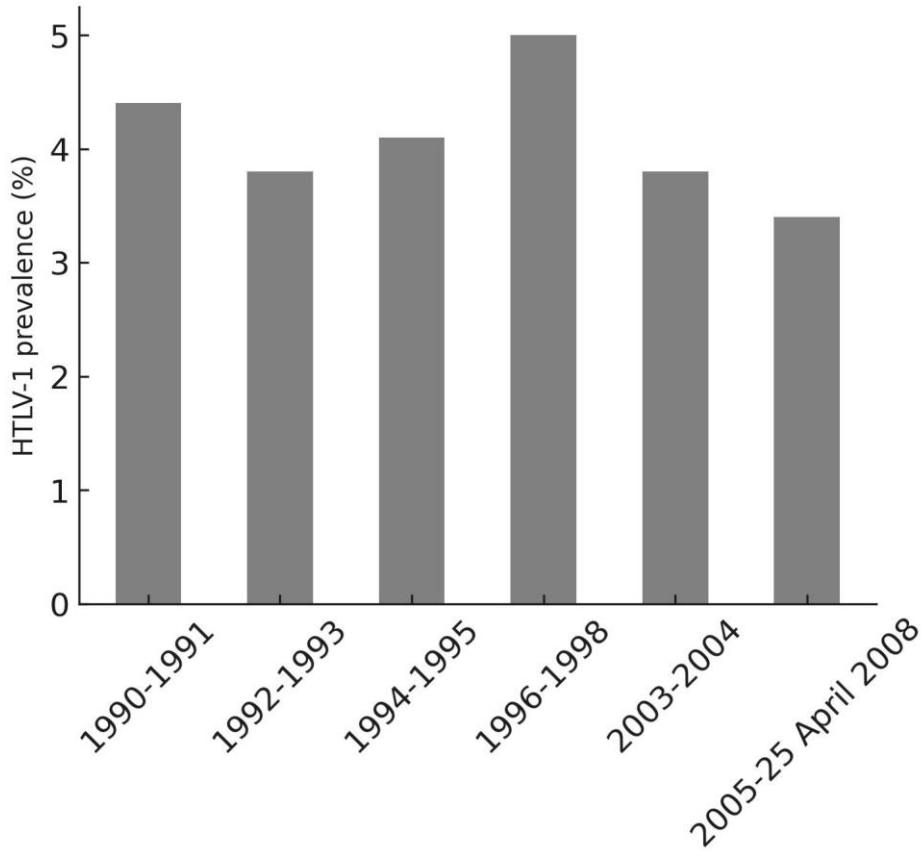

Figure 5. HTLV-1 prevalence 1990-2008 stratified by 2-3-year time periods.

HTLV-1 prevalence and incidence

The overall HTLV-1 prevalence was 4.0%, and the overall incidence was 0.25 per 100 PY. Trends in HTLV-1 prevalence and incidence by 2-3-year intervals are presented in Figure 5 and Figure 6, respectively.

HTLV-1 prevalence was 4.3% in 1990-1999 and 3.7% in 2000-2008, with no statistically significant difference after adjustment for age and sex (OR 0.80, 95% CI 0.58-1.10). In contrast, HTLV-1 incidence declined significantly, from 0.31 per 100 PY in 1990-1999 to 0.10 per 100 PY in 2000-2006 (IRR 2.32, 95% CI 1.13-4.75, adjusted for age and sex).

Risk factors for HTLV-1 infection at enrolment included age >45 years (OR 1.83, 95% CI 1.32-2.54), female sex (OR 1.86, 95% CI 1.23-2.73), HIV-2 infection (OR

2.35, 95% CI 1.54-3.58), history of blood transfusion (OR 2.01, 95% CI 1.09-3.72), and ever visited a FSW (OR 1.99, 95% CI 1.25-3.14).

HIV-2 infection at enrolment (IRR 2.48, 95% CI 1.13-5.45) and never using condoms compared to sometimes or always (IRR 2.21, 95% CI 1.14-4.30) were independently associated with increased risk of HTLV-1 seroconversion.

