Ph.D. Thesis

Late HIV presentation to care:

Earlier HIV diagnosis in Primary Health Care
&
Long-term survival
&
Tuberculosis co-infection

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Preface

This Ph.D. was born in the year 2017 out of an interest in understanding and improving the diagnosis and the lives of people living with HIV with late presentation to care. Late HIV presentation and diagnosis have important health consequences for patients and for the overall society at large. Despite the encouraging decrease in HIV incidence over the last years and since the introduction of preventive pre-exposure prophylaxis (PrEP) and the many efforts to bring down the number of late presenters, the problem continues across all of Europe and challenges us to think in new ways to impact the situation. Furthermore, and even though this is not within the scope of this thesis, the COVID-19 pandemic has adversely impacted HIV testing, earlier HIV diagnosis, and ART initiation in the last two years, due to disruptions of HIV services in many countries around the world, which is likely to result in an increase in late HIV presentation rates worldwide. It will require additional attention and actions to mitigate and reverse these consequences.

Besides the academic challenge and learnings, one of the best things about this Ph.D. has been the opportunity to collaborate so closely with several very inspiring people from whom I could draw inspiration not only for my research project but also as a person. I am very thankful to all of them.

The initial project about visits to primary health care and antimicrobial consumption was inspired by Court Pedersen's ideas and I am very thankful for his contribution. Court Pedersen has now retired, and I feel lucky to have been able to work with him both in my Ph.D. and in the clinical work in the Infectious Diseases department at Odense University Hospital.

I would like to thank my main tutor professor Isik S. Johansen. She offered me the opportunity to start this Ph.D., and she has believed in me and supported me all the way through. I am indebted to her for her trust. Isik is a very inspiring person, clinician, and researcher. She has an incredible work capacity, energy, academic enthusiasm, curiosity, resilience, and perseverance. I love her practical approach to finding solutions and her capacity for getting things done. She is also a very caring person.

I am also indebted to Josep M Llibre, my tutor from the Fight Infections Foundation in Barcelona, Spain, and clinically responsible for the Catalan HIV database PISCIS. Not only for his contagious positivity, his energy, and work capacity, his curiosity, and his ability to always see the clinical applicability of the findings, his guidance, and his always constructive suggestions but also for his way of being and for being a good friend.

There are many other people that have contributed to this Ph.D. I want to thank my colleague Line Dahlerup Rasmussen for her assistance in statistics and epidemiology, especially at the beginning of the Ph.D. She marked a big influence on my way of approaching statistics and epidemiology, always showing great enthusiasm, discipline, and rigor and I am very grateful for that. I also want to thank Niels Obel, for his support at the beginning of the Ph.D. and his many years of work dedicated to keeping the Danish HIV Cohort study, which allows the opportunity of performing high-quality HIV research in Denmark by crossmatching with other National population-based registries.

Throughout these years I have enjoyed the education in statistics and epidemiology at the University of Southern Denmark, Copenhagen University, and the London School of Hygiene and Tropical Medicine which has given me thorough insight into the methodological process and

granted me the opportunity to work with big datasets independently. I love (and dread) getting my hands on a dataset, working with the data, and understanding how the data behaves. Being a clinician, knowing statistics, and having built confidence give one an empowering feeling of freedom as a clinical researcher.

I had the enormous pleasure of working for 9 months at the Catalan center for epidemiological studies of sexually transmitted diseases and AIDS (CEEISCAT). It has been a very rewarding experience and CEEISCAT has a near-ideal epidemiological setting for infectious disease doctors to perform epidemiological studies. In collaboration with the Ministry of Health, they conduct epidemiological surveillance of HIV and sexually transmitted infections, as well as COVID-19. I was able to conduct two studies on late HIV presenters in their longitudinal population HIV cohort, PISCIS, crossmatching with the PADRIS registry.
I would particularly like to thank Jordi Casabona, the center's director, and Juliana Reyes Urueña, the previous unit coordinator, who has now moved to the European Center for Disease Control in Sweden.

I'd like to thank Henrik Nielsen, professor at Aalborg University Hospital, as well. Henrik was not involved in my Ph.D., but he had a great influence on my very first years in Denmark, for which I want to thank him. Henrik is an excellent doctor, researcher, and even better person and I still have him as a role model. I often remember many of his reflections: "infectious diseases specialty is the only specialty where the treatment of one individual has repercussions for the next" regarding the importance of appropriate antibiotic use.

Finally, I'd like to dedicate this Ph.D. to my family. Klaus, for your unwavering and encouraging support and seemingly unlimited patience. To my children, Lucia and Víctor, for their fortitude and strength when we moved to Spain during my Ph.D., as well as to my mother Pilar and brother Javier, for their unbending love. Tragically, the beginning of my Ph.D. was marked by my father Marino's passing, and I dedicate this Ph.D. to him in particular.

" **Success is walking from failure to failure with no loss of enthusiasm**"- Winston Churchill.

Abbreviations

ADE AIDS-defining events
aIRR Adjusted Incidence Rate Ratio
aMRR Adjusted Mortality Rate Ratio
aOR Adjusted Odds Ratio
AIDS Acquired Immunodeficiency syndrome
ART Antiretroviral therapy
DCRS The Danish Civil Registration System
DHCS The Danish HIV Cohort Study
DNHSR The Danish National Health Service Register
DNPR The Danish National Prescription Register
ICD-10 International Classification of Disease 10th revision
IDU Illicit drug use
IGRA Interferon-Gamma Release Assay
INSTI Integrase Strand Transfer Inhibitors
IR Incidence rate
IRR Incidence rate ratio
LP Late presenters
LPAD Late presenters with advanced disease
LTBI Latent tuberculosis infection
MR Mortality rate
MRR Mortality rate ratio
MSM Men who have sex with men
Non-LP Non-late presenters
OR Odds ratio
PADRIS Data Analysis for Health Research and Innovation Program
PHC Primary Health Care
PI Protease inhibitor
PLWH People living with HIV
SMR Standardized mortality rate
STI Sexually transmitted infections
TB Tuberculosis
WHO World Health Organization

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The studies were entirely investigator-driven and thus independent of any pharmaceutical company. The funding sources were not involved in study design, data collection, analyses, report writing, or decision to submit the papers.

List of publications

This Ph.D. thesis is based on 4 publications

(1) **Martin-Iguacel R**, Pedersen C, Llibre JM, Søndergaard J, Jensen J, Omland LH, Johansen IS, Obel N, Rasmussen LD. Primary health care: an opportunity for early identification of people living with undiagnosed HIV infection. HIV Med. 2019 Jul;20(6):404-417
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(2) **Martin-Iguacel R**, Pedersen C, Llibre JM, Søndergaard J, Ilkjær FV, Jensen J, Obel N, Johansen IS, Rasmussen LD. Prescription of antimicrobials in primary health care as a marker to identify people living with undiagnosed HIV infection, Denmark, 1998 to 2016. Euro Surveill. 2019 Oct;24(41).
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(3) **Martin-Iguacel R**, Reyes-Urueña J, Bruguera A, Aceitón J, Díaz Y, Moreno-Fornés S, Domingo P, Burgos-Cibrian J, Tiraboschi JM, Johansen IS, Álvarez H, Miró JM, Casabona J, Llibre JM and PISCIS study group. Determinants of long-term survival in late HIV presenters: a prospective cohort study. eClinicalMedicine. 2022;52:101600.
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(4) **Martin-Iguacel R**, Llibre JM, Pedersen C, Obel N, Stærke NB, Åhsberg J, Ørsted I, Holden I, Kronborg G, Mohey R, Rasmussen LD, Johansen IS. Tuberculosis incidence and mortality in people living with human immunodeficiency virus: a Danish nationwide cohort study. Clin Microbiol Infect. 2022 Apr;28(4):570-579.
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English summary

Delay in HIV diagnosis carries important consequences for both the individual and society. While there is an encouraging decreasing trend in the global HIV incidence, late HIV diagnosis remains a blot in the management of HIV, with still half of new HIV patients presenting at late stages of the disease at the time of diagnosis. Despite public health efforts, the magnitude of late HIV diagnosis has remained unchanged over the last decade. Late HIV diagnosis leads to increased morbidity and mortality, lower life quality, poorer immune reconstitution, persistent chronic inflammation, higher health care costs, and increased risk of onward transmission in the population before diagnosis. Individuals not belonging to the traditional HIV risk groups, such as heterosexual men, females, and older patients, as well as non-European individuals, are at a higher risk of late HIV diagnosis. The first group is primarily attributable to physicians' (and patients') low clinical suspicion of undiagnosed HIV, but the latter is counterintuitive given that many individuals from non-European countries come from HIV-endemic areas.

Various guidelines have been developed to better target patients for HIV testing. The European guidelines recommend routine HIV screening in pregnant women and targeted HIV testing in high-risk groups for HIV acquisition and in case of presenting to care with an HIV indicator condition. Despite these recommendations, many patients are not offered an HIV test in the latter two situations, so-called missed opportunities for earlier HIV diagnosis. These missed opportunities are frequent, and some studies have reported figures as high as 70-80% of newly diagnosed PLWH having had at least one missed opportunity for earlier HIV diagnosis. Primary health care (PHC) serves as the frontline service and is pivotal in facilitating early HIV diagnosis through provider-initiated HIV testing. Thus, engaging PHC could improve timely HIV diagnosis and, ultimately, HIV prognosis.

In the studies included in this thesis, we investigated behavioural aspects and risk factors related to PHC associated with HIV infection to improve the effectiveness of HIV testing strategies. For those PLWH where "the damage had already occurred" and were unfortunately diagnosed at a late stage of the infection, we evaluated determinants for long-term survival and incomplete immune recovery. Finally, we assessed TB risk and associated mortality in PLWH as TB is still one of the most common AIDS-defining opportunistic infections worldwide in late HIV presenters.

Studies I and II were designed as matched case-control studies. From the Danish HIV Cohort, we included all adult PLWH diagnosed with HIV between 1998-2015 and living in Denmark for ≥3 years at HIV diagnosis. From the population, we included age- and gender-matched controls (13:1). In total 2784 HIV cases and 36,192 population controls were included in the analysis. We found that PLWH frequently attended PHC in the three years preceding HIV diagnosis. Actually, 93% of PLWH had attended PHC at least once in this period, with a median number of visits of 14. Only 4% and 13% of Danish-born and non-Danish-born men and 3% and 26% of Danish-born and non-Danish-born women had had no contact with PHC in the previous three years of HIV diagnosis. Besides, we observed that PLWH had a higher prescription of antimicrobials compared to controls in the 3 years before HIV diagnosis. The antimicrobial drug prescription of any of the antimicrobial drug classes included in the study was associated with the risk of subsequent HIV diagnosis. This association was higher with higher levels of antimicrobial consumption. Although we did not dispose of indication information for the prescriptions, we assume that the use of antimicrobial drugs was in many cases a surrogate marker for missed HIV indicator conditions (e.g., community-acquired pneumonia, STIs, herpes zoster, or candidiasis).

Study III was designed as a cohort study. From the Catalan HIV PISCIS cohort, we included all adult PLWH initiating ART between 2005-2019, who were alive 2 years after, with available CD4 cell count at ART initiation and two years after (n=2719). We found that despite the late HIV diagnosis, 44% of 2-year survivors' late presenters achieved a CD4 cell count > 500cells/μL after ART initiation. Two-year CD4 count recovery was a strong independent early predictor for long-term all-cause mortality, independently of the CD4 cell count at ART initiation. Importantly, late HIV presenters with two-year CD4 count >500 cells/μL had similar survival to non-late presenters. Furthermore, initiating ART with an integrase-inhibitor (INSTI)-based regimen in the first two years was associated with a significantly better two-year immune recovery and better long-term survival.

Study IV was designed as a nationwide population-based cohort study. We included 6982 PLWH followed-for-HIV-care in Denmark between 1995-2017. Overall, 217 develop TB in the study period. Two different populations of HIV/TB coinfected individuals were identified: those presenting to care with concomitant HIV/TB infection (more frequently migrants, late presenters with a higher percentage of disseminated TB) and those diagnosed with TB >3 months from HIV diagnosis (median time from HIV diagnosis to TB 5.1 years (IQR 2.0-9.6), more frequently Danish-born individuals, with a higher social burden, higher rates of liver and non-TB AIDS-related comorbidities, a higher percentage of pulmonary TB and with a higher risk of suboptimal linkage to care). The latter had a worse TB outcome, with higher rates of treatment discontinuation and death. The incidence of concomitant HIV/TB coinfection remained high and unchanged over the study period, while the incidence of TB diagnosed >3 months after HIV diagnosis, declined over time, probably related to better ART, ART coverage, and earlier ART initiation. The risk of TB also declined with time from HIV diagnosis and with CD4 immune recovery. No individual had received treatment for latent TB infection (LTBI). Migration, history of IDU, not receiving ART, and HIV-induced immune suppression were identified as risk factors for developing TB. The overall mortality rate in PLWH co-infected with TB remained high over time and HIV-associated immunosuppression, disseminated TB, and social burden were found to be significant predictors of death in these patients.

Taken together, the loss of diagnostic opportunities for HIV diagnosis represents a critical turning point to further curb the HIV epidemic globally and improve HIV prognosis. PHC is frequently attended by individuals with undiagnosed HIV infection and should provide numerous opportunities for HIV testing. There is thus a need for more proactive testing in PHC, implementing existing HIV testing guidelines and other novel strategies. Some particular antimicrobial consumption is associated with a higher subsequent risk of HIV diagnosis and could be used as a marker to prompt proactive testing of HIV infection. Using electronic health record alerts, this strategy could be easily implemented in PHC helping reduce counselling time and contributing to the normalization of HIV testing and the reduction of HIV-related stigma.
In LP, two-year CD4 cell recovery is a good early predictor of long-term survival, much more precise than initial baseline CD4 cell counts, and this parameter could be used in routine clinical practice to early orient the patient's clinical trajectory. Furthermore, our findings support the positioning of INSTI-based ART as preferred regimens. INSTI were associated with a significantly better two-year immune recovery and, importantly, better outcomes including long-term survival in LP, though additional research is required to confirm our findings.
Finally, we found that TB rates in PLWH decreased over time and paralleled CD4 cell recovery, which emphasizes the importance of early HIV diagnosis and successful ART. Due to the

continually high incidence of late HIV presentation with concomitant TB, more proactive HIV testing, and TB prevention measures are required. Migration, IDU, and HIV-induced immunosuppression have all been identified as important risk factors for TB and underline the importance of routine screening and treatment of LTBI in these high-risk groups in low-TB incidence countries.

Danish summary

Trods en opmuntrende nedadgående tendens i den globale hiv-incidens, er sen hiv-diagnose fortsat et problem i håndteringen af hiv, hvor stadig halvdelen af nye hiv-patienter diagnosticeres i sene stadier af sygdommen. Til trods for en omfattende folkesundhedsindsats har omfanget af sen HIV-diagnose ligget stort set uændret igennem det sidste årti. Sen hiv-diagnose medfører øget sygelighed og dødelighed, lavere livskvalitet, dårligere immunrestitution, vedvarende kronisk inflammation, højere sundhedsudgifter og øget risiko for videre spredning af infektionen i samfundet. Personer, der ikke tilhører de traditionelle HIV-risikogrupper, såsom heteroseksuelle mænd, kvinder og ældre patienter, såvel som ikke-europæiske individer, har en højere risiko for sen hiv-diagnose. Den første gruppe kan primært tilskrives lægers (og patienters) lave kliniske mistanke om udiagnosticeret hiv-infektion, mens sidstnævnte forekommer kontraintuitiv, da mange individer fra ikke-europæiske lande kommer fra hiv-endemiske områder.

Der findes forskellige retningslinjer for hiv-testning. De europæiske retningslinjer anbefaler rutinemæssig hiv-screening hos alle gravide kvinder og målrettet hiv-testning i højrisikogrupper for hiv og i tilfælde af hiv-indikator sygdomme. På trods af disse anbefalinger tilbydes mange patienter ikke en hiv-test i de to sidstnævnte situationer, såkaldte ”missed oppurtunities” for tidligere hiv-diagnose. Disse ”missed oppurtunities” er hyppige, og i nogle undersøgelser er der rapporteret så høje tal som 70-80 % af alle nydiagnosticerede PLWH, der har haft mindst én ”missed oppurtunity” for tidligere hiv-diagnose. Almen praksis fungerer som frontlinje kontakt til sundhedsvæsnet og har en afgørende rolle for at diagnosticere disse patienter tidligt med proaktiv hiv-test.

I undersøgelserne inkluderet i denne afhandling kiggede vi på adfærdsmæssige aspekter og risikofaktorer, relateret til almen praksis, forbundet med hiv-infektion, med henblik på at kunne forbedre effektiviteten af hiv-test strategier. For de PLWH, der allerede blev diagnosticeret på et sent stadium af infektionen, undersøgte vi prognostiske faktorer for langsigtet overlevelse og ukomplet immunrestitution. Endelig undersøgte vi TB-risiko og tilhørende dødelighed i PLWH, da TB stadig er en af de mest almindelige AIDS-definerende opportunistiske infektioner på verdensplan hos patienter med sene hiv diagnose.

Studie I og II blev designet som nested matchede case-control undersøgelser. Fra den danske hiv-kohorte inkluderede vi alle voksne PLWH diagnosticeret med hiv mellem 1998-2015 og bosat i Danmark i ≥3 år ved hiv-diagnosen. Fra det Danske CPR-register inkluderede vi alders- og kønsmatchede kontroller (13:1). I alt 2784 hiv-smittede individer og 36.192 matchede kontroller blev inkluderet i analysen. Vi fandt, at PLWH hyppigt besøgt deres praktiserende læger i de tre år der gik forud for hiv-diagnosen. Faktisk havde 93% af PLWH besøgte almen praksis mindst én gang i denne periode, med et mediantal af besøg på 14. Desuden observerede vi, at PLWH havde et højere forbrug af antimikrobielle midler sammenlignet med kontroller i de 3 år før hiv-diagnose. Vi fandt en statistisk signifikant association mellem forbrug af alle antimikrobielle lægemidler inkluderet i studiet og risiko for senere hiv diagnose. Associationen var mere udtalt jo højere niveauet af antimikrobielt forbrug var. Selvom vi ikke havde adgang til information om indikation til recepterne, antager vi, at brugen af antimikrobielle lægemidler i mange tilfælde var en surrogatmarkør for hiv-indikator sygdomme (f.eks. samfundserhvervet lungebetændelse, STI'er, herpes zoster eller candidiasis).

Studie III var designet som et kohortestudie. Fra den catalanske hiv PISCIS-kohorte inkluderede vi alle voksne PLWH, der initierede ART mellem 2005-2019, som var i live 2 år efter, med

tilgængeligt CD4-celletal ved ART-opstart og to år efter (n=2719). Vi fandt at, på trods af den sene hiv-diagnose, opnåede 44% af dem der overlevede de første 2 år efter ART start et CD4-celletal >500 celler/µL. To-års CD4-tallet var en stærk uafhængig tidlig markør for langsigtet dødelighed, uafhængigt af CD4-celletallet ved ART-start. Hiv-smittede med sen diagnose og med to-års CD4-tal >500 celler/µL havde samme overlevelse som ikke-sen-diagnosticeret. Endvidere var opstart af ART med et integrase-hæmmer (INSTI)-baseret regime i de første to år forbundet med en signifikant bedre to-års immunrestitution og bedre langtidsoverlevelse.