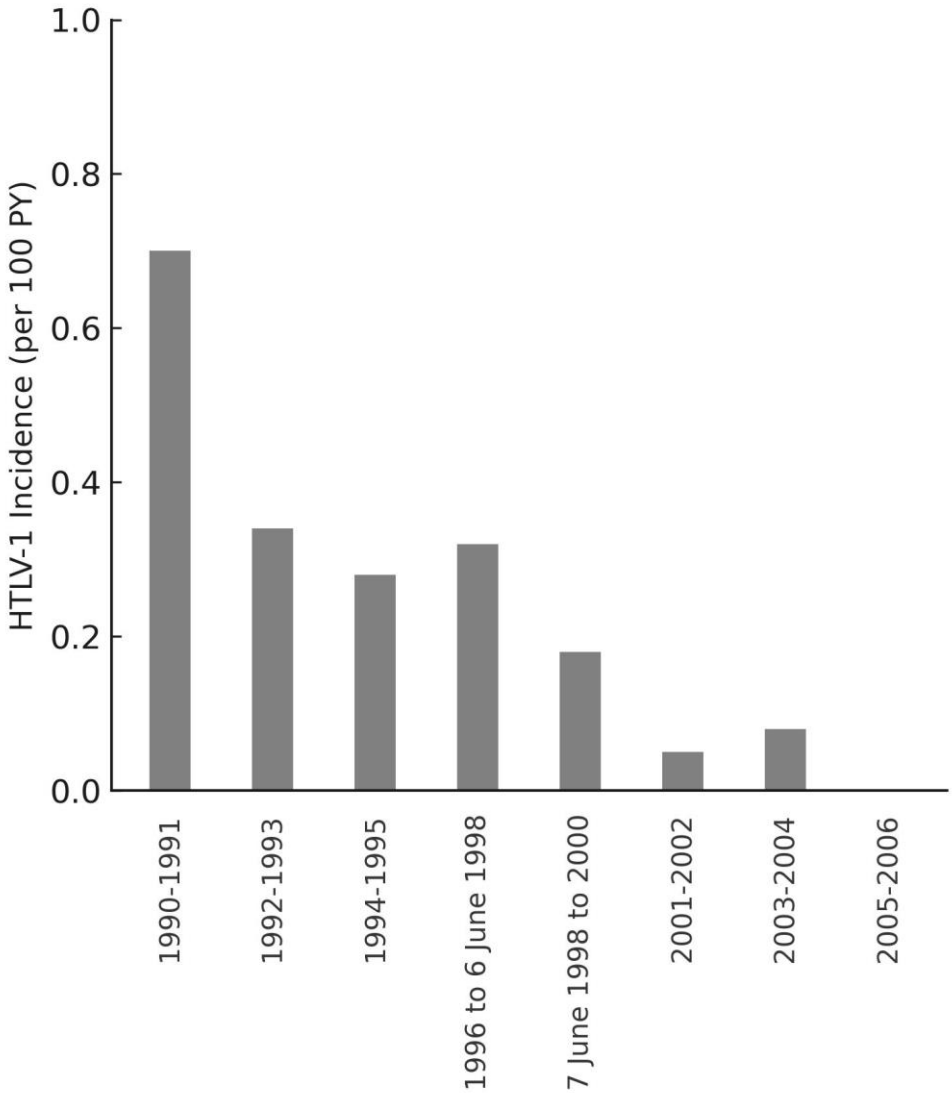

Figure 6. HTLV-1 incidence 1990-2006 stratified by 2-3-year time periods.

HTLV-1 associated mortality and impact of HIV co-infection

We observed a crude mortality rate of 2.90 per 100 PY (95% CI 1.30-6.46) among HIV-negative participants who acquired HTLV-1 during follow-up, compared to 0.96 per 100 PY (95% CI 0.84-1.09) among HIV-negative and HTLV-1 negative participants. In Cox proportional hazards model adjusting for age and sex, HTLV-1 seroconversion in HIV-negative participants was associated with an increased risk of death (hazard ratio (HR) 3.04, 95% CI, 1.35-6.88) (Figure 7). This association remained significant in a multivariable model additionally adjusting for education level, calendar period of cohort entry and self-reported urethral discharge (HR 3.37, 95% CI, 1.48-7.69). In contrast, no significant difference in mortality was observed between HIV-negative participants with HTLV-1 at enrolment and those who were HTLV-1 negative at enrolment.

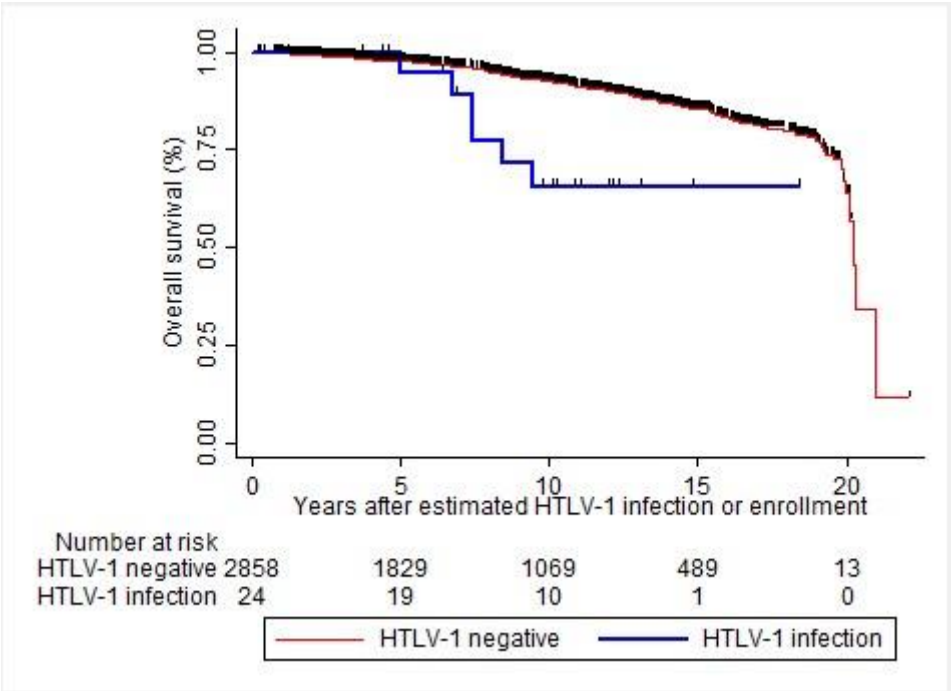

Figure 7. Kaplan-Meier survival curves for participants who acquired HTLV-1 during follow-up and for HTLV-negative participants. Tick marks indicate censored observations.

Among participants who acquired HIV-1 during follow-up, we compared outcomes between 17 participants with HTLV-1 co-infection and 208 participants with HIV-1 mono-infection. Median survival was shorter in participants with co-infection (6.8 years, 95% CI 5.6-8.7) compared to those with HIV-1 mono-infection (8.7 years, 95% CI 6.2-11.6, p=0.011) (Figure 8). In a Cox regression model adjusted for age and sex, co-infection was associated with increased risk of death (HR 1.88, 95% CI

1.06-3.34). Time to AIDS did not differ significantly between groups: 5.7 years (95% CI 5.7-8.1) among participants with co-infection and 4.3 years (95% CI 2.3-7.2) among participants with HIV-1 mono-infection (p=0.398). In a multivariable linear regression model with log-transformed $CD4^+$ counts, HTLV-1 and HIV-1 co-infection was independently associated with a 43% higher $CD4^+$ T cell count (β = 0.36, 95% CI, 0.10-0.62), adjusting for age, sex, and time since estimated HIV-1 infection. However, due to limited follow-up data (only six participants with co-infection had ≥2 $CD4^+$ measurements), $CD4^+$ T cell dynamics over time could not be evaluated.

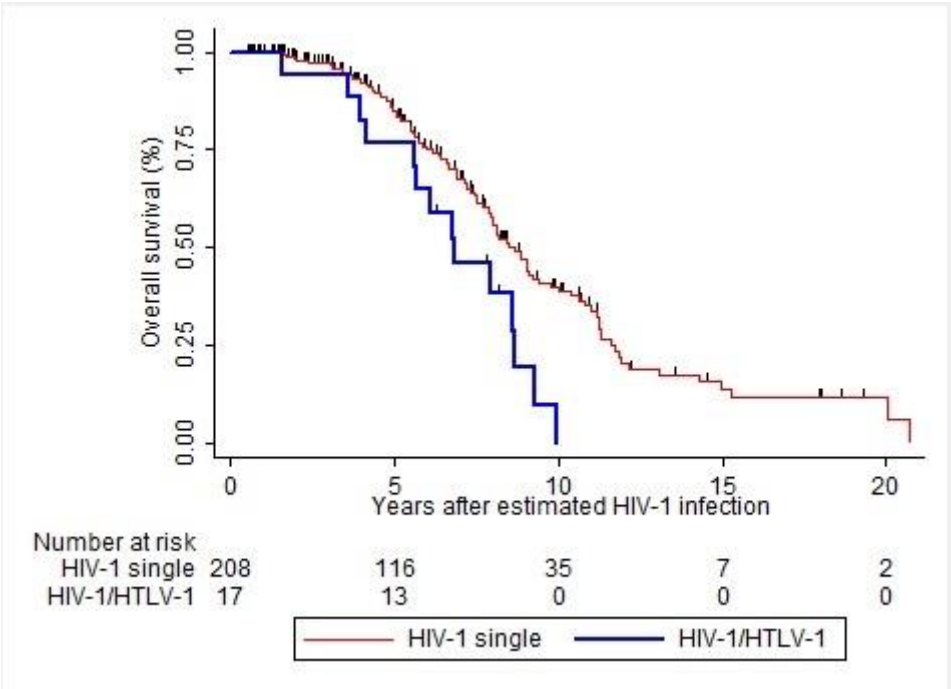

Figure 8. Kaplan-Meier survival curves for participants with HTLV-1 and HIV-1 co-infection, and for those with HIV-1 mono-infection. Tick marks indicate censored observations.