Studie IV var designet som et nationalt populations-baseret kohortestudie. Vi inkluderede 6982 PLWH fulgt for hiv i Danmark mellem 1995-2017. To hundrede og sytten patienter udviklede TB i studieperioden. Studiet identificerede to forskellige populationer af hiv/TB co-inficerede individer: individer med samtidig hiv/TB-infektion (hyppigere migranter, LP med en højere procentdel af dissemineret TB) og individer, der blev diagnosticeret med TB >3 måneder fra hiv-diagnosen (median tid fra hiv-diagnosen til TB 5,1 år, hyppigere danskfødte, med en højere social belastning, højere forekomst af lever- og ikke-TB AIDS-relaterede sygdomme, en højere procentdel af lunge-TB og med en højere risiko for suboptimal kobling til pleje). Sidstnævnte gruppe havde et værre TB-outcome med hyppigere behandlingsophør og død. Forekomsten af samtidig hiv/TB co-infektion forblev høj og uændret over undersøgelsesperioden, mens forekomsten af TB diagnosticeret >3 måneder efter hiv-diagnose faldt over tid, sandsynligvis relateret til bedre ART, ART-dækning og tidligere ART-initiering. Risikoen for TB faldt også med tiden fra hiv-diagnosen og med stigning i CD4-tal. Ingen individ modtog behandling for latent TB-infektion (LTBI). Migration, iv misbrug, ikke at være i ART og hiv-relateret immunsuppression blev identificeret som risikofaktorer for at udvikle TB. Dødelighed af co-inficerede individer forblev høj over tid, og hiv-associeret immunsuppression, dissemineret TB og social belastning blev fundet at være signifikante prognostiske faktorer for død hos disse patienter.

Mulighed for at minimere forekomsten af sen hiv-diagnose er et kritisk vendepunkt for yderligere at bremse hiv-epidemien globalt og forbedre hiv-prognosen. Almen praksis kan spille en vigtig rolle i rettidig hiv diagnose, da >90% af hiv patienter besøger almen praksis i årene før deres diagnose. Der er således behov for mere proaktiv testning i almen praksis, bedre implementering af eksisterende retningslinjer for hiv-testning samt andre og nye strategier. Opmærksomhed omkring et individs forbrug af antimikrobielle midler kunne være én mulighed og anvendes som et værktøj til vejledning for, hvornår en hiv-test bør udføres i almen praksis. Et sådant værktøj kunne lette integrationen i den daglige klinik og medvirke til normaliseringen af hiv-test i almen praksis og hermed undgå nogle af de kendte barrierer for hiv-test samt hjælpe til at mindske hiv-relateret stigma.
Hos LP er to-års CD4-tal en god tidlig markør af langtidsoverlevelse, meget mere præcis end det initiale CD4-tal, og denne parameter vil kunne bruges i klinikken til at orientere patientens prognose. Desuden peger vores resultater på at INSTI-baseret ART kan overvejes som første valg regimer. INSTI var forbundet med en signifikant bedre to-års immunrestitution og langtidsoverlevelse i LP.
Endelig fandt vi, at TB-raten i PLWH faldt over tid og sideløbende med stigning i CD4-tal, hvilket understreger vigtigheden af tidlig HIV-diagnose og vellykket ART. På grund af den høje forekomst af sen hiv-diagnose med samtidig TB, er en mere proaktiv hiv-testning og TB-forebyggende foranstaltninger påkrævet. Migration, IDU og hiv-induceret immunsuppression er alle blevet identificeret som vigtige risikofaktorer for TB og understreger vigtigheden af rutinemæssig screening og behandling af LTBI i disse højrisikogrupper i lande med lav TB-forekomst.

Introduction

With approximately 40 million PLWH worldwide, HIV infection is one of the world's most challenging public health issues. Since the introduction of ART in 1996, HIV-associated morbidity and mortality have decreased dramatically, and nowadays, PLWH who are timely diagnosed and begin treatment with CD4 cell count above 350 cells/μL have a life expectancy close to or comparable to the general population.[1–7]

Late HIV presentation remains a problem.

Despite the tremendous advances in the management of HIV and an encouraging decreasing trend in global HIV incidence driven by better ART options, immediate treatment as prevention and pre-exposure prophylaxis (PrEP), late diagnosis of HIV and late ART initiation remain an unresolved problem in the field. Approximately 50% of newly diagnosed HIV patients still present at a late stage of the disease at the time of diagnosis.[8,9] Late HIV presentation is defined as diagnosis with CD4 cell count <350 cells/μL or presenting with an AIDS-defining event (ADE) in the first 6 months, regardless of the CD4 cell count.[10,11]

Late HIV diagnosis and late ART initiation have many implications and represent a major challenge for the patients, the physicians, and society as it leads to increased AIDS- and non-AIDS related morbidity and mortality,[9,12–17] lower life quality,[18] poorer immune response to ART[19], higher risk of resistance mutation during ART,[20,21] persistent chronic inflammation,[22] increased health care costs,[23] and increased onward transmission in the population.[24,25]

Some groups have a higher risk of being diagnosed late, including heterosexual men, women, and older individuals, which often represent people not belonging to classical risk groups for HIV infection, with a low degree of risk awareness among the treating physicians and the patients.[9] In fact, a growing proportion of individuals with late presentation are diagnosed ≥50 years.[26] Other risk groups for late presentation include people with low-educational or economic status and migrants.[27,28] The last is paradoxical because many of them come from highly HIV-endemic areas, which should trigger an HIV test with the first contact with care in the overall migrant healthcare check-up.

Consequences of late HIV diagnosis

The CD4 cell count at which PLWH initiate ART is a strong predictor of short and long-term morbidity, and mortality and LP have shown to have higher mortality compared to non-LP.[1,2,29,30] The mortality is highest in the first year following diagnosis, and many deaths are due to AIDS-related diseases, strongly related to lower CD4 cell counts.[9,15] Late HIV diagnosis was associated with a 6-13 fold increased rate of ADE/death in the first year after HIV diagnosis, depending on the European region, in a COHERE study compared to non-late presenters.[9] Another study from the Spanish CoRIS database described that when compared to non-LP, the mortality risk in the first year was 10-fold higher for LP and 15-fold higher for LP with advanced disease (defined as CD4 cell count <200 cells/μL or with ADE).[15] However, LP have an increased risk of not only death or ADE but also of non-ADE. In the START study, starting ART with CD4 >500 cells/μL was associated with a lower risk of ADE, death, and non-ADE (which included cardiovascular disease, end-stage renal disease, non-AIDS defining cancer, and any death not attributable to AIDS).[31]

The association between low initial CD4 counts and increased mortality risk decreases over the years, and some studies have found that these patients can recover a life span similar to or close to that of non-LP or even the general population if they achieve a successful virologic suppression in the long term and are able to restore their CD4 cell count.[7,32–37] However, for PLWH beginning ART with very low CD4, it might be difficult to restore their CD4 cell count to more than 500 cells/μL.[38] Between 1-3 out of 10 LP, particularly those with a nadir CD4 cell count < 200 cells/μL, cannot restore their CD4 count >200 cells/μL despite virologically effective ART. These individuals referred to as immunological non-responders, experience a particularly high risk of mortality and ADE and increased risk of non-ADE compared to non-INR. [32,39–45]

Older age and low baseline CD4 cell counts have been identified as strong independent risk factors for incomplete immune recovery. These facts highlight two points: first, the need for early HIV diagnosis and ART initiation before cellular immunity is severely impaired; and second, the importance of early ART initiation in older individuals as they face a more jeopardized CD4 restoration due to a senescent immune system.

However, the knowledge of prognostic factors of long-term mortality and determinants of immune recovery in LP is scant, especially after the first high-risk 1-2 year period. Many studies were performed more than a decade ago, with exposure to older and less efficient ART regimens.

Modifiable factors to improve immune recovery and the role of INSTI-based regimens

Older studies have not been able to demonstrate effective interventions or consistent differences between drug classes to increase CD4 cell counts in individuals with poor CD4 cell count recovery.[46–48] However, more recently, three cohort studies, and two randomized controlled trials, have found that Integrase Strand Transfer Inhibitor (INSTI)-based regimens might be associated with improved immune recovery compared to other ART regimens [49–55]

Earlier HIV diagnosis

Despite the overwhelming negative impact of late HIV diagnosis on the life span of PLWH and associated comorbidities, and despite the extensive efforts in public health care policies, the rates of late HIV diagnosis in Europe have remained unchanged over the last decade, including in Western and Northern European countries. Nearly all HIV-associated deaths are attributable to late HIV diagnosis, late ART initiation, and incomplete CD4 restoration despite virologic response. Besides, early diagnosis would also prevent further HIV infection spread in the background population.

Various guidelines have been developed to better target patients for HIV testing. The EACS guidelines recommend routine HIV screening in pregnant women and targeted testing in high-risk groups for HIV acquisition and in the case of HIV indicator conditions or an ADE.[56] The HIV Indicator Diseases across Europe Studies (HIDES) 1 and 2, designed by the HIV in Europe initiative, identified a group of indicator conditions associated with an undiagnosed HIV prevalence of more than 0.1%.[57,58] In these cases, offering an HIV test has been proven cost-effective.[57,58] The BHIVA guidelines also recommend routine/universal testing for all individuals seeking health care services in areas with high HIV prevalence, defined as >0.2%

Despite these recommendations, many patients who present to care with one of these indicator conditions are not offered an HIV test, resulting in missed opportunities for earlier HIV diagnosis. Some studies have shown little awareness of HIV indicator conditions-testing guidelines across

many medical specialties, resulting in missed opportunities in PHC and the hospital setting. Sadly, according to some studies, the proportion of missed opportunities in newly diagnosed PLWH is as high as 70-80%.[59,60]

Avoiding the loss of HIV diagnostic opportunities and diagnosing these patients earlier may represent an essential factor in curbing the HIV epidemic, emphasizing the critical need for more proactive implementation of guidelines, and developing additional screening strategies.

Primary care is the frontline of care and could be a crucial scenario for improving provider-initiated HIV testing to improve early diagnosis. Furthermore, the continuity of care in PHC expands the possibility of dynamic risk assessment and repeated HIV testing.

Opportunistic infections

AIDS-related opportunistic infections and malignancies persist as significant causes of morbidity and death in the ART era, particularly in LP. [61–64] Tuberculosis (TB) is still one of the most common AIDS-defining opportunistic infections worldwide, ranking alongside HIV as the world's most deadly infectious disease. HIV and TB interact synergistically, and coinfection accelerates the progression of each infection, complicates both the diagnosis and treatment of TB, and increases the risk of death, treatment complications, and therapeutic failure.[65] TB continues to be an important cause of morbidity and mortality in PLWH in Denmark. Nonetheless, only a few reports of temporal trends in the incidence and patterns of TB in PLWH in the ART era, particularly in the last decade, have been published.

Objectives and expected impact of the Ph.D.

Objectives

Studies I and II

The aim of studies I and II was to investigate variables related to PHC in Denmark associated with occult HIV infection, in order to improve the effectiveness of testing strategies.

- To assess the fraction of individuals newly diagnosed with HIV infection who had attended PHC services in the 3-year period prior to HIV diagnosis compared with age and sex-matched controls from the population.

- To assess the fraction of individuals newly diagnosed with HIV infection who underwent some specific medical services/procedures in PHC in the 3-year period prior to HIV diagnosis compared with age and sex-matched controls from the population.

- To assess whether the risk of HIV diagnosis and the degree of immunodeficiency at diagnosis were associated with the frequency of visits PHC in the 3 years preceding HIV diagnosis.

- To assess whether the risk of HIV diagnosis was associated with some medical services/procedures provided in PHC.

- To assess specific antimicrobial drug consumption (i.e., antibiotics, antivirals, and antifungals) in the 3 years preceding the HIV diagnosis compared with age and sex-matched controls based on all redeemed antimicrobial drug prescriptions in the study period.

- To assess whether consumption of antimicrobial drugs was associated with an increased risk of subsequent HIV diagnosis.

Study III

The aim of study III was to investigate determinants for long-term survival and for incomplete immune restoration in LP.

- To assess prognostic factors for long-term survival in PLWH initiating ART and surviving the first two years.

- To assess whether the two-year CD4 cell count was a good early predictor of long-term survival in PLWH initiating ART and surviving the first two years.

- To assess risk factors for incomplete immune recovery two years after ART initiation

- To assess the effect of INSTI-based regimens as first-line ART regimens on immune recovery and long-term mortality.

Studies IV

The aim of study IV was to investigate the temporal trends in the incidence and mortality of TB in Denmark in the last 20-year period, the associated mortality rates (MR), and the associated predictive and prognostic factors.

- To assess TB IR over time and associated mortality rates in PLWH during 1995-2017 and evaluate predictive and prognostic factors.

- To assess the need for routine screening and treatment for latent TB infection (LTBI) in the HIV co-infected population in Denmark, as a country with a low TB incidence (0.05 per 1000 person-years) and with a high ART coverage rate.

Expected impact of the Ph.D.:

- If the hypothesis that PLWH frequently attend PHC in Denmark in the years preceding their HIV diagnosis is confirmed, it would imply that PHC may be an ideal setting for better implementing existing HIV testing guidelines and implementing novel strategies.

- If the hypothesis that some antimicrobial consumption is associated with a higher subsequent risk of HIV diagnosis is confirmed, antimicrobial consumption could be used as a surrogate marker of occult HIV infection or a proxy for HIV indicator conditions and to aid in proactive HIV testing in the PHC setting.

- If the hypothesis that two-year CD4 cell recovery is a good early predictor for long-term survival in LP confirms, this parameter could be used in routine practice to early orientate the patient clinical trajectory.

- If the hypothesis that initiating ART with INSTI-based regimens helps to achieve an early robust immunological recovery in this fragile population of LP, and an impact on survival is confirmed, INSTI-based regimens should be positioned as the preferred first-line therapy in this specific subpopulation.

- To understand the characteristics of the HIV-infected population developing TB in Denmark and elucidate temporal trends regarding incidence and mortality rates.

Earlier HIV diagnosis: Studies I and II

a) Data sources for studies I and II

These two studies are based on data from the Danish HIV Cohort Study and from different administrative Danish population-based registries shown in figure 1. All the included registries contain high-quality data with high validity and completeness and long follow-up, covering the whole Danish population.

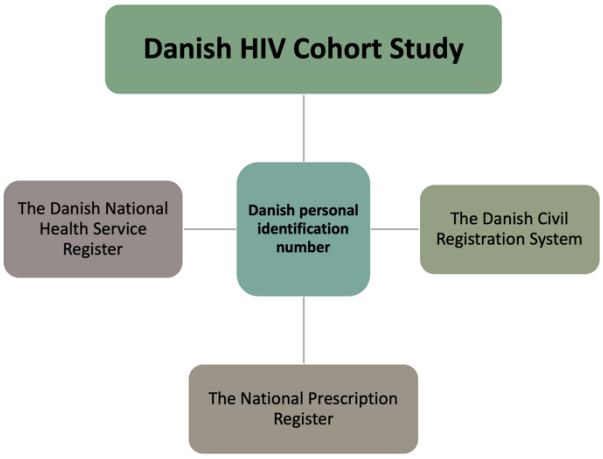


Figure 1. Registries used in studies I and II.

A pivotal feature of register-based research in Denmark is the possibility to link individuals in different registries using the unique 10-digit Danish personal identification number assigned to all residents at birth or upon immigration by the Danish Civil Registration System. Table 1 displays the data extracted from the registries used in the studies.

Danish National Registries	Purpose of the information extracted from the registries
The Danish HIV Cohort Study	To identify all adults ≥ 18 years diagnosed in Denmark with HIV between 1 January 1998 and 31 December 2016
The Danish Civil Registration System	To identify age- and sex-matched controls from the population To identify those cases and controls that had been living in Denmark during the 3 years before study inclusion (the date of HIV diagnosis)
The Danish National Health Service Register	To assess the number of contacts to PHC and specific medical services/procedures provided in primary health care
The Danish National Prescription Register	To assess all redeemed prescriptions of oral antimicrobial in the study period.

Table 1. Information extracted from the registries

The Danish HIV Cohort Study (DHCS)

The DHCS is an ongoing nationwide, prospective population-based cohort covering close to all diagnosed PLWH in Denmark. Individuals are included from January 1, 1995, the date of HIV diagnosis, or at immigration when known HIV diagnosis. Data are collected prospectively with the aim of quality and clinical care and are updated yearly. The validity and completeness of the registry are very high.[66]

Primary care in Denmark and The Danish National Health Service Register (DNHSR)
Denmark has a universal tax-financed health care system that guarantees free access to PHC and hospital care to all Danish residents. Residents have the right to choose between two types of health insurance, group I and II. Members of group I represent more than 99% of the population and they have the right to choose a general practitioner (GP) from a list of GPs registered with the social security system and receive free medical assistance in PHC and from private medical specialists with a referral from their GP. Residents in group II can seek medical assistance from any GP and visit private medical specialists without referral but requiring a co-payment. In both groups, a referral from GPs is required to access hospital care, except in case of emergencies. Services in both groups are registered in the DNHSR.

The average number of physician consultations per person-year in Denmark was 4.5 in 2014 and the vast majority (90%) have at least one contact with the PHC system every year.[67] Thus, PHC is the main entry point into the health care system and has a pivotal role to play in the early detection of health conditions.

Category	Information
Patients	Personal Identification number Name Address Region Age Gender Present general practice prover identification number Previous general practice prover identification number Health Insurance group
Health provider	Provider identification number Name of practice Address Date of entering and leaving practice Number of patients on the list Referrals to specialist Fees
Services provided	
Consultation type (Basic services)	Ordinary consultation Telephone consultation Home visit Preventive consultation E-mail consultations
Additional services[1]	Blood test Rapid throat antigen Group A Streptococcus test Urinalysis for urinary tract infections Anoscopy Lung function test Biological samples other than blood to reference laboratories Biopsy Conversation therapy Self-measured blood pressure monitoring Involuntary psychiatric hospitalizations Counselling for pregnancy prevention Counselling for abortion Counselling for sterilization

Table 2. Main information registered in the Danish National Health Service Register for primary health care (Adapted from Schmidt M, 2011)
[1] Provided additional medical services relevant to the present studies. The original service list contains more than 200 individually priced services.

The DNHSR for PHC is a national, administrative, population-based database that contains detailed individual-level data on all patients, healthcare providers, and services reimbursed by the National Health Service (Table 2). All GPs have electronic health records. The clinics weekly send an electronic invoice with information about the patient, the healthcare provider, and the type of healthcare services provided to the Regional Health Administration, which sends it to the National Health Service. The data in the registry is generated automatically through the invoice. The database's main weakness is that it contains limited information on clinical data and diagnoses. There are no validation studies available but the validity and completeness of the data are regarded as high because of the economic incentive for the providers as reimbursement of the health care services provided is dependent on its registration through the invoice.[68] The risk of overreporting because of the economic incentive is reduced by the fact that the GPs are specifically asked for further details if their invoices exceed 25% of the regional average for GPs. Data are available from 1990.

The Danish National Prescription Register (DNPR).