In contrast to the significant effect of HTLV-1 on HIV-1 disease progression, survival was similar in participants with HTLV-1 and HIV-2 co-infection (n=35) (16.5 years, 95% CI 8.2-21.9) and those with HIV-2 mono-infection (n=330) (18.8 years, 95% CI 9.3-20.8, p=0.937). This analysis was restricted to participants HTLV-1 and HIV-2 co-infection at enrolment, as only four participants with HTLV-1 acquired HIV-2 during follow-up.

Discussion

Discriminating between HIV-1, HIV-2, and HIV-1/2 dual infection: a diagnostic challenge

In paper I we demonstrated that the Geenius assay performs better than Immunocomb in discriminating between HIV-1, HIV-2 and HIV-1/2 dual infections, and both the Geenius Reader and visual reading of the Geenius assay showed good concordance with INNO-LIA. However, visual reading of the Geenius assay appeared to overestimate the number of HIV-1/2 dually reactive samples, a pattern also reported in previous studies (185, 193). Although ImmunoComb has likewise been shown to overestimate HIV-1/2 dual reactivity (171, 396, 397), the extent of misclassification in our material was greater. This likely reflects inter-reader variability, which has previously been reported (179). In contrast, in our study the Geenius assay was read by two independent observers with complete agreement, supporting its reliability when read visually.

The most frequent misclassification involved blood samples from individuals with HIV-2 being incorrectly classified as HIV-1/2 dually reactive. While this may not affect initial ART selection, it has clinical implications, as individuals with HIV-1/2 dual infection have been shown to progress to AIDS faster than those with HIV-2 (124, 128). Beyond individual treatment decisions, diagnostic misclassification also has broader implications for epidemiological surveillance and programmatic planning.

The selection of an appropriate diagnostic test requires careful consideration of each assay`s strengths and limitations. In 2003, the World Health Organization's Special Programme for Research and Training in Tropical Diseases outlined a set of criteria for diagnostic tests intended for use in low-resource settings. These criteria, summarized by the acronym ASSURED (Affordable, Sensitive, Specific, User-friendly, Rapid and Robust, Equipment-free, and Deliverable to end-users), have since been widely recognized as the gold standard for POC diagnostics in low-income countries (398, 399). When evaluating the Geenius assay against the ASSURED criteria, several factors merit consideration. In terms of affordability, the cost per sample is comparable to that of INNO-LIA but exceeds that of other rapid diagnostic tests currently used in Guinea-Bissau. Moreover, in settings like Guinea-Bissau where HIV-1/2 dual reactivity is relatively common, our findings

support the use of the Geenius Reader. However, compared to visual interpretation alone, its implementation increases financial costs, which could limit feasibility in resource-constrained settings.

Regarding sensitivity and specificity, both our findings and previous evaluations have demonstrated that the Geenius assay performs well in differentiating HIV-1, HIV-2, and HIV-1/2 dual infections. In terms of user-friendliness, the assay is relatively simple to operate and requires only minimal laboratory training. Nevertheless, a study conducted in South Africa revealed that only 3% of HIV rapid diagnostic tests were correctly performed (400), emphasizing the importance of continuous and adequate training for healthcare personnel. Concerning turnaround time (rapid) and robustness, the assay produces results within 30 minutes and can be stored at room temperature, aligning well with these criteria. As for the equipment-free criterion, the Geenius assay can be interpreted visually, eliminating the need for a Reader in certain contexts. Finally, with regard to deliverability to end-users, this aspect is highly dependent on the efficiency of national procurement and distribution systems rather than the test characteristics.

More recently, two additional criteria have been proposed to complement the original ASSURED framework: real-time connectivity and ease of specimen collection and environmental friendliness, forming the acronym REASSURED (401). In terms of real-time connectivity, the Geenius Reader supports electronic storage of results, which supports centralized reporting and surveillance. Regarding ease of specimen collection, the Geenius assay requires invasive sampling through fingerstick or venous blood, which can present challenges in certain community settings. Environmental friendliness is more difficult to assess, but it is notable that the test cartridges are composed of non-renewable plastic materials.

Due to the risk of serological cross-reactivity, HIV NAAT is considered the gold standard for confirming HIV-1/2 dual infection. However, this method has limitations in diagnosing HIV-2, as many PWH-2 may have undetectable plasma HIV-2 RNA even without ART (378). In some cases, proviral HIV-2 DNA is also undetectable (172). As such, a negative NAAT result does not exclude HIV-2 infection, highlighting the diagnostic challenges in settings lacking molecular testing and relying on potentially suboptimal discriminatory serological assays

In conclusion, the Geenius assay outperforms Immunocomb in differentiating between HIV-1, HIV-2, and HIV-1/2 dual infections, and offers diagnostic accuracy comparable to that of INNO-LIA. Diagnostic performance is further improved when used with the Geenius Reader. However, the cost associated with both the test and the Geenius Reader may limit its feasibility in low-resource settings such as West Africa, where HIV-2 and HIV-1/2 dual infections still remain relatively common.