The DNPR is a national, administrative, population-based database that gathers detailed individual-level data on the patient, the prescribing physician, the community pharmacy, and all the medical prescriptions redeemed at community pharmacies since January 1, 1995 (Table 3). The data is generated from the electronic dispensing systems at the community pharmacies. In our study II, we used the registry to assess antimicrobial consumption in the study population. The registry covers redeemed rather than issued prescriptions, which has proven to be a more accurate surrogate measure for actual drug consumption as approximately 10% of issued prescriptions in Denmark are not subsequently redeemed in the pharmacies.[69] Primary non-adherence to antimicrobials Is reported to be low in Denmark (6.5%).[70]

The registry does not contain data on medications dispensed during hospital stays or over the counter. The latter is not a limitation of our study as antimicrobials in Denmark are only available on prescription from registered practicing physicians in Denmark. Furthermore, most of the prescriptions in Denmark are issued by GPs.[71]

The registry does not include information on the disease indication for the prescribed drugs and the intended duration and dosage, which represents the main limitation of the database. The data registered is of high validity and completeness. The use of bar codes in community pharmacies and economic incentives for complete registration minimizes the risk of coding errors and incomplete registration.

Variables related to the dispensed drugs	Information
Date	Date of the dispensation of the medication
Packages	Number of packages dispensed
Product code	Product code of the medication dispensed
Name	Product name
ATC	The Anatomical Therapeutic Chemical Code defined by the WHO
DDD	Number of defined daily doses per package
Amount	Number of tablets pre package
Strength	The numerical strength per tablet
Form	Formulation of the drug

Adapted from Pottegård A. International Journal of Epidemiology. 2017

Table 3. Overview of the main variables related to the dispensed drugs included in the Danish National Prescription Register

b) Study design for study I and II

Analytical epidemiology aims to measure the associations between exposures and the occurrence of an outcome and involves a comparison group. Analytical epidemiologic studies can be further classified as experimental (i.e., randomized control trials) and observational. The two most important analytical designs used in observational studies are the cohort and case-control designs.

The studies I and II were designed as nested case-control studies, which is a variation of a case-control design, in which cases and controls are sampled from an established prospective cohort. The studies were conducted using nationwide population-based registries and were nested within the entire Danish population. Cases included all adult individuals diagnosed with HIV in Denmark between 1998-2016, who had been living in Denmark for at least 3 years before HIV diagnosis. Cumulative (or survivor) sampling was used to select the control group from those in the Danish population who did not develop the outcome (HIV infection) during the follow-up (1998-2016) and who had been living in Denmark 3 years before the study inclusion. Controls were individually matched on age and sex to each case by random sampling from the set of individuals at risk at the time that each case was diagnosed with HIV infection (Figure 2).
As controls were selected from those who were disease-free at the end of the follow-up period the measure of association used was odds ratios (ORs). Based on the rare disease assumption and given the cumulative sampling method, these ORs provide a valid relative risk ratio estimate.

A strength of this design is that the cases were included from a population-based nationwide prospective registry including all diagnosed HIV patients in the study period in Denmark and controls were sampled from the entire Danish population.

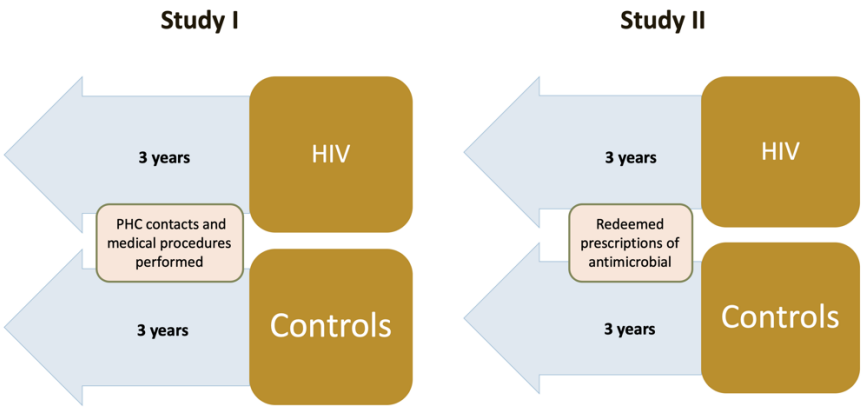


Figure 2. Study design: nested case-control study assessing contacts to primary health care (PHC), medical procedures, and redeemed prescriptions of antimicrobials in the 3 years preceding HIV diagnosis in cases compared to age- and sex-matched controls.

Selection of controls in nested case-control studies

Only a sample of the prospective cohort (in our studies the whole adult Danish population) is analysed to estimate the exposure distribution in the source population. Thus, the selection of controls in (nested) case-control studies is crucial. They should be selected at random and independently of the exposure and represent the population that gave rise to the cases (the population at risk).

In studies I and II we chose cumulative sampling but there are two other approaches to select controls:

Incidence density sampling (or risk-set sampling). In this sampling approach, controls are selected across the study from the at-risk population at the point in time when each case occurs. The controls may be eligible to become a case later during the follow-up. This sampling method allows the estimation of the incidence rate ratio, regardless of the outcome frequency.

Case-based sampling (case-cohort design). In this design, the control are selected at random from the source population at the start of the follow-up period, regardless of whether they develop the outcome later in the study. This sampling method allows for the estimation of the risk ratio, without the need of assuming that the disease under evaluation is rare in the source population.

Cohort and case-control studies both aim to compare exposed and unexposed groups. In case-control studies, the individuals are selected based on the outcome of interest. Thus, they are more efficient because the sampling, especially for studying rare diseases, is less costly and usually less time-consuming. However, they do not allow the estimation of incidence (absolute risk) and may be more susceptible to bias. These design issues are addressed in the next section.

c) Methodological considerations: measures of association, random error, and systematic error (bias) in nested case-control studies

Measure of association
In (nested) case-control studies the controls are sampled from the source population from where the cases were drawn. The control group's purpose is to estimate the relative size of the source population's exposed and unexposed individuals. Thus, the denominator does not include the total number of exposed and unexposed subjects in the source population. For this reason, case-control studies cannot estimate measures of disease frequency (rate or risk). Instead, ORs can be estimated, which represents a valid estimate of the risk ratio if the disease of study is rare in the source population. This is the case of HIV infection in Denmark, where the HIV prevalence among the adult population is estimated to be 0.1%.

Random error
Random error refers to the fact that the effect estimate can variate due to chance (or random variation). The main source of random error is sampling error, which cannot be eliminated totally but can be quantified statistically. There are two approaches to assessing the effect of random variation or chance in our observations:

1) Hypothesis testing assessing the p-value. When we test the null hypothesis there is the risk of rejecting the true null hypothesis (Type 1 (α) error or "false positive") or accepting the null hypothesis when it is false (type 2 (β) error or "false negative"). P-value refers to Type 1 (α) error and is used to estimate the probability that the observed differences in comparison are merely due to chance. P-values <0.05 are usually accepted as statistically significant (if there was no real difference the probability of observing a difference if the study was repeated many times would be less than 1 in 20).

The probability of type II error should be considered when no differences are observed in the comparison. This is related to the sample size and the study's power (Table 4).

2) Assessing the point estimate of the effect and its confidence intervals. The effect estimate of a particular comparison is the best estimate of the true population value based on the observations made in our sample. The statistical precision of the point estimate is expressed as a confidence interval and refers to the range of values likely to include the true population effect estimate (usually expressed as the 95% confidence interval around the true point estimate).

The confidence interval and the statistical significance are two ways of reporting the same association, but the confidence intervals provide additional information regarding the size of the effect, the range of plausible values, and the power of the study. Therefore, confidence intervals are becoming increasingly preferred in clinical research when reporting results.

An unbiased sample's estimates tend to cluster around the true population value. By increasing the size of the study population, the risk of sampling error can be reduced. The statistical power of case-control studies can be increased by including more controls than cases. However, the increase in power diminishes, and a ratio greater than 4:1 offers only a minor additional impact on power. We chose 13 controls per case in our studies to ensure statistical power because controls were easily identifiable from the population, and information on exposure was easily gathered.

		After carrying out the study	
		Concluded difference	**Concluded no difference**
True values	**True difference**	Correct POWER	Type II error
	Absent difference	Type I error	Correct

Table 4. Type I and type II errors.

Bias

Bias refers to systematic errors affecting the estimate of the association between exposure and disease/outcome. Bias can be classified into three types: selection bias, information bias, and confounders. Selection and information bias are related to study design, and cannot be controlled for in the analysis, whereas confounding variables can be controlled in the analysis if they are measured.

Bias causes results to be skewed in one direction or the other (Figure 3).

Selection bias refers to when the cases and controls sampled for the study are not truly representative of their source population. Thus, the key to minimizing selection bias is to meticulously sample controls so that they are representative of the exposure status in the source population. To reduce the risk of selection bias, two requisites must be met:

1) the control group should be representative of the population from which the cases originated.
2) the controls must be sampled independently of the exposure.

Because cases and controls are drawn from the same defined cohort population, the nested case-control design reduces the risk of selection bias. In studies I and II the cases were selected from a nationwide population-based registry including all subjects with HIV diagnosis in Denmark during the study period. Controls were selected at random from the source population, the entire Danish population, which minimized the risk of selection bias.

Information bias refers to the inaccuracy in the collection of information on exposure, outcome, and potential confounders. As previously stated, information on the outcome and the exposure variables were extracted from national population-based registries with high validity and completeness in studies I and II. The same data source was used for both cases and controls, avoiding the risk of differential misclassification of exposure status or potential confounders.

Confounders refer to an inherent relationship between the variables in the study. The confounding variable is a factor that is related to the exposure while also being an independent risk factor for the disease/outcome, and it can mask a true association or distort its magnitude (Figure 4). Age and sex are classical confounding variables.

There are different approaches to dealing with confounders:

In the design phase:

- Matching of cases and controls on potential confounder variables
- Restricting the study to only a single value of a potential confounder
- Stratification

In the analysis phase:

- Statistical adjustment in the analysis

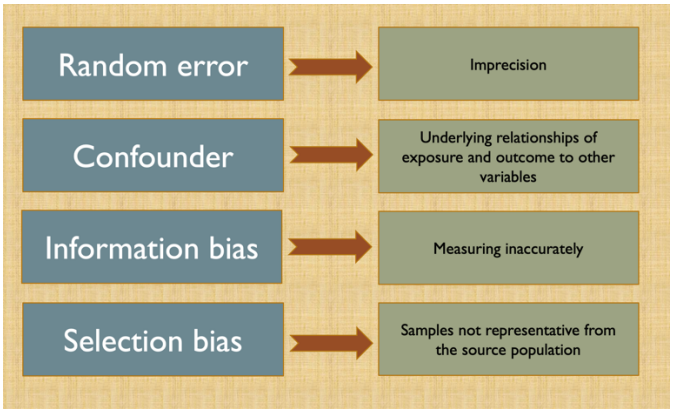


Figure 3. Methodological considerations.

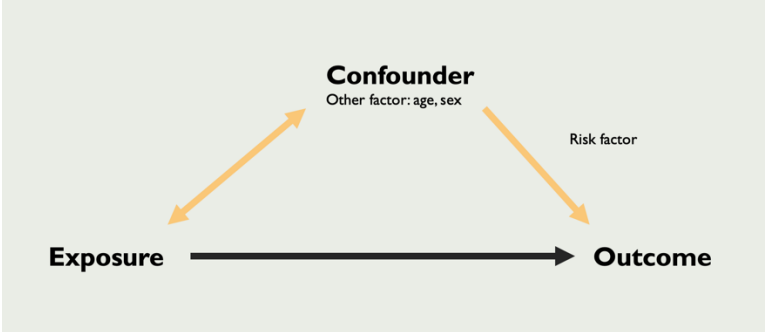


Figure 4. Exposure, outcome, and confounder.

These techniques can eliminate confounding by known risk factors. However, the possibility of **residual (unmeasured) confounding** by unknown risk factors can never be ruled out.

In case-control studies, statistical **overmatching** refers to controlling for factors that are risk factors for the disease/outcome but are strongly associated with exposure status in the source population, resulting in a loss of statistical power.

In our studies, controls were individually matched to cases on age and gender to minimize the effect of confounding by these variables. Besides, in sensitivity analysis, we stratified by sex, age groups, and ethnicity (Danish and non-Danish) because of clinical and statistically significant interactions. We further stratified analysis by HIV subgroups (early, late, and very late HIV diagnosis) and mode of infection.

Despite the nationwide design, individual matching for age and sex, and stratification for the different variables, residual confounding cannot be ruled out.

The nested case-control design increases efficiency and flexibility, allows matching, and reduces selection and information bias compared to classical case-control studies (Figure 5).

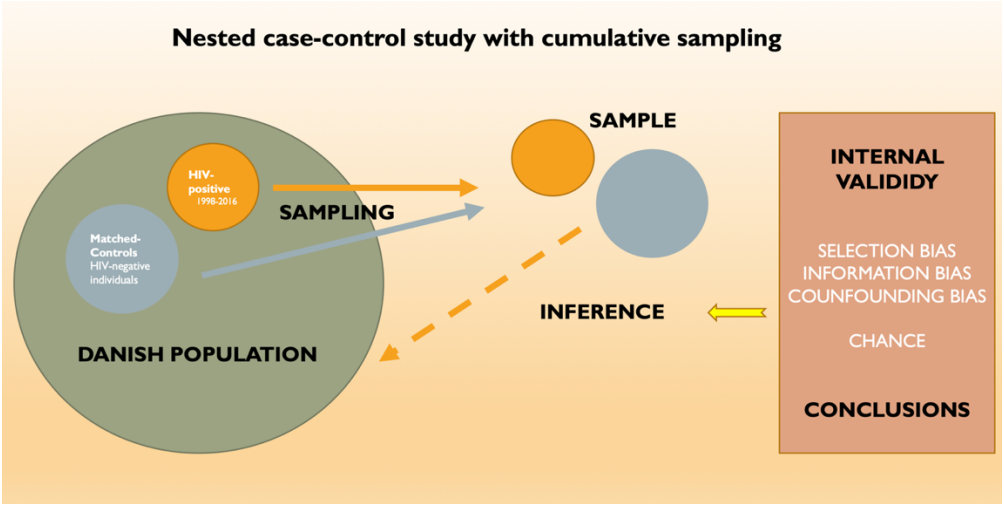


Figure 5. Nested case-control study with cumulative sampling.

d) Statistical methods

Study I

To explore risk factors (exposures) for subsequent HIV diagnosis or severe immunodeficiency we performed conditional logistic regression to compare different cut-offs of numbers of visits to PHC and exposure to different medical procedures. We calculated the OR and its 95% CI. Based on the rare disease assumption, these ORs were considered estimates of the relative risk of being diagnosed with HIV infection or with advanced disease.

Because of statistical evidence of interaction (likelihood ratio test, LRT, <0.0001), we further stratified the results for gender and Danish/non-Danish origin.

Study II
The exposure was the prescription of antimicrobials in the three years before study inclusion (date of HIV diagnosis of the matched case). Consumption was assessed based on all redeemed prescriptions of oral antimicrobial drugs in this period. The consumption was measured as the number of defined daily doses (DDD) per person-year.
To use estimates that were more applicable to the clinical practice, we categorised consumption for each antimicrobial drug class based on different DDD cut-offs, which were chosen based on clinical criteria as the average estimated number of DDD for one standard treatment for the most common infections.

Consumption of each antimicrobial class was assessed according to:

- Total DDD/PY, in the three-year period before HIV diagnosis.
- Percentage of patients in each DDD/PY category, both per year and in the three-year period before HIV diagnosis.

We used conditional logistic regression analysis to compare exposure to different levels of antimicrobial drug consumption between cases and controls to identify exposures associated with the risk of subsequent HIV diagnosis, providing OR and 95% CI.

To analyse the trend of increase in consumption of antimicrobial drugs in the different years before HIV diagnosis, we used the Cochran–Armitage test.

Because of statistical evidence of interaction (likelihood ratio test, LRT, <0.01), we further stratified the results for gender.

Effectiveness factor. In both studies, we calculated a fraction of exposed controls and cases in the study to estimate the percentage of people being tested for HIV and the percentage being diagnosed based on the assumption that we will test all exposed individuals above a defined cut-off regarding the number of visits to PHC or antimicrobial consumption. The effectiveness factor was calculated by dividing the percentage of cases that would be diagnosed by the percentage of controls that would be tested, and it provided an estimate of how effective the different interventions would be compared to testing at random if the population had the same demographical pattern as that of our sample. A factor > 1 was considered effective.

e) Findings

Study I
We included 2,784 cases and 36,192 age- and gender-matched controls from the population.
Older age, heterosexual mode of transmission, and non-Danish origin were all independent risk factors for late or very late presentation to care. Late presentation (16.9%) and very late presentation (30.7%) remained common in the most recent calendar period (2010-2016)
Only 49% of the female cases were of Danish origin compared to 81% of male cases and 86% of the controls.

The most important finding in our study was that individuals with a new HIV diagnosis frequently attended PHC in the three years preceding HIV diagnosis. A higher percentage of non-Danish individuals had no contact with PHC in this three-year period: 4% and 13% of Danish-born and

non-Danish-born men and 3% and 26% of Danish-born and non-Danish-born. Overall, the median number of PHC visits among cases was high, and the differences between cases and controls were more pronounced among non-Danish individuals and Danish men (Table 5).

	Percentage of individuals having ≥1 contact with PHC		Median number of visits to PHC in the 3-year period before HIV diagnosis	
	Cases	Controls	Cases	Controls
Danish men	96.1%	92.2%	14	8
Non-Danish men	86.9%	53.7%	10	2
Danish women	97%	98.5	22	17
Non-Danish women	74.3%	53%	13	3

Table 5: Percentage of individuals having at least one contact with primary health care in the three years preceding HIV diagnosis and the median number of visits to primary health care in this period.

We observed a positive association between the number of visits to PHC ≥1 compared to 0 and the risk of subsequent HIV diagnosis in men and non-Danish individuals. This association increased with the increasing number of visits and a shorter time to HIV diagnosis. A U-shaped association was found between the number of visits to PHC and the risk of subsequent HIV infection in Danish women (Figures 6 and 7).