HIV, STI burden and care gaps in female sex workers in Guinea-Bissau

In these studies (paper II and III) among FSW in Guinea-Bissau, we found a high burden of HIV, curable STIs, and HIVDR, highlighting persistent gaps in both prevention and care.

Total HIV-1 prevalence (23.6% in paper II and 27.0% in paper III) was approximately four times higher than total HIV-2 prevalence (6.4% in paper II and 6.2% in paper III) when including women with HIV-1/2 dual-infection in each group. The relatively low HIV-2 prevalence aligns with the declining trend observed in previous studies (62-69). Compared to adult women in the most recent community-based study from Bissau, which reported HIV-1 and HIV-2 prevalence of 5.2% and 3.4% respectively (69), the prevalence of HIV-1 was more than four times higher, while HIV-2 prevalence was nearly twice as high. At the time the study presented in paper II was published, no data on the HIV care continuum were available for the general population in Guinea-Bissau. Since then, a study conducted in Bissau during a similar period (2014-2016 vs 2014-2017) has reported on these indicators (6). While viral suppression rates among PWH-1 were comparable (29.8% in our study vs 26.1%), awareness of HIV status (58.7% vs 21.5%) and reported ART use (41.4% vs 8.5%) were both notably higher among FSW in our study compared to the general population. However, the similar rate of viral suppression despite higher awareness of HIV status and ART use among FSW highlights the need for future interventions to improve linkage to care, adherence, and retention.

Younger age (OR 2.7, 95% CI 1.2-6.5) and inconsistent condom use (OR 3.0, 95% CI 1.04-8.4) were associated with undiagnosed HIV infection, suggesting ongoing transmission among younger FSW and highlighting the need for targeted prevention. Due to these findings, and the high HIV prevalence, low ART coverage, and limited viral suppression observed in our study, we explored the feasibility of conducting a PrEP study in Guinea-Bissau, as no national PrEP programme existed or was planned at the time. We assessed PrEP awareness and interest among 147 HIV-negative FSW and found that while 98.0% had never heard of PrEP, 86.4% expressed interest in using it. Importantly, 94.4% reported that they would continue to use condoms as before if taking PrEP, which was reassuring and supports the feasibility of introducing PrEP without compromising existing prevention strategies (unpublished data). In 2019, based on these findings, we planned an open-label randomized trial including 150 FSW in Bissau, to evaluate whether text message reminders could improve retention in care and adherence to PrEP, using plasma tenofovir levels as an objective adherence measure. The study was unfortunately delayed due to the COVID-19 pandemic and was ultimately cancelled due to funding constraints.

Nonetheless, since the study presented in paper II, ENDA has scaled up services for FSW in Guinea-Bissau. According to personal communication with Kátia Ribeiro Barreto, Program Director at ENDA Guinea-Bissau, a dedicated HIV clinic for FSW was established in recent years and as of April 2025 provides ART to 37 FSW in addition to 23 MSM. Between April and December 2023, a pilot PrEP programme was initiated, enrolling 50 FSW and 65 MSM. No incident HIV infection was reported among participants, though the small sample size and short follow-up duration limit the interpretation. The programme was cancelled due to lack of funding, but ENDA has applied for renewed support with plans to resume PrEP distribution in 2026.

The prevalence of *C. trachomatis* (11.8%), *N. gonorrhoeae* (10.1%), *M. genitalium* (21.9%) and T. *vaginalis* (26.3%) observed in our study was higher or comparable to previously ranges in systematic reviews of FSW across sub-Saharan Africa, which estimated genital prevalence between 4.2% to 7.3% for *C. trachomatis* (287), 5.4% to 11.0% for *N. gonorrhoeae* (287), 13.5% to 18.9% for *M. genitalium* (289) and 7% to 46% for *T. vaginalis* (288). As expected the prevalence of curable STIs in our study was higher than what was reported in the most recent community-based study in Guinea-Bissau which reported a prevalence of 5.7% for *C. trachomatis*, 3.9% for *N. gonorrhoeae*, 2.8% for *M. genitalium*, 9.9% for *T. vaginalis* and 0.8% for *T. Pallidum* (295).

The high prevalence of curable STIs and HIV observed in this study suggests ongoing transmission within sex work networks and likely reflects suboptimal condom use and insufficient clinical interventions. Several strategies could help to reduce STI burden in this context. First, STI services must be able to detect both symptomatic and asymptomatic infections. Second, condom interventions should address the structural challenges that limit consistent use in sex work (402). As transmission in key populations can drive broader community spread, targeted interventions and investments in prevention among FSW may have wider public health benefit and can contribute to wider STI control efforts (403). Interestingly, our study indicated a protective effect of female condom use against curable STIs. This aligns with evidence from a systematic review, which found that combined use of female condoms and male condoms was more effective in reducing the risk of *C. trachomatis* (RR 0.59, 95% CI 0.41-0.86) and *N. gonorrhoeae* (RR 0.67, 95% CI 0.47-0.94) compared to male condoms alone (404). Despite this, uptake in our study was low, with only 7.9% of women reporting ever using female condoms. As a female-initiated method potentially offering greater autonomy and demonstrated effectiveness, expanding access to female condoms within existing programmes warrants further consideration.