(a) Contacts with primary health care in the 3 years before HIV diagnosis

NVPC	HIV	Controls	OR (95%CI)
Danish men	*N* = 1808	*N* = 25 050	
Median (IQR)	14 (7–25)	7 (3–14)	
0	70 (3.9)	1947 (7.8)	Ref (1)
1–2	85 (4.7)	3554 (14.2)	0.66 (0.48–0.92)
3–6	271 (15.0)	6412 (25.6)	1.16 (0.89–1.52)
7–12	402 (22.2)	5808 (23.2)	1.95 (1.50–2.53)
13–24	512 (28.3)	4656 (18.6)	3.17 (2.45–4.10)
>24	468 (25.9)	2673 (10.7)	5.37 (4.13–6.98)
Non-Danish men	*N* = 429	*N* = 4031	
Median (IQR)	10 (4–19)	2 (0–10)	
0	56 (13.1)	1865 (46.3)	Ref (1)
1–2	34 (7.9)	272 (6.8)	4.26 (2.39–7.61)
3–6	59 (13.8)	548 (13.6)	3.81 (2.28–6.36)
7–12	89 (20.8)	525 (13.0)	5.61 (3.46–9.11)
13–24	120 (28.0)	507 (12.6)	6.89 (4.37–10.85)
>24	71 (16.6)	314 (7.8)	5.52 (3.27–9.30)
Danish women	*N* = 267	*N* = 6014	
Median (IQR)	22 (12–41)	17 (9–27)	
0	8 (3.0)	90 (1.5)	3.49 (1.52–8.01)
1–2	10 (3.8)	196 (3.3)	1.79 (0.85–3.77)
3–6	21 (7.9)	624 (10.4)	1.27 (0.72–2.24)
7–12	32 (12.0)	1282 (21.3)	Ref (1)
13–24	76 (28.5)	1995 (33.2)	1.55 (1.01–2.37)
>24	120 (44.9)	1827 (30.4)	2.52 (1.68–3.77)
Non-Danish women	*N* = 280	*N* = 1097	
Median (IQR)	13 (0–25)	3 (0–18)	
0	72 (25.7)	516 (47.0)	Ref (1)
1–2	6 (2.1)	22 (2.01)	1.50 (0.53–4.26)
3–6	22 (7.9)	61 (5.6)	2.42 (1.13–5.19)
7–12	36 (12.9)	119 (10.9)	1.87 (1.09–3.20)
13–24	74 (26.4)	192 (17.5)	2.64 (1.70–4.12)
>24	70 (25.0)	187 (17.1)	2.83 (1.79–4.48)

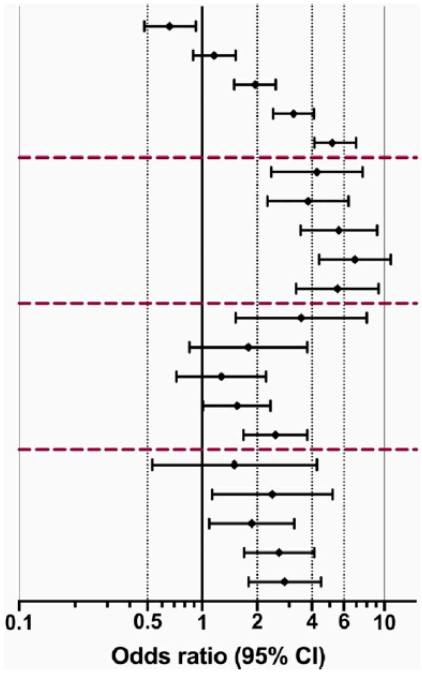


Fig. 1 Association between the total number of annual contacts with primary care in the 3 years before HIV diagnosis and being diagnosed with HIV infection in men and women stratified by Danish or non-Danish origin (HIV-infected individuals versus matched controls). (a) Men; (b) women. CI, confidence interval; IQR, interquartile range; OR, odds ratio; PHC, primary health care.

Figure 6. Association between the total number of contacts with primary care in the 3 years before HIV diagnosis and risk of being diagnosed later with HIV infection.

NVPC	Cases	Controls	OR (95% CI)	EF
Danish men	*N* = 1808	*N* = 25 050		
≥1 visits (*vs.* 0)	1423 (78·7)	18142 (72·4)	1·42 (1·26–1·60)	1·09
≥2 visits (*vs.* <2)	1212 (67·0)	14002 (55·9)	1·62 (1·46–1·80)	1·20
≥3 visits (*vs.* <3)	995 (55·0)	10778 (43·0)	1·64 (1·49–1·81)	1·28
≥4 visits (*vs.* <4)	837 (46·3)	8442 (33·7)	1·72 (1·56–1·90)	1·37
≥5 visits (*vs.* <5)	700 (38·7)	6618 (26·4)	1·80 (1·62–1·99)	1·47
Non-Danish men	*N* = 429	*N* = 4031		
≥1 visits (*vs.* 0)	295 (68·8)	1752 (43·5)	2·41 (1·80–3·21)	1·58
≥2 visits (*vs.* <2)	230 (53·6)	1382 (34·3)	1·73 (1·33–2·26)	1·56
≥3 visits (*vs.* <3)	192 (44·8)	1091 (27·1)	1·67 (1·28–2·19)	1·65
≥4 visits (*vs.* <4)	153 (35·7)	863 (21·4)	1·78 (1·32–2·39)	1·67
≥5 visits (*vs.* <5)	120 (28·0)	702 (17·4)	1·44 (1·05–1·97)	1·61
Danish women	*N* = 267	*N* = 6014		
≥1 visits (*vs.* <1)	225 (84·3)	5460 (90·8)	0·52 (0·37–0·76)	0·93
≥2 visits (*vs.* <2)	210 (78·7)	4923 (81·9)	0·86 (0·62–1·17)	0·96
≥3 visits (*vs.* <3)	191 (71·5)	4374 (72·7)	0·94 (0·71–1·25)	0·98
≥4 visits (*vs.* <4)	172 (64·4)	3861 (64·2)	1·02 (0·78–1·33)	1·00
≥5 visits (*vs.*<5)	160 (59·9)	3351 (55·7)	1·20 (0·93–1·57)	1·08
Non-Danish women	*N* = 280	*N* = 1097		
≥1 visits (*vs.* 0)	184 (65·7)	521 (47·5)	2·19 (1·55–3·09)	1·38
≥2 visits (*vs.* <2)	163 (58·2)	473 (43·1)	1·90 (1·36–2·65)	1·35
≥3 visits (*vs.* <3)	146 (52·1)	421 (38·4)	1·78 (1·29–2·46)	1·36
≥4 visits (*vs.* <4)	127 (45·4)	369 (33·6)	1·57 (1·14–2·17)	1·35
≥5 visits (*vs.* <5)	111 (39·6)	319 (29·1)	1·51 (1·09–2·09)	1·36

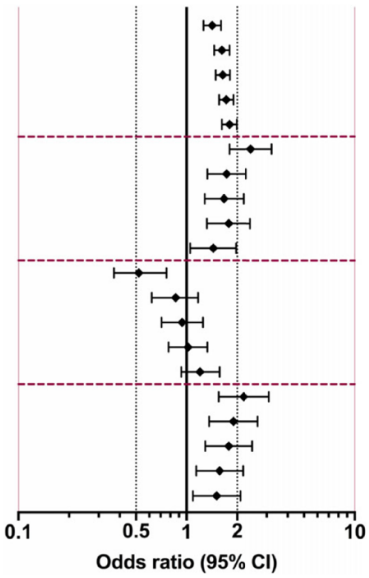


Fig. 2 Estimated percentage of HIV-infected patients being diagnosed, percentage of patients in the community being tested for HIV and the effectiveness factor according to the number of visits to primary care for men and women stratified by non-Danish/Danish origin in the third year before diagnosis.

Figures 7. Estimated percentage of HIV-infected patients being diagnosed, percentage of patients in the community being tested for HIV, the odds of being diagnosed with HIV, and effectiveness factors according to the number of visits to primary care.

However, we found no significant association between the number of visits to primary care and the degree of immunodeficiency.

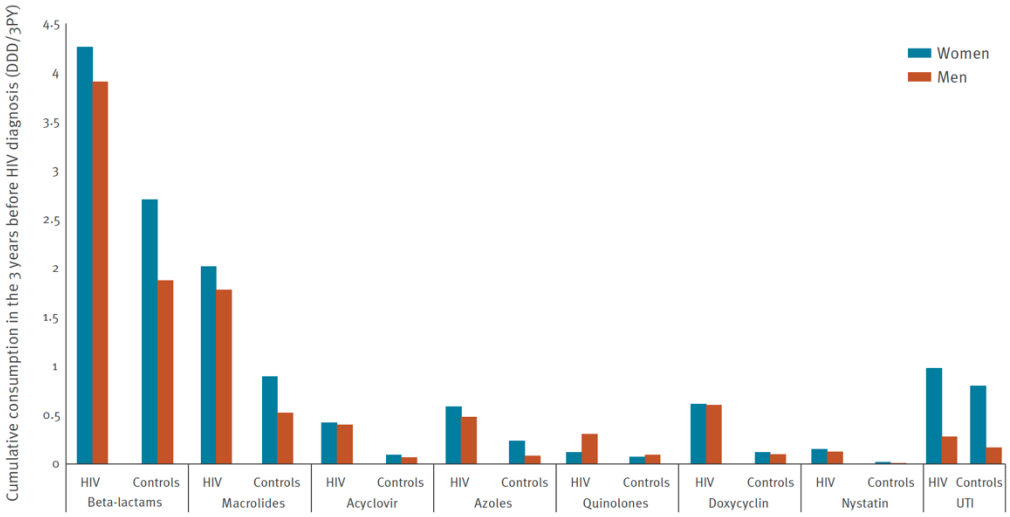
Regarding the type of procedures performed, only some were related to the risk of HIV diagnosis: anoscopy (OR 2.87; 95% CI 1.67–4.95) (particularly in men <50 years OR 4.15; 95% CI 2.22–7.77), sending biological samples to reference laboratories (OR 2.41; 95%CI 2.13–2.72), counselling for abortion and/or sterilization in women (OR 2.13; 95% CI 1.22–3.70), and to a lesser extent urinalysis for urinary tract infection, throat antigen Group A *Streptococcus* tests, blood tests for infection/inflammation and other blood tests.

Study II

The study population is the same as in study I. During the three years preceding HIV diagnosis, the antimicrobial prescription was higher in cases than in controls, with 72.4% of cases and 46.3% of controls receiving at least one prescription for any of the antimicrobial drugs studied (p=0.001). The trend was independent of gender, although consumption was higher in women than in men. For both cases and controls, beta-lactams and macrolides were the most commonly prescribed antimicrobial drug classes (Figure 8).

FIGURE 1

Antimicrobial drug prescription in the 3 years before HIV diagnosis, stratified by sex, Denmark, 1998–2016 (n = 38,976)



DDD/3PY: defined daily dose per person in the 3-year study period; HIV: human immunodeficiency virus; UTI: urinary tract infection.

Figure 8. Antimicrobial drug prescription in the 3 years preceding HIV diagnosis

For all antimicrobials, we found a significantly higher fraction of cases than controls with antimicrobial drug prescriptions (Figure 8). Antimicrobial drug prescription increased in cases as the date of HIV diagnosis approached, while it remained stable among controls. This increased association over the years was statistically significant for beta-lactams, macrolides, quinolones acyclovir, azoles, and nystatin ($p<0.01$, Cochran-Armitage trend test) (Figure 9).

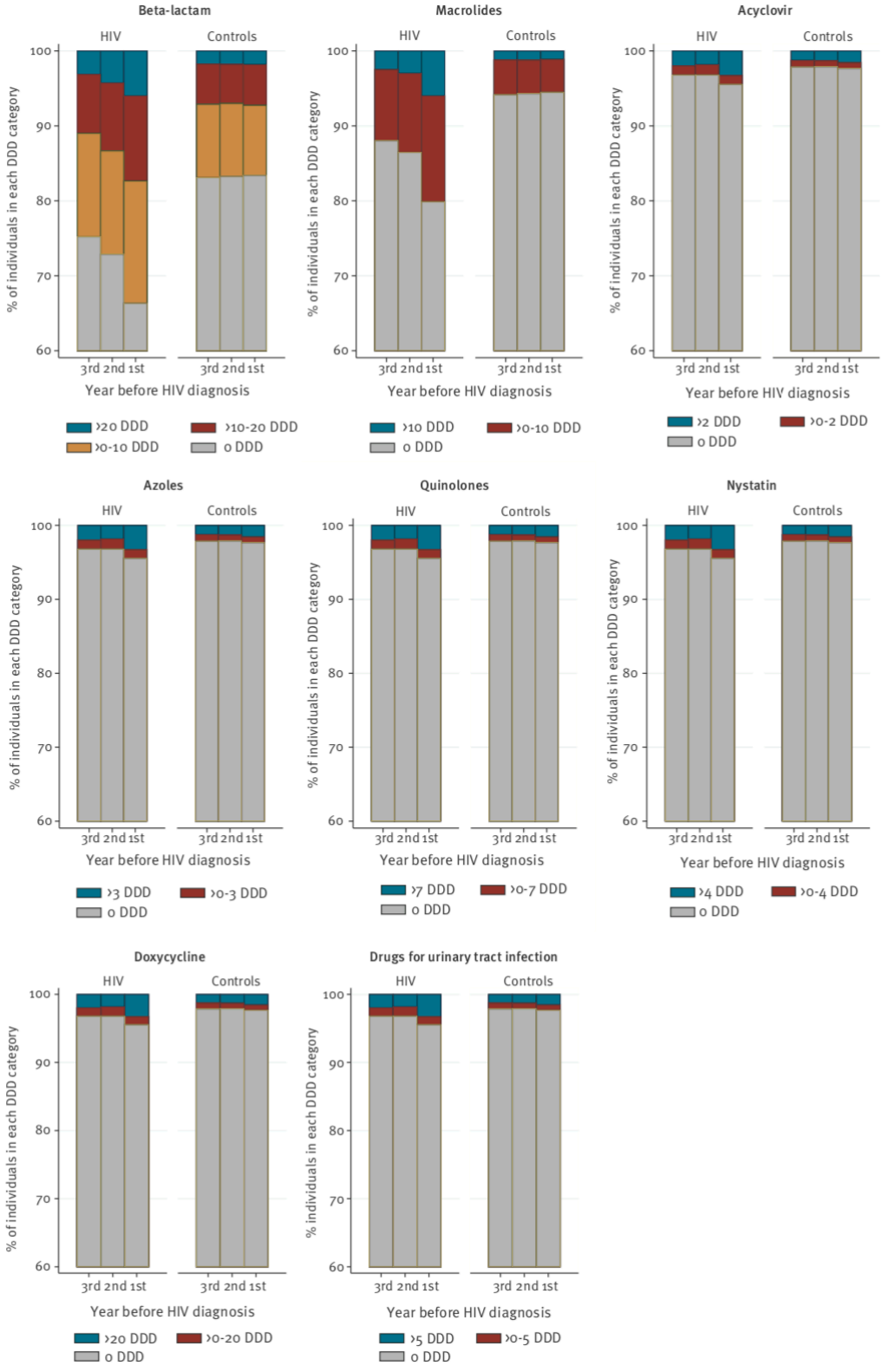


Figure 9. Prescription of antimicrobials for cases and controls, by year before HIV diagnosis of the matched case, Denmark, 1998–2016.

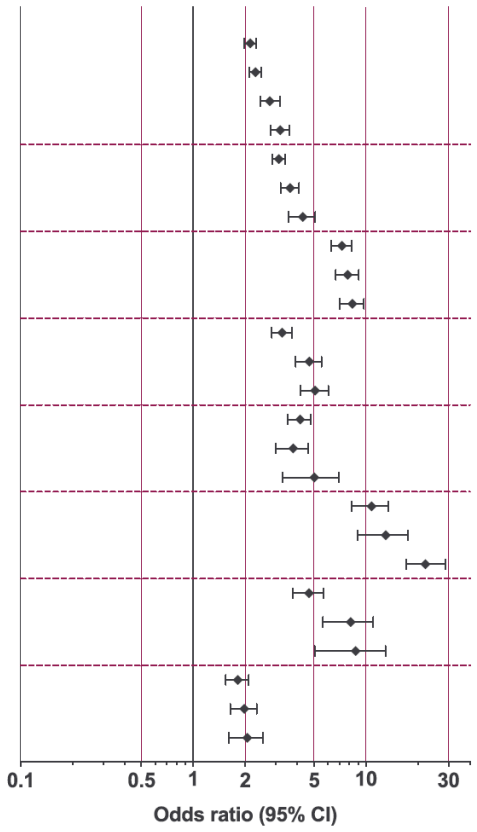
A higher fraction of cases than controls with a high drug prescription based on the predefined DDD levels per person-years ($p < 0.01$). Increasing drug prescriptions in HIV cases the closer the date was to the HIV diagnosis. This was statistically significant for beta-lactams, macrolides, quinolones, acyclovir, azoles, and nystatin ($p < 0.01$, Cochran-Armitage trend test).

We found a positive association between the degree of consumption or the number of treatments for any of the studied antimicrobials and the risk of subsequent HIV diagnosis, which increased with higher levels of consumption and a shorter time to the date of HIV diagnosis (Figure 10). Except for antibiotics used to specifically treat urinary tract infections, there was a linear association between the degree of consumption and the risk of subsequent HIV diagnosis for all antimicrobials.

FIGURE 3

Association between different antimicrobial prescriptions in the 3 years before an HIV diagnosis and the risk of being subsequently diagnosed with HIV infection, Denmark, 1998–2016 (n = 38,976)

DDD/3 PYR	HIV n	HIV %	Controls n	Controls %	OR (95% CI)	EF
Beta-lactams						
>0 vs 0	1,533	55.1	13,315	36.8	2.13 (1.97–2.30)	1.50
>10 vs ≥ 10	1,032	37.1	7,485	20.7	2.28 (2.11–2.48)	1.79
>20 vs ≤ 20	557	20.0	3,104	8.6	2.70 (2.44–2.98)	2.33
>30 vs ≤ 30	333	12.0	1,508	4.2	3.17 (2.79-3.60)	2.86
Macrolides						
>0 (vs 0)	932	33.5	5,037	13.9	3.12 (2.87-3.40)	2.41
>10 (vs ≤ 10)	373	13.4	1,494	4.1	3.62 (3.20-4.08)	3.27
> 20 (vs ≤ 20)	170	6.1	551	1.5	4.26 (3.56-5.08)	4.07
Acyclovir						
>0 (vs 0)	353	12.7	736	2.0	7.20 (6.29–8.25)	6.35
>2 (vs ≤ 2)	279	10.0	525	1.4	7.78 (6.67–9.08)	7.14
>4 (vs ≤ 4)	255	9.2	444	1.2	8.27 (7.03–9.72)	7.67
Azoles						
>0 (vs 0)	310	11.1	1,448	4.0	3.25 (2.84–3.72)	2.78
>3 (vs ≤ 3)	190	6.8	574	1.6	4.65 (3.92–5.52)	4.25
>6 (vs ≤ 6)	160	5.8	440	1.2	5.01 (4.16–6.04)	4.83
Quinolones						
>0 (vs 0)	252	9.1	869	2.4	4.14 (3.52–4.81)	3.79
>7 (vs ≤ 7)	110	4.0	403	1.1	3.72 (3.00–4.63)	3.64
>14 (vs ≤ 14)	38	1.4	106	0.3	4.79 (3.29–6.97)	4.67
Nystatin						
>0 (vs 0)	122	4.4	164	0.5	10.55 (8.27–13.46)	8.80
>4 (vs ≤ 4)	67	2.4	72	0.2	12.53 (8.94–17.57)	12.0
>8 (vs ≤ 8)	33	1.2	25	0.1	17.16 (10.21-28.85)	12.0
Doxycycline						
>0 (vs 0)	132	4.7	386	1.1	4.61 (3.77–5.65)	4.27
>20 (vs ≤ 20)	56	2.0	95	0.3	7.87 (5.63–10.99)	6.67
>40 (vs ≤ 40)	28	1.0	45	0.1	8.09 (5.05–12.97)	10.0
Drugs for urinary tract infection						
>0 (vs 0)	230	8.3	1,834	5.1	1.80 (1.54–2.09)	1.63
> 5 (vs ≤ 5)	165	5.9	1,190	3.3	1.95 (1.64–2.33)	1.79
>10 (vs ≤ 10)	94	3.4	635	1.8	2.02 (1.61–2.53)	1.89



CI: confidence interval; DDD/3PYR: defined daily dose per person in the 3-year study period rate; EF: effectiveness factor; HIV: human immunodeficiency virus; OR: odds ratio.

Percentages refer to the totals of all cases/all controls in this study.

Figure 10. Association between the different antimicrobial prescriptions in the 3 years preceding HIV diagnosis and the risk of being subsequently diagnosed with HIV infection.