In Guinea-Bissau, management of curable STIs currently relies on syndromic approaches, a strategy that fails to identify many infections and leave many FSW untreated (285). In our study the prevalence of curable STIs was nearly identical among symptomatic and asymptomatic women (47.0% and 46.4%), even after

symptom prompting, highlighting the limitations of the syndromic management approach. This results in a diagnostic gap that sustains high prevalence and supports ongoing transmission. At the same time, most symptomatic women did not have a laboratory-confirmed curable STI (53.0%) illustrating that syndromic management also leads to frequent overtreatment, thereby contributing to the growing problem of antimicrobial resistance (405). The GyrA S91F mutation, a marker of ciprofloxacin resistance, was detected in 83.9% of *N. gonorrhoeae*-positive samples, a substantially increase compared to the 10% resistance reported in 2006-2008 in Guinea-Bissau (406). The high resistance level in our study demonstrates that ciprofloxacin is no longer appropriate for gonorrhoea treatment in Guinea-Bissau, in line with resistance trends reported globally (255).

The Global HIV Prevention Coalition advises quarterly clinical visits as part of "trusted access platforms" for key populations, a recommendation shaped by experiences from countries such as Kenya and India (407). While routine screening could be an alternative to syndromic management, resource constraints and delayed results presents major barriers, particularly in outreach contexts like evening mobile clinics. In such settings, POC tests that do not require electricity, are simple to use, provide a result within 30 minutes and are affordable would be required. Periodic presumptive treatment has shown effectiveness in other settings (408), and may be worth considering though it risks substantial overtreatment and could drive antimicrobial resistance. Doxycycline post-exposure prophylaxis might offer a potential strategy to prevent curable STIs after unprotected sex. However, a recent trial from Kenya found no reduction in STI incidence among cisgender women receiving doxycycline post-exposure prophylaxis compared to those who did not (409). These findings suggest that the protective effect observed in MSM and transgender women may not extend to cisgender women (410)

These findings highlight persistent gaps in HIV and STI prevention and care among FSW in Guinea-Bissau, including high prevalence, ongoing transmission, limited viral suppression, and rising antimicrobial resistance. Targeted, evidence-based interventions, such as PrEP, condoms including female condoms, improved diagnostic tools, and tailored outreach, are urgently needed to address these challenges and reduce the burden of disease in this key population

Trends and risk factors for HTLV-1 infection in Guinea-Bissau

In paper IV, we reported a non-significant decline in HTLV-1 prevalence from 4.3% between 1990 and 1999 to 3.7% between 2000 and 2008 (OR 0.80, 95% CI 0.58-1.10). Furthermore, we observed a significant decrease in HTLV-1 incidence during the same period, from 0.3 per 100 PY to 0.1 per 100 PY (IRR 2.32, 95% CI 1.13-

4.75). These findings are in line with previous studies from Guinea-Bissau, which reported declining HTLV-1 prevalence over time in both urban Bissau (from 3.6% in 1996 to 2.3% in 2006) (353, 354) and in rural Caió (from 5.9% in 1997 to 4.5% in 2007) (356). However, a more recent study conducted in Bissau between 2014 and 2016, reported an increase in HTLV-1 prevalence to 2.8%, which the authors attributed to ongoing sexual and vertical transmission (355).

Consistent with earlier research, we found that older age, female sex and HIV-2 infection were associated with prevalent HTLV-1 infection (353-356). The association with female sex is well established and likely reflects the higher efficiency of male-to-female transmission. In addition, older women, may be more susceptible due to an increased mucosal fragility and atrophy associated with age (411). However, in our study female sex was not associated with HTLV-1 seroconversion during follow-up, which may reflect the limited number of female participants and insufficient power to detect an association.

Older age as risk factor likely represents cumulative exposure to risk over time, particular to unprotected sex, although parenteral transmission during the independence war may also have contributed. HIV-2 infection was associated to both prevalent and incident HTLV-1 infection, and inconsistent condom use was a risk factor for seroconversion. These findings likely reflect shared modes of transmission and overlapping risk factors for retroviral infections. As described in the introduction, the spread of HIV-2 in Guinea-Bissau is thought to have coincided with independence war and that parenteral rather than sexual transmission likely was playing a major role (34, 77, 78). This could also help explain the higher HTLV-1 prevalence observed in the 1990s.