Sensitivity analysis

Several sensitivity analyses were conducted, i.e., stratifying results according to sex, age, mode of infection
and HIV subgroups, for the data with statistically significant interactions and/or clinical interest.

- Similar association for men and women, although for most of the drugs, the association was stronger for men (Figure 11 and Supplementary Table S3 in the article).

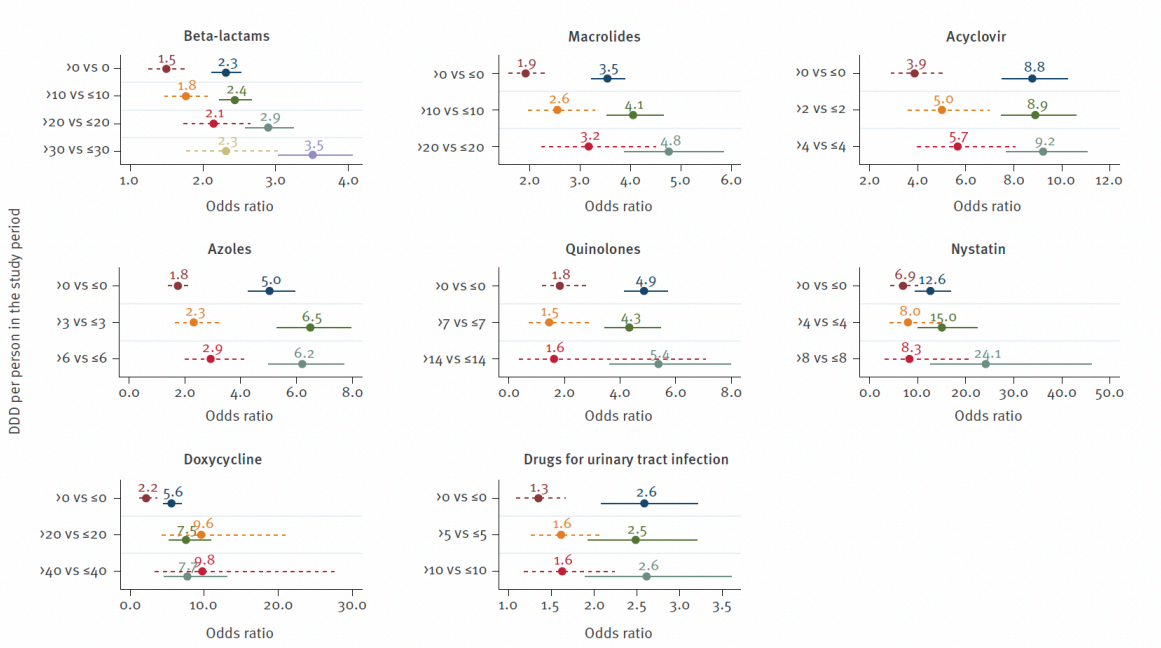


Figure 11. Association between antimicrobial prescription in the 3 years before HIV diagnosis and the risk of HIV diagnosis, stratified by sex.

- The association with acyclovir, was stronger for middle-aged men (40-59 years; >2 DDD/3 PY: OR: 13.22; 95% CI: 10.20–17.13).
- The association with azoles increased with older age.
- The association with quinolones was strongest in younger men (Supplementary Tables S4 and S5 in the article).
- The association between the prescription of antimicrobials and subsequent HIV diagnosis was stronger for MSM compared to heterosexuals, especially for macrolides, quinolones, and doxycycline (Supplementary Tables S6 and S7 in the article).
- The association with macrolides, acyclovir and azoles was stronger for cases presenting at a late stage of HIV infection (Supplementary Table S8 in the article).

f) Strengths and limitations

Strengths

- Both studies are nested case-control studies conducted in a well-established nationwide population. To obtain data, we had complete access to high-quality national Danish registries.
- We were able to estimate the contact pattern with PHC and antimicrobial consumption in both controls and cases three years back from the date of HIV diagnosis of the matched case.
- Because primary non-adherence is low in Denmark (6.5%), prescription drug redemption data in community pharmacies is a good proxy for drug consumption.[70] We used data on redeemed rather than on issued prescriptions, because it is a more accurate surrogate measure for actual antimicrobial consumptions, as approximately 10% of issued prescriptions in Denmark are not subsequently redeemed in the community pharmacies.[69]

Limitations

- No validation of DNHSR has been performed. However, due to the economic incentive in the DNHSR, we believe that data completeness and accuracy are high.
- The DNHSR primarily contains administrative information, with little information on clinical data or diagnoses. As a result, we had to rely on the number of visits to PHC or the coded procedures performed.
- Because this is an observational study, we cannot rule out the possibility of residual confounding. Lifestyle factors (smoking, alcohol, drug abuse), socio-economic status, educational level, comorbidities, and sexual orientation, may differ between controls and cases, all of which may influence their healthcare-seeking behaviour.
- We lacked access to the disease indications for the different drug prescriptions.
- Effectiveness factor: based on the assumption that the general population had the same demographic pattern as in our study population, the effectiveness factor was used to estimate how effective an intervention (e.g., testing all the population with a specific number of visits to primary care in one year or with a specific level of drug consumption) was compared to testing at random. This isn't an exact estimate, but it is the best approximation our data allows.

Prognosis of late presenters: study III

a) Data sources for study III

This study is based on data from the Catalan and Balearic Islands HIV cohort PISCIS, the Public Data Analysis for Health Research and Innovation Program (PADRIS) in Catalonia, and the Spanish national mortality registry (Table 6).

The PISCIS Cohort
In this study III, only data from Catalonia were used as we crossmatched these data with PADRIS, which contains only information about Catalan citizens.

PISCIS is an ongoing, prospective, multicentre, population-based cohort ongoing from January 1, 1998 that includes all individuals aged ≥16 years diagnosed with HIV and followed in one of the 16 collaborating Catalan hospitals. The database includes 84% of all diagnosed PLWH in Catalonia. Data are collected prospectively and updated yearly. These data include demographic information, date of HIV diagnosis, AIDS-defining events, ART information, CD4 cell count, and HIV-RNA measurements performed throughout the follow-up period.

Public Data Analysis for Health Research and Innovation Program (PADRIS)
PADRIS is an analytic data program for health research, which uses, and cross matches real-world health data generated by the public health system in Catalonia (SISCAT) in accordance with the current ethical, legal and regulatory framework. These data are provided by the Catalan Agency for Health Quality and Evaluation (AQuAS). The program provides detailed administrative and clinical data based on PHC and hospital contacts. It is a source of information on diagnosed diseases or procedures from hospital discharge records and from electronic health records from PHC using the International Classification of Disease 10th revision (ICD-10) medical coding system. In addition, it collects data on laboratory results and drug prescriptions dispensed by the hospital or community pharmacies. The program covers the period from 2005 to the present.

Registries	Purpose of the information extracted from the registries
The Catalan PISCIS Cohort	To identify all treatment naïve PLWH aged ≥18 years, who initiated ART between January 1, 2005, and June 30, 2019, in Catalonia. Individuals were followed until June 30, 2021.
PADRIS	To obtain comorbidity and mortality data on PLWH. Comorbidity data included: cardiovascular disease (including myocardial infarction, Congestive heart failure, cerebrovascular disease, and peripheral vascular disease), diabetes mellitus type 2, arterial hypertension, dyslipidaemia, chronic lung disease, chronic kidney disease, chronic liver disease, solid organ malignancy, haematological malignancy, dementia, and depression.
Spanish national mortality registry	To assess mortality data, cross-matching it with data from PISCIS and PADRIS

Table 6. Registries used in the study

Comorbidity data were retrieved from PADRIS. We used ICD-10 codes to define comorbidities according to the coding used in the Swedish National Study of Aging and Care in Kungsholmen (SNAC-K) cohort (www.snac-k.se), and for some specific comorbidity groups, additional clinical, laboratory and prescription information was used to define the groups. This is described in detail in Table S1 and S2 in the appendix.

Mortality data were obtained by cross-matching data from PISCIS, PADRIS, and the Spanish national mortality registry.

b) Study design for study III

The study III is designed as a cohort study. Cohort studies are observational (non-experimental) studies. The groups are defined based on their exposure status (e.g., late HIV presentation), which precedes the outcome of the evaluation. These individuals are then tracked over time to see who develops the outcome (e.g., death). The rates of the outcome in the exposed can then be compared to the rates of the outcome in the non-exposed.

We included all treatment naïve adult PLWH (≥18 years) from the PISCIS cohort, who initiated ART between January 1, 2005, and June 30, 2019. Individuals were followed until June 30, 2021.

We defined LP (or "late ART initiators") as those initiating ART with CD4 cell count ≤350 cells/µL and non-LP as those initiating ART with CD4 cell count >350 cells/µL. LP were further stratified according to their CD4 cell count at ART initiation (<100, 100-199, and 200-350 cells/µL) and to two-year CD4 cell count recovery (<200, 200-500, and >500 cells/µL).
Non-LP were also stratified according to the two-year CD4 cell count recovery, in non-LP with two-year CD4 cell count ≤500 cells/µL, and those with CD4 cell count >500 cells/µL (the latter was considered the reference group).

Only those PLWH who started ART during the study period, survived the first two years after starting ART, and had an available CD4 cell count at ART initiation and two years later were included in the main analysis.

The primary outcome was time to all-cause mortality and it was evaluated in the different groups of LP according to their two-year immune recovery after ART initiation (Figure 12).

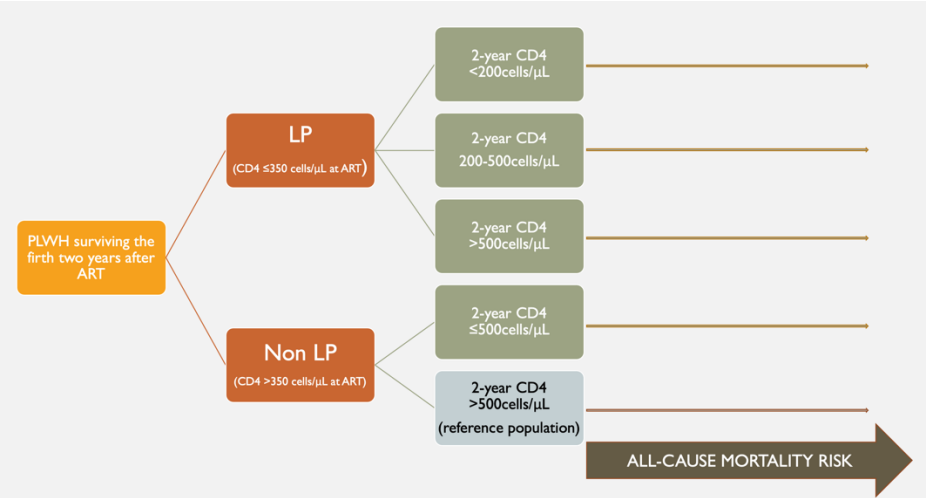


Figure 12. Study design in study III.

The secondary outcome was incomplete immune recovery, defined as two-year CD4 cell count ≤500 cells/µL. This analysis was carried out as a nested case-control study (Figure 13).

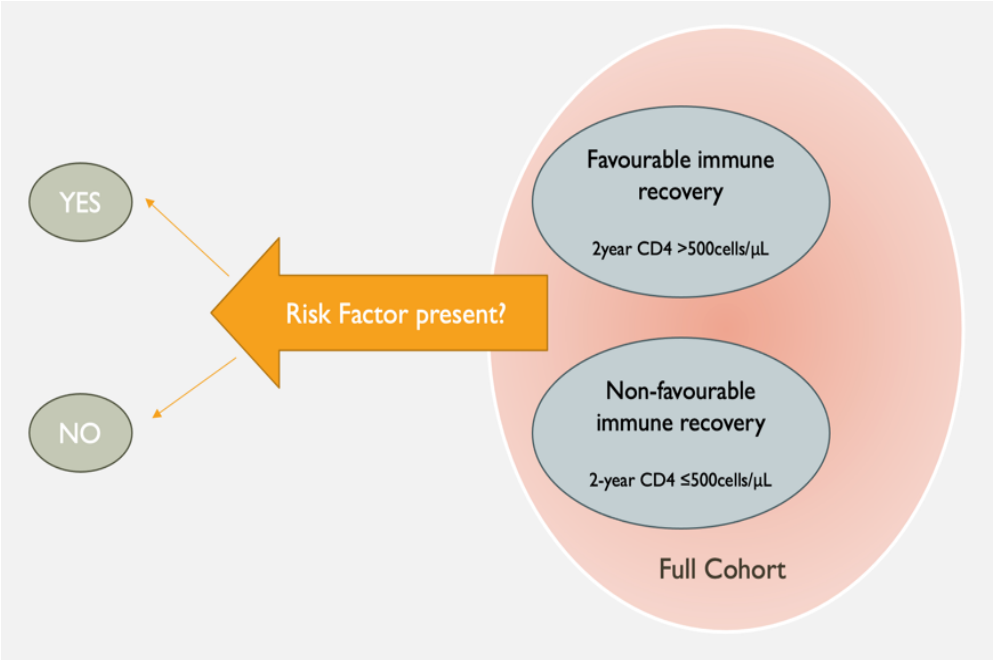


Figure 13. Study design in sub-analysis in study III. Risk of incomplete immune recovery (defined at 2-year CD4 cell count ≤ 500 cells/µL in late presenters.

We also performed a sensitivity analysis to evaluate if the results were concordant when including all PLWH initiating ART, thus including also those PLWH not surviving the first two years. This was done to evaluate the potential introduction of selection bias in the principal analysis.
In this analysis, the baseline was at ART initiation and the mortality risk was assessed in the different groups of LP according to their CD4 cell count at ART initiation. Non-late presenters were the comparison group.

c) Methodological considerations (measures of association, random error, bias)

Measure of association
We calculated MR as a measure of disease frequency. The denominator represents the sum of each individual's time at risk (person-time at risk), while the numerator represents the number of new cases developing the outcome in our population during the study period (death).

We calculated the mortality rate ratio as a relative measure of the exposure effect. The comparison of disease frequency between the groups was calculated by the ratio of risk in the exposed groups (LP) and the risk in the unexposed group (i.e. non-LP).

Random error
The significance threshold for all analyses was set at a two-sided alpha value of 0.05.
We provided the point estimate of the different risk analyses as the best estimate of the true value based on our observations and its 95% CI as an expression of the statistical precision of the estimate with a 5% significance level.

Bias and confounders
As restricting the study population to those surviving the first two years could introduce **selection bias** in our study design, we performed a sensitivity analysis including all PLWH initiating ART, thus also those not surviving the first two years, to check if the findings were consistent.

In this study, we assessed if initiating a regimen with a specific antiretroviral drug class (INSTI) compared to the others, was associated with better long-term survival or achieving a better immune response at two years. However, the availability of ART has varied throughout the study period. The first available INSTI drug (Raltegravir) was available in Europe from 2009 on. Besides, treatment guidelines have also varied over time, particularly from 2015 when the Strategic Timing of AntiRetroviral Treatment (START) study showed the benefit of starting ART early, when CD4 cell count is >500 cells/µL, instead of waiting until CD4 cell count fell <350 cells/µL. We adjusted our analysis for calendar time considering these periods (2005-2009, 2010-2014, 2015-2021) but we cannot rule out residual confounder. As there was a risk of confounding by indication or disease severity bias, where INSTI-based regimens are preferably prescribed to individuals with less advanced disease, we performed a survival analysis after performing **propensity score matching** on the following covariates reflecting important patient characteristics at ART initiation: age, gender, CD4 cell count, modified Charlson comorbidity score and ADE. The application of these matching helped to generate more balanced subpopulations regarding these potential confounders and thus eliminate or significantly reduce the risk of confounding by indication.

We also analysed the **interaction** between the association of the exposure and the outcome according to exposure to other factors (age, transmission mode, calendar time, basal CD4 cell count) without finding significant evidence of effect modification.

As in all observation studies, we cannot rule out the possibility of **residual confounding** by unknown or unmeasured risk factors (for example frailty, smoking, etc).

d) Statistical methods

Continuous variables were described as the median and the interquartile range (IQR), and categorical variables were presented as frequency and percentage. We used the chi-squared test, t-test and Mann-Whitney U test to compare covariates between groups.

Survival analysis
In the survival analysis right censoring was considered at the time of the event, death, loss-to-follow-up, or end of the observation period, whichever occurred first.

Kaplan-Meier survival curves were used to plot the estimated cumulative survival probability of the specific event (death) against time.

Poisson regression models were used to estimate MR, crude rates ratios, and rate ratios adjusted for potential confounding variables.

Age and calendar time were introduced in the model as time-updated/time-varying variables.

Propensity score matching

To create more balanced populations regarding confounding covariates across treatment groups when assessing the impact of initiation of INSTI as first-line regimen on long-term mortality, we performed Poisson regression analysis after using propensity score matching (1:1 nearest-neighbour matching with caliper 0.2 on each sample). We did a matching for the following variables at ART initiation: gender, age, CD4 cell count, modified Charlson comorbidity index, and ADE. The same analysis was conducted including instead all PLWH who initiated ART, regardless of survival in the first two years.

To create more balanced populations when assessing the effect of INSTI-based regimen as first-line regimen on incomplete CD4 cell count recovery 2 years after ART initiation (≤500 cells/μL), we conducted a logistic regression analysis after propensity score matching as described above matching for gender, age, CD4 cell count, modified Charlson comorbidity index, and ADE prior or within 90 days after ART initiation. We provided the ORs and a 95% CI.

e) Key findings

We included 2,719 PLWH who initiated ART between 2005-2019 and survived the first two years after ART initiation, with a median follow-up time of 5.8 years [IQR 3.2-8.9]) (16593.1 person-years).

Of the study population 1,441 (53%) were diagnosed at a late stage, being more frequently older, heterosexual men, women, with a history of IDU, of African origin, with lower educational levels, and higher economic deprivation.

Of LP, 44% (n=637) managed to achieve a favourable immune recovery two years after ART initiation (>500 cells/μL) (Figure 14).

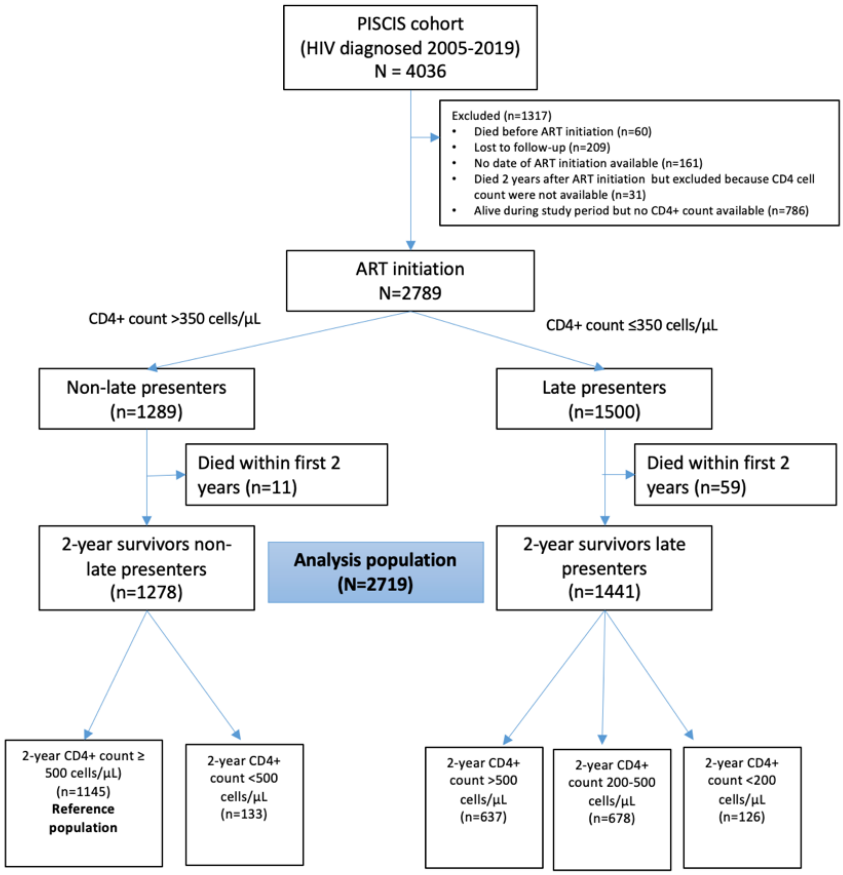


Figure 14. Flowchart of cohort construction.