We also found that a history of blood transfusion was associated with HTLV-1 infection at enrolment, supporting the role of parenteral transmission. This has been documented previously (353, 355). Moreover, having ever visited a FSW was identified as a risk factor for HTLV-1 infection at enrolment further supporting the role of sexual transmission. However, data on HTLV-1 prevalence among FSW in Guinea-Bissau are lacking. An older study from The Gambia reported an HTLV-1 prevalence of 10.4% among FSW in the 1990s, highlighting the potential role of sexual transmission in HTLV-1 epidemiology and the need for targeted prevention efforts (412).

Taken together, our findings support a decline in HTLV-1 prevalence and incidence from the 1990s to the 2000s, consistent with earlier studies from Guinea-Bissau. This may reflect improved public health measures or shifts in sexual behaviour. Risk factors for HTLV-1 infection largely overlapped with those for other retroviruses, underscoring the role of shared transmission routes. The potential involvement of sex work in ongoing transmission highlights the need for further investigation among key populations.

HTLV-1 and mortality: outcomes in PWH and HIV-negative individuals

In Paper IV, we demonstrated that individuals with both HTLV-1 and HIV-1 had an increased risk of death compared to those with HIV-1 alone, as estimated by Cox proportional hazards models. We also found that HIV-negative individuals who acquired HTLV-1 during follow-up had an increased risk of death compared to those who remained HTLV-1-negative. These findings are based on longitudinal data from individuals with estimated dates of HTLV-1 or HIV-1 infection. Furthermore, to minimize the confounding effect of ART, participants with HIV were censored at the date of ART initiation.

Median survival was nearly two years shorter among participants with both HTLV-1 and HIV-1 compared to those with HIV-1 alone. This association remained statistically significant after adjusting for age and sex in a Cox proportional hazards model (HR 1.88, 95% CI 1.06-3.33). This finding is consistent with previous studies, although those lacked known infection dates and included individuals with varying ART exposure (359-361). Interestingly, participants with co-infection had higher $CD4^+$ T cell counts at first measurement despite worse outcomes, a paradox also noted in other studies (364, 368). While delayed ART initiation due to higher $CD4^+$ T cell counts has been proposed as one explanation, our study took place largely in the pre-ART era, and participants were censored at ART initiation, limiting this source of bias. Functional impairment of $CD4^+$ T cells, including reduced naïve T cells and increased activation have been reported in individuals with both HTLV-1 and HIV-1, and may partly explain the poorer prognosis in this group (367). Although further mechanistic studies are lacking, given that chronic HTLV-1 infection is associated with persistent immune activation, it is plausible that HIV acquisition occurs within a pre-activated immune environment, potentially accelerating disease progression. Encouragingly, in the context of universal ART initiation regardless of $CD4^+$ T cell count, comparable survival outcomes have been observed among individuals with HTLV-1 and HIV-1 and those with HIV-1 mono-infection, provided they are on ART and achieve viral suppression (VL <50 copies/mL) (359).

Among HIV-negative participants, we found that those who acquired HTLV-1 during follow-up had more than a threefold increased risk of death compared to HTLV-1-negative individuals (HR 3.37, 95% CI, 1.48-7.69) independent of age, sex, educational level, calendar period of enrolment and a proxy indicator of sexual risk behaviour (self-reported-urethral discharge). In contrast, HTLV-1 infection at enrolment was not significantly associated with an increased risk of death. The mechanism underlying these divergent findings remain unclear. Primary HTLV-1 infection is generally considered asymptomatic, and HTLV-1-associated diseases

such as HAM/TSP and ATL typically emerge only after years of chronic infection (413).

One possible explanation relates to high HTLV-1 PVL, which has been linked to increased mortality (342). Additionally, high HTLV-1 antibody titres, which are correlated with elevated HTLV-PVL (346), have also been shown to be associated with increased all-cause mortality (338). In a study that stratified the mortality risk by age, individuals aged 15-29 years, presumably with a shorter duration of infection, had a RR of 3.80 (95% CI 1.70-8.50), compared to 1.20 (95% CI 0.90-1.50) among those ≥60 years (341). A high HTLV-1 PVL has also been associated with increased risk for both communicable diseases (348, 349) and non-communicable diseases (332, 333, 350, 351). Moreover, HTLV-1 PVL tends to decline over time during the chronic phase, suggesting that the viral burden may be higher shortly after infection (310). This raises the possibility that heightened immune activation and inflammation during the early phase of infection may contribute to the increased risk of death observed in HTLV-1 seroconverters, but not in participants with HTLV-1 at enrolment.

An alternative explanation is that the risk of death may vary by mode of transmission. Most HTLV-1 infections acquired during follow-up are likely to have been sexually transmitted, while vertical transmission may have been more common among those with HTLV-1 at enrolment. Differences in route of transmission could influence initial PVL, immune response, and long-term health outcomes.