Mortality risk

Overall, 113 (4.2%) died during the study period (crude all-cause MR 6.8/1000 person-years [95%CI: 5.7-8.2]). We found that CD4 cell count recovery two years after ART initiation predicted better long-term mortality than CD4 counts at ART initiation. Those LP achieving CD4 cell >500 cells/µL had comparable MR to the reference group (non-LP with two-year CD4 count >500 cells/µL), irrespective of their initial CD4 cell count (aMRR 1.05 [95%CI: 0.50-2.21], test for interaction p=0.48). On the contrary, individuals achieving CD4 cell counts ≤500-200 and <200 cells/µL had a 2-fold and 4-fold increased MR compared to non-LP, respectively (Figure 15, Table 7).

Other factors associated with increased mortality included detectable viremia at two years (>200 copies/ml), multimorbidity, and some HIV transmission groups (heterosexual men and IDU compared to MSM) (Table 7).

On the contrary, exposure to an INSTI-based regimen in the first two years was associated with better survival (aMRR 0.54 [95%CI: 0.31-0.-93]).

We conducted a series of different sensitivity analyses finding concordant results:

- Kaplan-Meier analysis excluding those with unsuppressed or unknown viremia two years after ART, and we found similar results.

- Survival analysis comparing non-LP with two-year ≥700 cells/μL and CD4 >500-699 without significant differences in MR (aMRR 0.55 [95%CI: 0.21-1.47]).

- Survival analysis to assess the effect of INSTI on mortality, including the above-described study population. We performed this analysis after conducting propensity score matching (on age, gender, CD4 count, Charlson comorbidity index, and ADE, all at ART initiation) to address the risk of potential prescription bias finding similar results (INSTI versus non-INSTI, aMRR 0.48 [95%CI: 0.25-0.90]).

- Survival analysis to assess the effect of INSTI on mortality, including all PLWH initiating ART, thus also those not surviving the first two years. We performed this analysis after conducting propensity score matching (on age, gender, CD4 count, Charlson comorbidity index, and ADE, all at ART initiation). This analysis was carried out to address the risk of selection bias. We found an association only when comparing INSTI versus PI (aMRR 0.41 [95%CI: 0.23-0.72]).

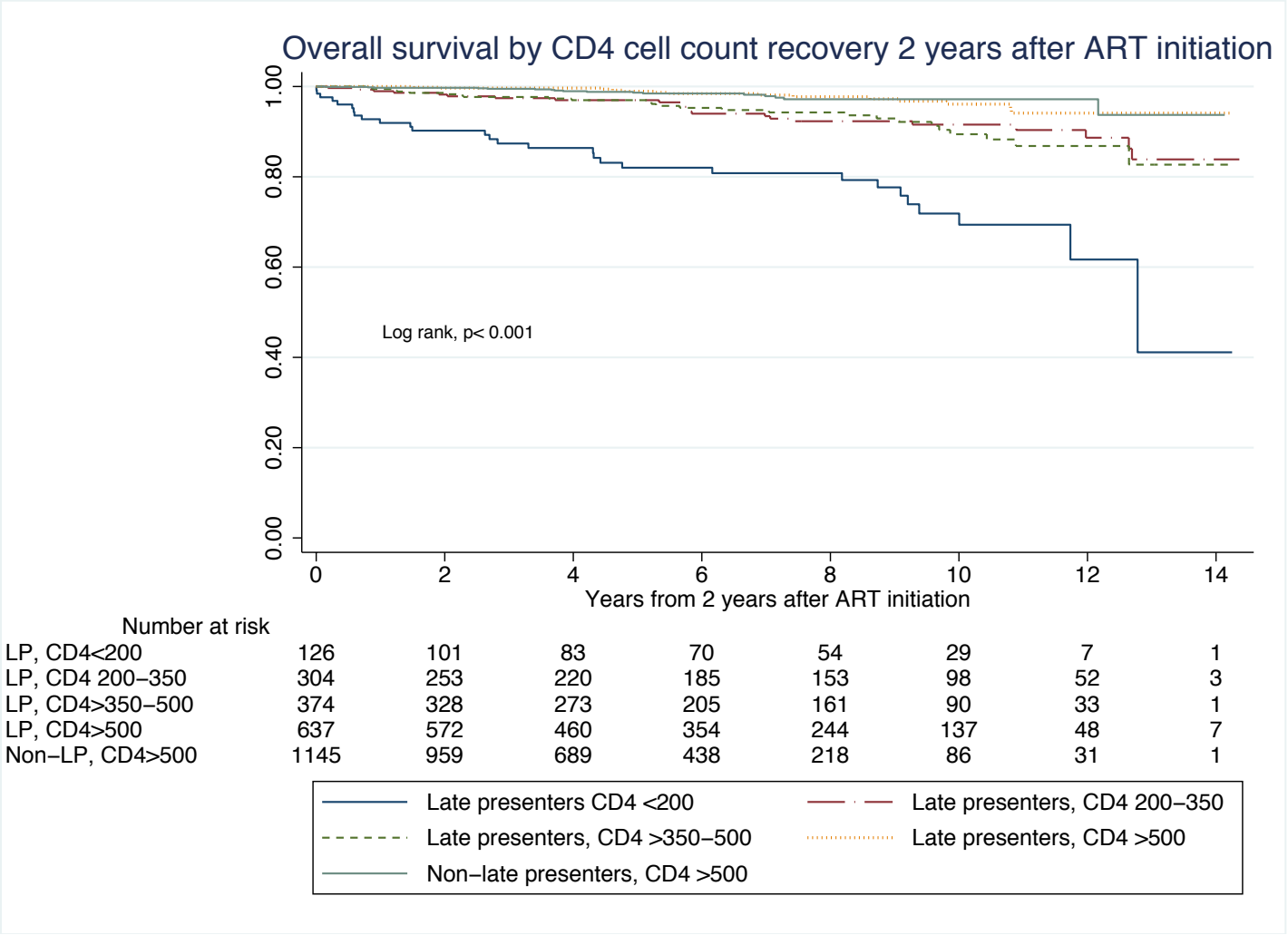
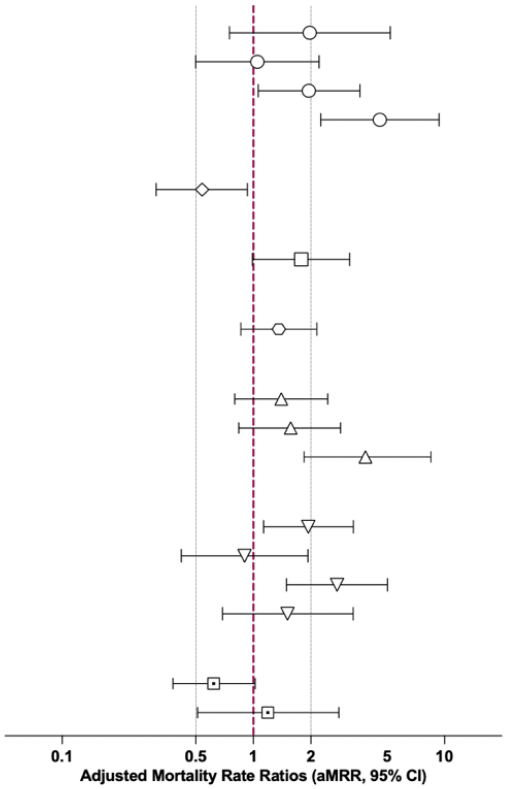


Figure 15. Kaplan-Meier curves for overall survival by immune recovery 2 years after ART initiation (non-LP with CD4 counts >500 cells/µL versus LP with CD4 counts >500 cells/µL, LP with CD4 counts >350-500 cells/µL, LP with CD4 counts 200-350 cells/µL and LP with CD4 counts <200 cells/µL at 2 years after ART initiation.

Table 7. Crude and adjusted mortality rate ratios in treatment-naive individuals surviving the first 2 years after ART initiation.

	Deaths (n, %)	*MRR (95% CI)[1]*	*aMRR (95% CI)[2]*
All individuals, N=2719	113 (4·2)		
CD4 cell count 2 years after ART initiation (cells/ µL)			
Non-late presenters, CD4 >500 cells/µL	17 (1·5)	Ref (1)	Ref (1)
Non-late presenters, CD4 ≤ 500 cells/µL	6 (4·5)	2·55 (1·00-6·46)	1·97 (0·75-5·21)
Late presenters, CD4>500 cells/µL	14 (2·2)	1·16 (0·57-2·35)	1·05 (0·50-2·21)
Late presenters, CD4 200-500 cells/µL	22 (7·2)	3·45 (1·98-6·00)	1·95 (1·06-3·61)
Late presenters, CD4 <200 cells/µL	29 (23·0)	12·66 (6·96-23·03)	4·59 (2·25-9·37)
INSTI as first line ART-regimen vs non-INSTI			
No	92 (5·3)	Ref (1)	Ref (1)
Yes	21 (2·2)	0·76 (0·47-1·21)	0·54 (0·31-0·93)
HIV viral load >200 c/ml 2 years after ART initiation			
No	89 (3·8)	Ref (1)	Ref (1)
Yes	15 (9·7)	2·28 (1·32-3·94)	1·78 (0·99-3·19)
AIDS defining event in the first 2 years after ART initiation			
No	85 (3·4)	Ref (1)	Ref (1)
Yes	28 (12·4)	2·96 (1·93-4·54)	1·36 (0·86-2·15)
Modified Charlson comorbidity score at ART start			
0	63 (2·8)	Ref (1)	Ref (1)
1	20 (7·6)	2·91 (1·76-4·81)	1·40 (0·80-2·45)
2-3	17 (10·1)	3·90 (2·28-6·66)	1·57 (0·86-2·86)
≥ 4	13 (28·3)	14·86 (8·18-27·00)	3·96 (1·84-8·50)
Transmission mode			
MSM	30 (1·8)	Ref (1)	Ref (1)
Heterosexual men	37 (9·4)	4·74 (2·93-7·67)	1·94 (1·13-3·34)
Women	10 (3·1)	1·53 (0·75-3·13)	0·90 (0·42-1·93)
IDU	25 (13·4)	6·46 (3·80-10·98)	2·74 (1·49-5·03)
Unknown/other	11 (6·7)	3·74 (1·87-7·45)	1·51 (0·69-3·33)
Calendar time (time-updated)			
2015-2020	79 (3·1)	Ref (1)	Ref (1)
2010-2014	28 (21·1)	1·03 (0·67-1·59)	0·62 (0·38-1·02)
2005-2009	6 (37·5)	2·13 (0·93-4·89)	1·19 (0·51-2·80)



ART, antiretroviral therapy; INSTI, integrase strand transfer inhibitor; MSM, men who have sex with men; IDU, injection drug use.
[1] MRR, mortality rate ratio.[2] aMRR, adjusted mortality rate ratio
[2] aMRR, adjusted mortality rate ratio. The multivariate analysis was adjusted for age at baseline, transmission mode, CD4 cell count recovery at 2 years after ART initiation, HIV viral load > 200 c/ml vs ≤200 c/ml at 2 years, INSTI-based regimen as the first-line regimen in the first 2 years, modified Charlson comorbidity score at baseline, history of AIDS-defining event at baseline, and calendar time (time-updated).

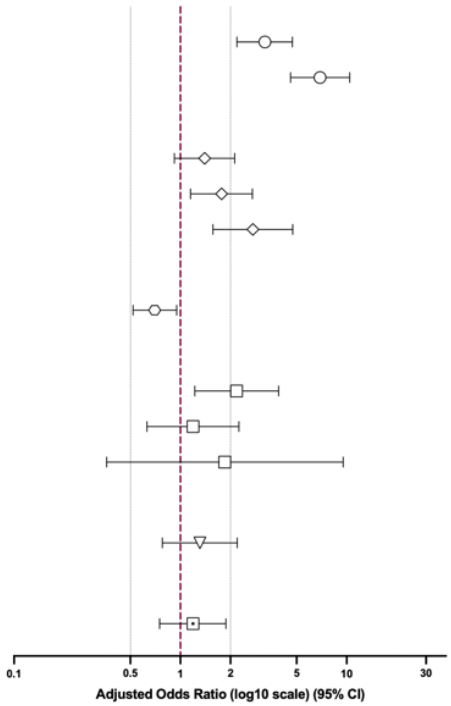
Risk factors for incomplete immune recovery

This analysis was conducted as a nested case-control study, using logistic regression after propensity score matching (on age, gender, CD4 count, Charlson comorbidity index, and ADE prior to or within 90 days after ART initiation). We defined incomplete immune recovery as two-year CD4 cell count ≤500 cells/µL, based on our results in long-term mortality.

We found that exposure to an INSTI-based regimen as first-line ART was associated with a lower risk of incomplete immune recovery (aOR 0.70 [95%CI: 0.52-0.95]). On the other hand, factors associated with a higher risk of incomplete immune response included: lower CD4 cell count, older age, and a worse Charlson comorbidity index at ART initiation (Table 8).

Table 8. Variables associated with incomplete immune restoration, defined as CD4 cell count ≤500 cells/µL, 2 years after ART initiation, in individuals with CD4 cell count ≤350 cells/µL at ART initiation.

	Incomplete immune recovery (n, %)	OR (95% CI)	*aOR (95% CI)*[1]
Individuals with CD4 cell count nadir ≤350 cells/µL (n=1441)	804 (55·8)		
CD4 cell count nadir at ART initiation (cells/ µL)			
CD4 200-350 cells/µL	283 (38·0)	Ref (1)	Ref (1)
CD4 100-199 cells/µL	205 (65·3	3·33 (2·31-4·83)	3·22 (2·19-4·72)
CD4 <100 cells/µL	316 (82·5)	8·13 (5·54-11·92)	6·92 (4·60-10·41)
Age at ART initiation (years)			
<30	123 (43·6)	Ref (1)	Ref (1)
30-39	279 (51·9)	1·75 (1·20-2·56)	1·40 (0·92-2·12)
40-49	257 (61·1)	2·22 (1·51-3·28)	1·77 (1·15-2·71)
≥50	145 (72·5)	4·40 (2·68-7·22)	2·73 (1·57-4·74)
INSTI as first line ART regimen			
No	567 (56·4)	Ref (1)	Ref (1)
Yes	237 (54·5)	0·81 (0·62-1·05)	0·70 (0·52-0·95)
Modified Charlson comorbidity score at ART initiation			
0	679 (53·8)	Ref (1)	Ref (1)
1	76 (70·4)	2·91 (1·76-4·80)	2·18 (1·22-3·89)
2-3	40 (66·7)	3·91 (2·29-6·68)	1·19 (0·63-2·25)
≥4	9 (81·8)	15·03 (8·27-27·30)	1·85 (0·36-9·54)
AIDS-defining event at ART initiation			
No	663 (53·0)	Ref (1)	Ref (1)
Yes	141 (74·2)	3·16 (1·99-5·03)	1·31 (0·78-2·20)
Gender			
Men	653 (54·3)	Ref (1)	Ref (1)
Women	151 (63·2)	1·51 (1·01-2·26)	1·19 (0·75-1·88)



AIDS, Acquired Immune Deficiency Syndrome; ART, antiretroviral therapy; INSTI, integrase strand transfer inhibitor; MSM, men who have sex with men; IDU, injection drug use.
[1] Multivariate analysis adjusted for age (categorical), gender, CD4 cell count at ART initiation, INSTI-based regimen, Charlson comorbidity score at ART initiation, and ADE at ART initiation
Propensity score weighting on age, gender, CD4 cell count at ART initiation, and Charlson comorbidity score at ART initiation.

Additional sensitivity analysis:

- To assess the effect of INSTI in assessing incomplete immune recovery using a two-year CD4 count ≥700 cells/µL as a threshold. We performed logistic regression analysis after propensity score matching as described above, finding similar results (aOR 0.59 [95%CI: 0.46-0.76]).

f) Strengths and limitations

Strengths:

- The study is strengthened by the prospective, population-based, multicentre cohort design, including 84% of all PLWH followed in Catalonia, and the long observation time. We had access to high-quality data on comorbidities and mortality generated by the public health system (PADRIS).
- We performed diverse additional analyses adjusting with propensity score matching, obtaining more comparable treatment risk groups in the cohort regarding INSTI-exposure,

reducing the risk for prescription bias. Besides, we conducted the analysis including all PLWH initiating ART to address the potential risk of selection bias in the initial study population

Limitations:

- The lack of information on the cause of death hampers the detailed interpretation of the mortality figures.
- We excluded 817 individuals because of unavailable initial (n=223) or two-year CD4 cell count (n=708). Of the latter, 41% corresponded to individuals whose two-year blood samples had to be performed during the years 2020-2021 during the COVID-19 pandemic, where hospital closure for non-COVID-19 patients prevented them from accessing the hospital. Of those missing CD4 counts at ART initiation, the majority (n=137, 61%) belonged to the initial calendar period 2005-2009, with a high proportion of IDU and mortality. This subpopulation differs from our overall study population, but given the small absolute numbers, it is unlikely to have influenced our finding.
- There is always a risk of confounding by indication, in this case, disease severity bias, when assessing drug exposure in an observational study. We tried to address this by performing a propensity score matching with relevant baseline variables at ART initiation. In consequence, matched individuals were more similar regarding these covariates at the moment of initiation of ART and had thus more similar risk of the endpoint, to better assess the effect of the exposure to INSTI. However, we cannot rule out some degree of residual confounder.
- ART availability and prescribing practices have varied over time. To adjust for these differences, we added calendar time to our model. We cannot, however, rule out residual confounders.
- Other residual or unknown confounders, such as viral resistance, lifestyle factors, or comorbidities not included in the study, are possible. These factors, however, are unlikely to have a major impact on our results.

Tuberculosis incidence and outcomes in people living with HIV in Denmark: study IV

a) Data sources

Study IV is based on data from the Danish HIV Cohort, different administrative Danish population-based registries, and a review of medical records. As mentioned previously, all the registries used are characterized by high data quality with high validity and completeness and long follow-up, covering the whole Danish population (Figure 16).

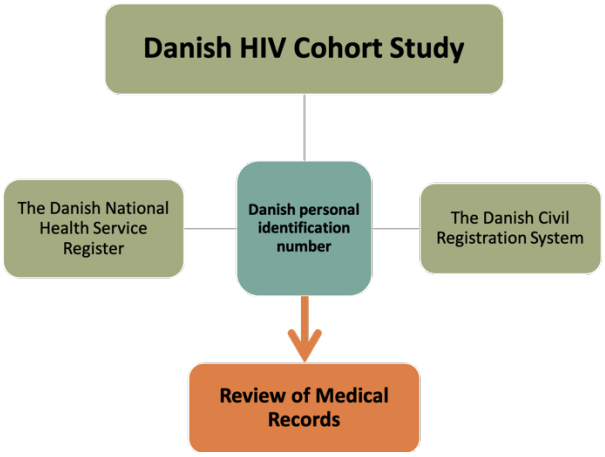


Figure 16. Data sources in study IV.

The information extracted from the different sources is shown in table 9.

	Purpose of the information extracted from the registries
Danish National Registries	
The Danish HIV Cohort Study[1]	To identify all adults ≥ 18 years diagnosed in Denmark with HIV between 1 January 1995 and 1 May 2017 To identify all patients diagnosed with a mycobacterial infection during the study period (including both hospitalized and outpatient diagnosis).
The Danish Civil Registration System	We extracted data on emigration, immigration and date of death
The Danish National Hospital Registry[1]	To identify all patients diagnosed with a mycobacterial infection during hospitalization (according to ICD-10 codes at discharge, supplementary data)
Medical records	To validate TB diagnosis To classify TB as definitive and presumptive diagnoses. To gather additional clinical data relevant to TB diagnosis To validate the cause of death

[1] Both databases were included to improve case detection.
Table 9. Registries used in the study IV.

The Danish National Hospital Registry (*DNHR).*
The DNHR was established in 1977 and contains information about hospitalization from non-psychiatric Danish hospitals, including discharge diagnosis codes according to the ICD-10 from 1994 onwards.

Review of medical records.
Five infectious diseases specialists reviewed all medical records from patients coded with a mycobacterial diagnosis in DHCS or DNHR between January 1, 1995, and May 1, 2017. They

validated the TB diagnosis and gathered additional clinical information, including death causes (Figure 17).

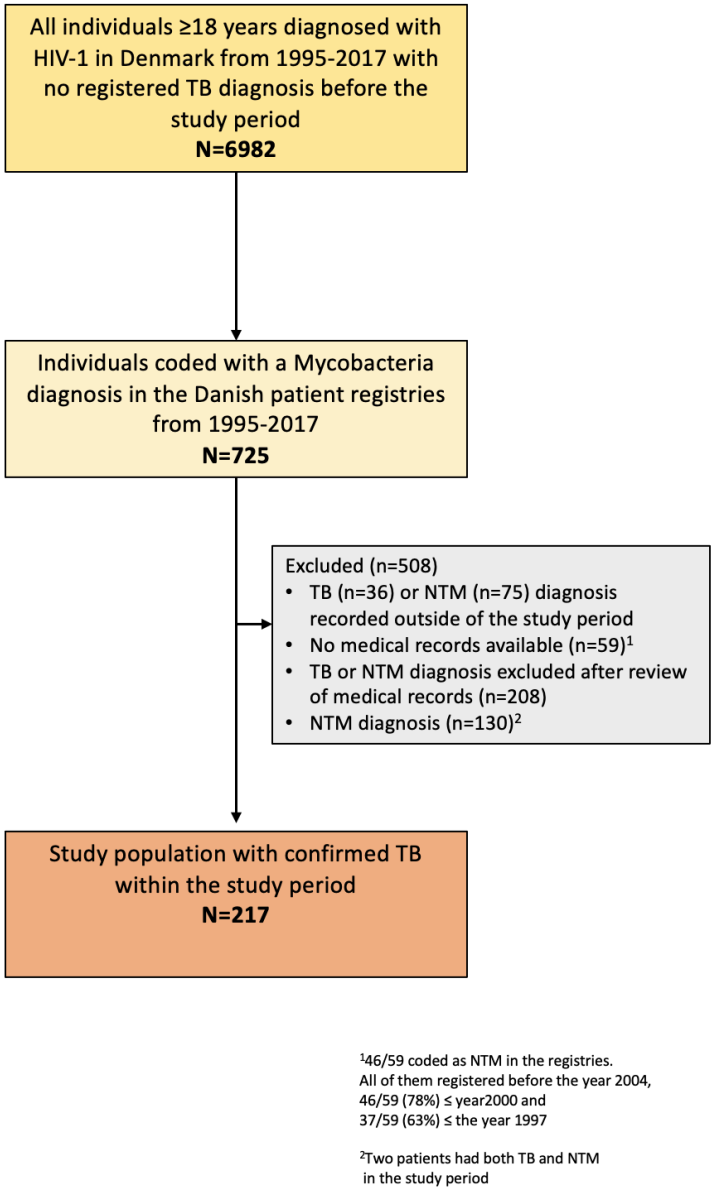


Figure 17. Flowchart of cohort construction in study IV.

b) Study design

Study IV was designed as a cohort study.
We included all adult PLWH from the DHCS followed for HIV care between 1995-2017 without a previous TB diagnosis before study inclusion (Figure 18).
The study had two parts. In the first part, the primary outcome was time to TB diagnosis. In the second part, the primary outcome was time to all-cause death for the subpopulation of HIV/TB coinfected individuals. We explored associated risk and prognostic factors.

TB was classified as:

- Concomitant: diagnosed within 1 month before and up to 3 months after HIV diagnosis
- Diagnosed > 3 months after HIV diagnosis

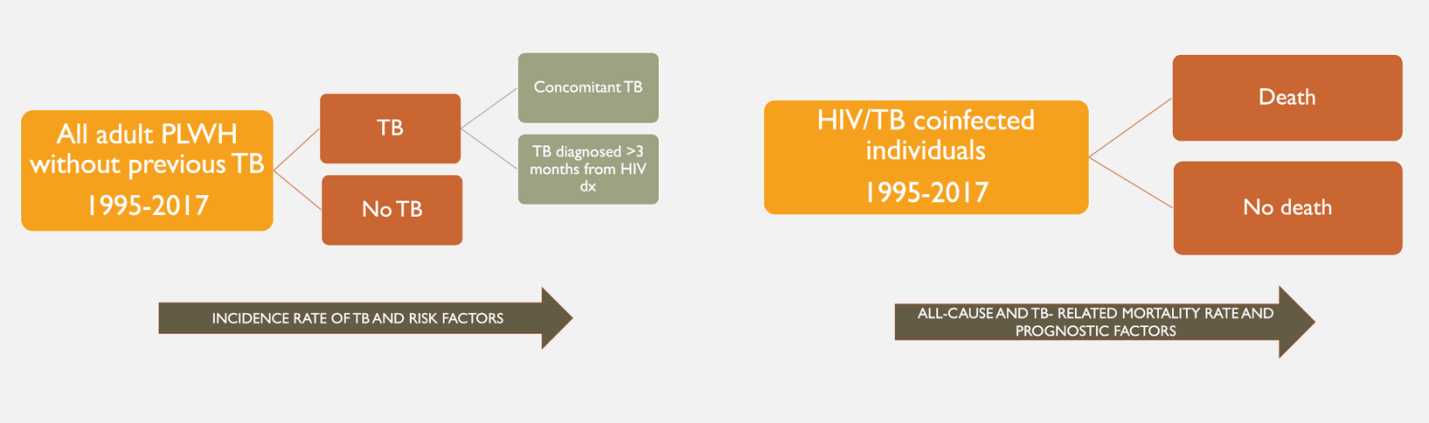


Figure 18. Study design in study IV.

c) Methodological considerations

Measure of association
We calculated IR or MR as a measure of disease frequency and the incidence or mortality rate ratio as a relative measure of the exposure effect.

Random error
We provided the point estimate of the different risk analyses as the best estimate of the true value based on our observations and its 95% CI as an expression of the statistical precision of the estimate with a 5% significance level.

The absolute number of deaths was low and type II errors in this analysis cannot be ruled out.

Bias and confounders
Fifty-nine older medical records of patients coded with any mycobacterial infection were sent to shredding in the archives, 78% of them before the year 2000, which could have resulted in an underestimation of TB incidence in the first period (1995-2002), and thus the true decline in TB incidence over time could have been even steeper.

Our study's social burden definition has not been validated, but it summarizes risk factors for TB exposure that reflect social vulnerability.

As in all observation studies, there is the possibility of **residual confounding** by unknown or unmeasured risk factors.

d) Statistical methods

Continuous variables were described as median and the IQR and categorical variables were presented as frequency and percentages. Chi-squared test, t-test, and Mann-Whitney U test were used to compare variables between groups.

For TB incidence, the date of study inclusion was the most recent of the following: January 1, 1995, the date of HIV diagnosis, or the date of immigration for individuals with known HIV infection. We estimated the time from study inclusion to the first of the following: TB diagnosis, death, emigration, loss to follow-up, or May 1, 2017. We calculated the crude IR and 95% CI. We further assess incidence by i) calendar time, ii) time after HIV diagnosis and iii) by CD4 cell count recovery.

For mortality, the baseline was the date of TB diagnosis. We estimated the time from TB diagnosis to the first of the following: death, emigration, loss to follow-up, or May 1, 2017. We calculated crude all-cause MR and 95%CI.

We used Poisson regression to control for potential confounders both in the incidence and mortality analyses.

Age, ART initiation, calendar time, and time from TB diagnosis were introduced in the model as time-updated/time-varying variables.

Kaplan-Meier analyses were used to estimate the cumulative survival of coinfected individuals. Curves were stratified by i) social burden, ii) TB localization, and iii) HIV viremia copy-years[72,73] before TB diagnosis.

We defined social burden as at least one of the following risk factors: alcohol consumption >7/14 drinks per week, history of IDU, homelessness, stay in a shelter or in prison facilities, and/or prostitution.

e) Key findings

We included 6982 PLWH during 1995-2017 (73,596 person-years, with a median time of follow-up of 9.6 years (IQR 3.8-16.9)).

Overall, 217 developed TB in the study period, with an IR of 2.9/1000 person-years (95% CI 2.6-3.4): 6.7/1000 person-years (95% CI 5.7-7.9) for migrants and 1.4/1000 person-years (95% CI 1.1-1.7) for Danish-born individuals (excess IR for migrants 5.3/1000 person-years, $p < 0.001$). A high proportion had a definitive TB diagnosis (84%) and very few were lost to follow-up (1%).

Among HIV/TB co-infected patients, there were fewer males, a higher percentage of heterosexuals, and more non-European individuals (mainly from Africa and Asia) compared with mono-infected individuals. The median time from arrival in Denmark to HIV diagnosis was 5.9 years (IQR 2.8-9.1) for the migrants.

Two different HIV/TB coinfected populations
Individuals who developed TB >3 months after their HIV diagnosis (median time from HIV to TB diagnosis of 5.1 years) differed in their characteristics from those who were diagnosed with concomitant TB/HIV (within 3 months from HIV diagnosis) (Figure 19 and Table 10):

- Demographic characteristics
    - More often Danish origin (42% *versus 23%)*
    - Older age at TB diagnosis (median age 40 *versus* 35 years)

        - Longer median time from arrival to Denmark to TB diagnosis (5.9 *versus* 2.7 years)
    - Behavioural characteristics
        - Higher prevalence of history of IDU (25% *versus 8%)*, social burden (38% *versus* 26%), and smoking (52% *versus 37%)*
    - HIV-related characteristics
        - Higher CD4 cell count at TB diagnosis (median CD4 count 260 *versus* 80 cells/μL)
        - Lower HIV viral load at TB diagnosis (median log10 3.0 *versus* 5.2 copies/ml)
        - Only half were on ART or with suppressed viremia at TB diagnosis despite known HIV infection
    - Comorbidity
        - Higher prevalence of high Charlson comorbidity scores ≥2 (35% *versus* 10%) (mainly driven by liver- and AIDS-defining illnesses (22% *versus* 7%)
    - TB outcomes
        - Higher rates of pulmonary TB (58% versus 34%) and lower rates of disseminated TB (20% versus 44%)
        - Higher rates of interruption of TB treatment (5% *versus* 0)
        - Higher mortality (36% *versus 13%)*

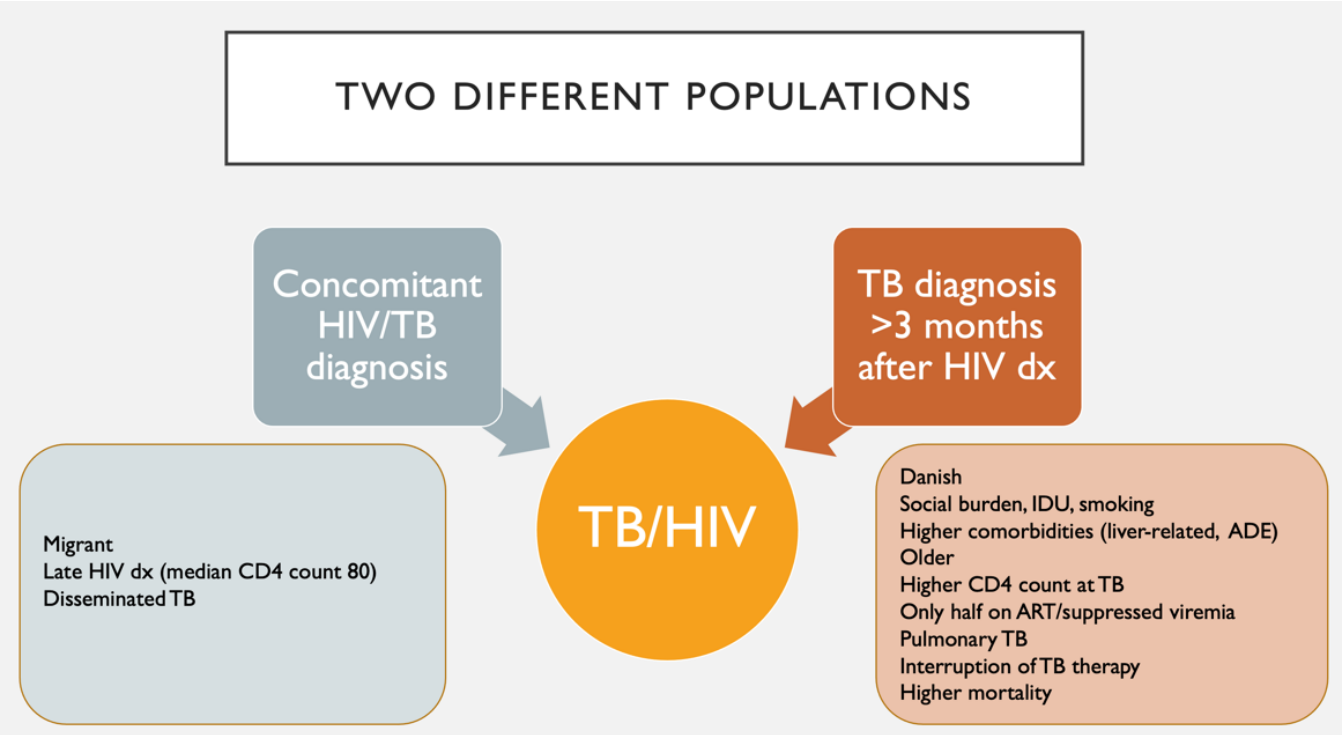


Figure 19. Two distinct populations with TB/HIV co-infection.

	All TB cases 1995-2017 n=217	Concomitant TB/HIV n=99		TB diagnosed >3 months after HIV n=118		p-value
TB diagnosis based on:						
Microbiology (PCR or culture)	180 (83.7)					
Histopathology	33 (15.4)					
Clinical suspicion	2 (0.9)					
Autopsy	2 (0.9)					
Performance of Interferon-gamma release assays	67 (30.9)	24 (24.2)		43 (36.4)		0.05
Positive	40 (59.7)	15 (62.5)		25 (58.1)		0.25
Negative	25 (37.3)	9 (37.5)		16 (37.2)		0.30
Performance of mantoux test	32 (14.7)	11 (11.1)		21 (17.8)		0.17
Positive	21 (65.6)	7 (63.6)		14 (66.7)		0.23
Negative	11 (34.4)	4 (36.4)		7 (33.3)		0.53
Tb localization						
Pulmonary	102 (47.0)	34 (34.3)		68 (57.6)		0.001
Extrapulmonary	47 (21.7)	21 (21.2)		28 (22.0)		0.88
Disseminated	68 (31.3)	44 (44.4)		24 (20.3)		<0.0001
Treatment outcome[b]						
Cure	5 (2.3)	1 (1.0)		4 (3.4)		0.25
Treatment completed	187 (86.2)	94 (95.0)		93 (78.8)		0.001
Died in the study period	55 (25.4)	13 (13.1)		42 (35.6)		<0.0001
Treatment failure	0	0		0		-
Treatment interrupted	6 (2.8)	0		6 (5.1)		0.02
Relapse	16 (7.4%)	8 (8.1)		8 (6.8)		0.72
Median time from TB to death, years (IQR)	2.1 (0.3-8.4)	5.8 (1.4-8.7)		1.8 (0.1-7.3)		0.16
Causes of death	Total	First ½ year	Beyond ½ year	First ½ year	Beyond ½ year	
TB-related n, (%)	16 (29.0)	2[c]	0	12[d]	2[e]	
HIV-related n, (%)	3 (5.5)	0	1	0	2	
Not TB- or HIV-related n, (%)[f]	27 (49.1)	0	9	2	16	
Unknown n, (%)	9 (16.4)	0	1	0	8	

TB, Tuberculosis; IQR, interquartile range; MSM, men having sex with men; IDU, illicit drug use; HCV, hepatitis C virus infection; HBV, hepatitis B virus infection; ART, antiretroviral therapy, NA, data not available.

Table 10. Clinical characteristics in HIV/TB coinfected individuals.