Taken together, our findings suggest that recent HTLV-1 infection may be associated with increased risk of death among HIV-negative individuals, while co-infection with HTLV-1 and HIV-1 is linked to poorer survival compared to HIV-1 mono-infection. However, given the observational nature of the study, limited number of HTLV-1 seroconverters and individuals with HTLV-1 and HIV-1 co-infection, these findings should be interpreted with caution. Further studies are needed to confirm these results and clarify the underlying mechanisms.

Future perspectives

The global HIV epidemic has evolved dramatically since the virus was first identified in the early 1980s. Over the past decades, major progress has been made through the scale-up of ART, improved access to HIV testing, and prevention efforts. However, challenges remain, particularly in resource-limited settings and among key populations as highlighted in this thesis. The results presented add further evidence to several areas in need of continued attention, and highlights the need of targeted and sustained interventions.

In Guinea-Bissau, our findings highlight the importance of tailored interventions that address the specific needs of FSW, a group disproportionately affected by HIV and curable STIs. A recent modelling study suggested that even low-level interventions can improve outcomes along the HIV care continuum and reduce HIV incidence among FSW in sub-Saharan Africa and in extension in general population. Scaling up to a higher intensity programme had a considerably higher effect (414). A recent trial among FSW in Zimbabwe assessed the effect of adding peer-led interventions and participatory self-help groups to an already comprehensive standard care (including PrEP, condoms, peer-support and more) and found no overall benefit on the combined risk of HIV transmission or acquisition (415). This illustrates the gap that sometimes exists between modelling and real-world outcomes, where behavioural, structural, and contextual factors may limit the effectiveness of even well-designed interventions.

Moving forward, correct HIV diagnosis remains a cornerstone of effective care and treatment. This thesis has highlighted the diagnostic challenges in differentiating between HIV-1, HIV-2, and HIV-1/2 dual infection. Future efforts should focus on improving diagnostic capacity at both centralized and decentralized levels. Continued training of laboratory personnel and access to validated discriminatory assays will be essential. It is equally important that discriminatory assays in use have been evaluated using samples from individuals with confirmed HIV-1/2 dual infection. A relatively recent evaluation of the Alere q HIV-1/2 Detect POC molecular assay for HIV-1 and HIV-2 RNA detection suggested that it may serve as a useful diagnostic option in resource-limited settings (176).

The high HIV prevalence, low ART coverage, and limited viral suppression observed among FSW in our studies suggest ongoing transmission and gaps in the care continuum. Introduction of PrEP may offer an important additional tool to

reduce HIV incidence in this group. Although our assessments showed limited awareness of PrEP, interest in its use was high. Future research should explore optimal PrEP delivery models in Guinea-Bissau, including integration with existing outreach services and adherence support strategies.

The burden of curable STIs among FSW in our study further highlights the need for improved STI prevention and control. Syndromic management remains the standard approach in Guinea-Bissau but has clear limitations. Scaling up access to accurate and affordable POC tests could enable detection of asymptomatic infections and reduce both transmission and long-term morbidity. The introduction of multiplex STI diagnostics into routine services, if feasible, would also reduce overtreatment and possibly antimicrobial resistance. In parallel, condom interventions must also be strengthened and adapted to reflect the realities of sex work, taking into account structural barriers.

Regarding HTLV-1, our findings of increased risk of death among HIV-negative HTLV-1 seroconverters and among participants with HTLV-1 and HIV-1 co-infection highlight the need for further public health and research attention. Although HTLV-1 is currently not included in routine screening in Guinea-Bissau (355), targeted prevention strategies should be considered. These include screening of blood donations to prevent parenteral transmission and antenatal screening to prevent vertical transmission. The potential role of HTLV-1 in ongoing transmission within sex work networks warrants further investigations through prevalence studies among FSW. Given the observed association between HTLV-1 and increased risk of death in PWH-1, integration of HTLV-1 screening into the national HIV programme should be evaluated. While this thesis does not explore the mechanisms underlying the impact of HTLV-1 on mortality in PWH-1, further studies are needed to better understand this relationship.

Continued surveillance of HIV and STI prevalence, incidence, and treatment outcomes is essential for monitoring progress and guide future interventions. Such studies should include both the general population and key populations such as FSW and MSM. Monitoring the HIV care continuum across different groups continues to be important, particularly in the context of the UNAIDS 95-95-95 targets. To date, the HIV care continuum indicators have only been assessed among FSW and the general population in urban Bissau, and there is a need for data from more rural regions as well. The continued surveillance of HIVDR will also be important, particularly following the introduction of the new DTG-based regimens. A recent report from Africa highlights a concerning prevalence of high-level DTG resistance among PWH-1 experiencing virological failure on DTG-based ART (416).

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