TB screening and treatment for latent TB infection (LTBI)

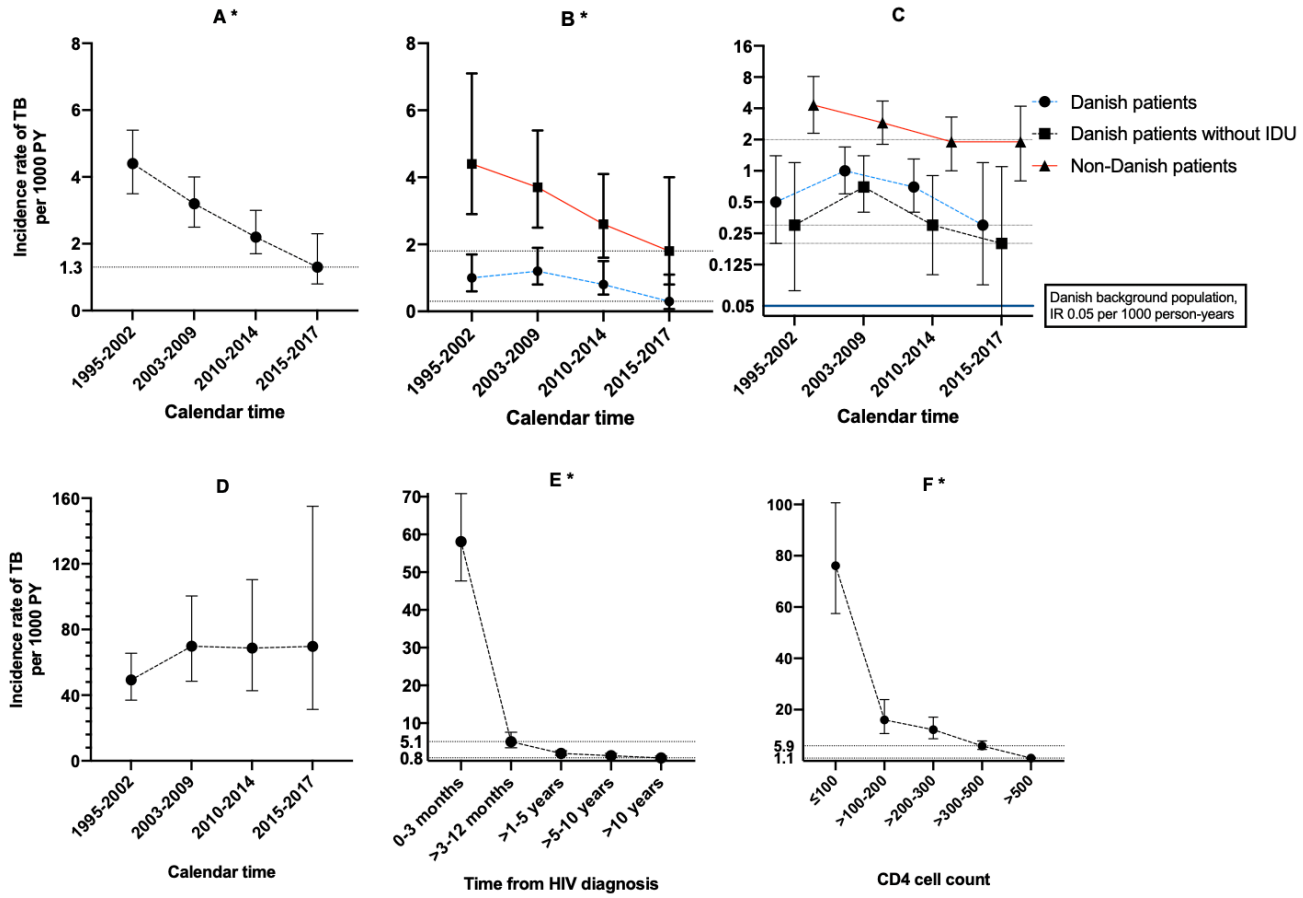
Only 45% of individuals with known HIV infection (TB developed >3 months after HIV diagnosis) had either a Mantoux test and/or IGRA performed before TB diagnosis (mainly migrants (62%)), finding a positive result in 54% of cases. However, none of these patients had received treatment for LTBI (Table 10).

TB incidence

The IR of concomitant TB/HIV infection remained high and unchanged during the whole study period. A decline in incidence over calendar time was observed for those individuals diagnosed with TB >3 months from HIV diagnosis. The risk of TB declined also with time from HIV diagnosis and inversely with CD4 cell count recovery (Figure 20).

Risk factors associated with increased risk of TB included (Table 11):

- African/Asian/Greenland origin
- History of IDU or heterosexual route of transmission compared to MSM
- CD4 cell count < 200 cells/μL
- Not being on ART

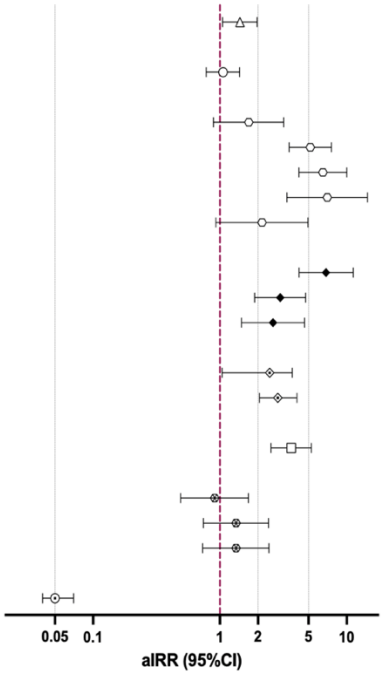


Incidence rates (IR) of TB are defined as incident TB cases per 1000 person-years of follow-up (PY). IDU, illicit drug use.
A. TB IR over calendar time. B. TB IR in patients with known HIV infection over calendar time stratified by Danish origin. C. TB IR in patients with known HIV infection on antiretroviral therapy (ART) over calendar time stratified by Danish origin (incidence rate in log scale base 2). The blue line represents TB incidence in the Danish background population (source www.ssi.dk). D. TB IR diagnosed concomitantly with HIV infection over calendar time. E. TB IR by time after HIV diagnosis. F. TB IR by CD4 cell count recovery (time updated). The dotted grey lines in every figure represent the point IR in the recent calendar period.
* Statistically significant test for trend (<0.001)

Figure 20. TB IR stratified by calendar time, time after HIV diagnosis, and CD4 cell count recovery.

Table 11: Predictive factors for developing tuberculosis in Danish HIV-infected patients.

	TB events	aIRR[a] (95% CI)
TOTAL	217	
DEMOGRAPHICAL FACTORS		
Gender		
Female	90	Ref (1)
Male	127	1.44 (1.05-1.97)
Age (time-updated)[b]		
≥40 years	93	Ref (1)
< 40 years	124	1.06 (0.78-1.43)
Region of birth		
Denmark	74	Ref (1)
Europe	11	1.69 (0.89-3.18)
Africa	82	5.16 (3.52-7.57)
Asia	36	6.49 (4.20-10.01)
Greenland	8	7.03 (3.38-14.59)
other	6	2.15 (0.93-4.96)
Route of HIV transmission		
MSM	30	Ref (1)
IDU	37	6.87 (4.21-11.24)
Heterosexual	127	2.99 (1.88-4.74)
other	23	2.62 (1.48-4.64)
HIV FACTORS		
CD4 cell count at baseline		
CD4 cell count >=200 cells/µl	138	Ref (1)
CD4 cell count <200, >=100 cells/µl	30	2.47 (1.64-3.72)
CD4 cell count <100 cells/µl	48	2.86 (2.05-4.05)
ART initiation (time-updated)		
Time before ART initiation	133	3.65 (2.53-5.26)
Time after ART initiation	84	Ref (1)
TIME RELATED FACTORS		
Calendar time (time-updated)		
1995-2002	82	0.91 (0.49-1.68)
2003-2009	75	1.34 (0.74-2.42)
2010-2014	46	1.34 (0.73-2.44)
2015-2017	14	Ref (1)
HIV-status at TB diagnosis		
Concomitant diagnosis[c]	99	Ref (1)
Known HIV infection[d]	118	0.05 (0.04-0.07)



TB mortality

Of the TB/HIV coinfected patients, 55 (25.4%) died during the study period (27,866 person-years, crude all-cause MR 27.9/1000 person-years, 95% CI 21.4-36.3).
From 2007-2017, the MR was 56% higher in PLWH who developed TB compared to HIV mono-infected. A decline in mortality was observed for mono-infected PLWH (from 34.2/1000 person-years to 14.6/1000 person-years before and after 2007, respectively; IRR 0.43, 95% CI 0.39-0.47), whereas mortality remained unchanged during the study period in TB/HIV co-infected patients.

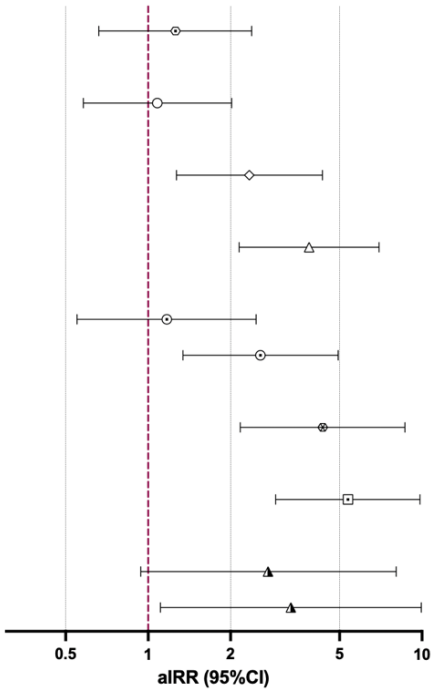
Prognostic factors for mortality in TB/HIV coinfected patients included (Table 12, Figure 21):

- Danish-origin
- Social burden
- Higher viraemia/copy years
- Disseminated TB
- TB diagnosed >3 months after HIV, despite the higher rates of late HIV diagnosis in those with concomitant TB/HIV diagnosis (13% versus 36%, $p < 0.001$). In this group, 33% of the deaths occurred within the first 6 months and were mainly TB-related (compared to 15% in the concomitant group).

TB-related deaths accounted for 29% of all deaths and 88% of them occurred in the first ½ year after TB diagnosis.

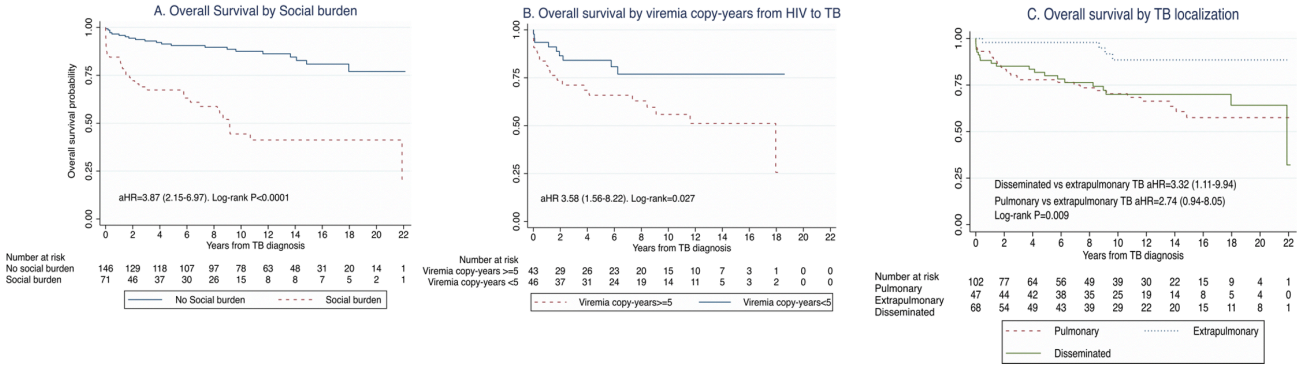
Table 12. Prognostic factors for mortality in HIV-infected patients developing TB

	Deaths	aMRR[a] (95% CI)
Total	55	
Gender		
Female	15	Ref (1)
Male	40	1.26 (0.66-2.39)
Age (time-updated)[b]		
>= 40 years	38	Ref (1)
< 40 years	17	1.08 (0.58-2.02)
Migrants		
No	38	2.34 (1.27-4.33)
Yes	17	Ref (1)
Social burden[e]		
No	21	Ref (1)
Yes	34	3.87 (2.15-6.97)
CD4 cell count at TB diagnosis		
≥200 cells/µl	25	Ref (1)
<200, >=100 cells/µl	10	1.17 (0.55-2.48)
<100 cells/µl	20	2.57 (1.34-4.94)
HIV-status at TB diagnosis		
Concomitant diagnosis[g]	13	Ref (1)
Known HIV infection[h]	42	4.34 (2.17-8.66)
Time from TB diagnosis (time-updated)		
First 6 months	16	5.37 (2.92-9.84)
Beyond 6 months	39	Ref (1)
TB localization		
Extrapulmonary	4	Ref (1)
Pulmonary	31	2.74 (0.94-8.05)
Disseminated	20	3.32 (1.11-9.94)



PYR, person-years at risk.
[a] Adjusted for age, gender, migrant, social burden, CD4 cell count, HIV status at TB diagnosis, first ½ year from TB diagnosis (time-updated), and TB localization. [b] Age, ≥ 40 years vs < 40 years at the time of TB diagnosis (time-updated); [c] Alcohol consumption defined as >7/14 drinks per week for women and men, respectively; [d] Tobacco consumption defined as ≥10 cigarettes daily; [e] Social burden defined as alcohol consumption >7/14 drinks per week, IDU, homelessness, stay in prison or shelter facilities and/or prostitution; [f] Viraemia copy-years from HIV to TB diagnosis, median Log10 copies/mL in those patients with known HIV-infection, ≥log10 5 copies xy/mL vs <log10 5 copies xy/mL; [g] TB was diagnosed at, up to 1 month before or within 3 months after HIV diagnosis; [h] TB was diagnosed >3 months after HIV diagnosis.

Figure 21. Kaplan-Meier curves for overall survival after tuberculosis diagnosis in HIV-infected individuals stratified by social burden, viremia copy-years, and TB localization



f) Strengths and limitations

Strengths
The design is a nationwide, population-based cohort with high coverage, a long observation period, and nearly complete follow-up.

We had access to national registries with high-quality data on vital status and migration at the individual level.

All medical records of individuals with mycobacterial infections coded in the registries were reviewed across the country to confirm the diagnosis of TB and obtain additional clinical data.

Limitations
Fifty-nine older medical records of patients diagnosed with a mycobacterial infection were transferred to the archives for shredding and could not be checked, 78% of them before the year 2000. This may have resulted in an underestimation of the TB incidence between 1995 and 2002. As a result, the true reduction in TB incidence over time could have been greater.

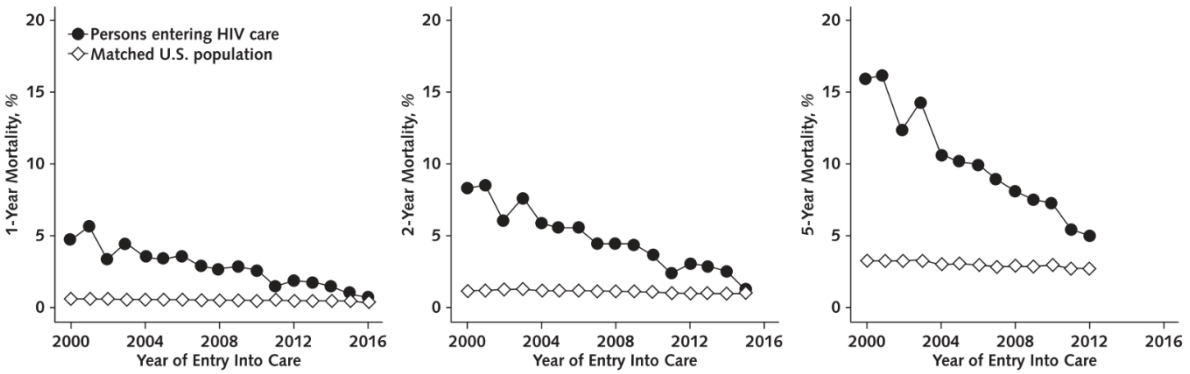
The absolute number of deaths among HIV/TB co-infected individuals was low. Therefore, conclusions should be drawn with caution as type II errors cannot be ruled out.

Discussion

Increasing life expectancy over the years, but the survival gap remains

The survival of PLWH has improved substantially over the years, which responds to an interplay of multiple factors such as advances in HIV management and linkage to care, earlier initiation of ART, increasing CD4 cell count at entry into care, better prevention, screening, and management of chronic comorbidities, new and more efficient ART drugs and drug-classes, and higher rates of and shorter time to viral suppression. In fact, some studies have shown that PLWH starting ART with CD4 cell count ≥350 cells/µL and without major risk factors has a life expectancy comparable[1,2] or close[4–6] to the general population.

Figure 2. Mortality at 1 year (*left*), 2 years (*middle*), and 5 years (*right*) after entering HIV care among 82 766 persons entering care at a U.S. NA-ACCORD clinical site between 1999 and 2017 compared with a matched subset of the general U.S. population, stratified by year of entry into care.



The gap in 1-year mortality seemed to close by the later years examined (*left*), but 5-year mortality remained elevated over the entire period (*right*), suggesting that some factors may influence the effectiveness of care (e.g., treatment discontinuation or disengagement from care) and, therefore, increase mortality among persons with HIV after the first year. NA-ACCORD = North American AIDS Cohort Collaboration on Research and Design.

Figure from Edwards JK, et al. Annals of Internal Medicine 2021;174:1197-1206.

Despite this improvement in survival, studies on life expectancy show that low CD4 cell count at ART initiation is still the main driver of the persistent disparity in life expectancy observed in PLWH compared to the general population and particularly in LP (i.e., with CD4 cell count <350 cells/µL or with an ADE) compared to non-LP. The mortality risk among these patients diagnosed late is highest in the year following HIV diagnosis, driven mainly by AIDS-defining illnesses and complications present at this time, and decreases thereafter. Some studies have shown that, in fact, the occurrence of ADE relates to a persistent survival impact over the years despite attaining high CD4 cell counts and suppressed viremia.[15,35] In a study from COHERE analysing 84,524 PLWH enrolled in the cohort from 2000 to 2011, Mocroft et al found that late HIV presentation was associated with a high risk of AIDS/death in the first year (13-fold increase in southern Europe and a 9-fold increase in North-Europe).[9] Similar findings were reported by Sobrino-Vegas et al, in the Spanish CoRIS cohort study including 7,165 newly diagnosed HIV-infected individuals from 2004 to 2013, finding a mortality risk of a 10-fold increase in the first year after HIV diagnosis, highest in those with an ADE and in those with CD4 <200 cells/µL at HIV diagnosis (22-fold and 5.6-fold increased risk, respectively).[15]

Table 3. Life expectancy at age 20 years in the Swiss HIV Cohort Study, by treatment era.

	Life expectancy (95% CI)				
	Monotherapy (1988–1991)	Dual therapy (1992–1995)	Early cART (1996–1998)	Later cART (1999–2005)	Recent cART (2006–2013)
Overall life expectancy	11.8 (11.2–12.5)	20.8 (19.4–22.2)	44.7 (42.2–47.3)	50.8 (48.5–53.3)	54.9 (51.2–59.6)
Education					
Higher education	–	26.7 (22.2–31.6)	53.0 (46.9–59.0)	58.2 (53.4–63.2)	60.0 (53.4–67.8)
Vocational training	–	25.3 (23.0–27.7)	44.4 (41.4–47.7)	49.2 (46.5–52.1)	52.6 (48.3–57.9)
Compulsory school	–	24.3 (21.3–27.5)	38.9 (35.1–43.0)	46.5 (43.1–50.3)	52.7 (46.4–60.1)
Main source of income					
Work	–	32.8 (29.2–36.5)	55.0 (50.9–58.9)	63.9 (59.3–68.4)	62.9 (56.2–70.9)
Welfare benefits	–	15.8 (13.6–18.4)	31.5 (29.0–34.1)	39.4 (36.9–42.0)	48.0 (43.4–53.0)
HIV transmission group					
MSM	8.9 (8.1–9.8)	22.9 (20.5–25.4)	52.7 (48.6–57.1)	57.3 (53.5–61.5)	56.8 (51.8–63.6)
Heterosexual contact	15.3 (13.7–17.21)	29.6 (26.1–33.3)	49.5 (45.8–53.6)	53.1 (50.2–56.2)	56.7 (51.7–62.8)
Injection drug use	12.4 (11.5–13.3)	15.7 (14.2–17.4)	27.3 (25.3–29.5)	31.3 (28.8–33.4)	35.8 (30.6–41.5)
Injection drug use					
Never	11.3 (10.4–12.2)	25.1 (23.1–27.2)	51.9 (49.0–55.1)	54.6 (52.2–57.1)	57.2 (53.1–62.5)
Former	12.2 (11.4–13.1)	16.9 (15.0–18.9)	29.9 (27.4–32.5)	33.5 (30.9–36.3)	39.6 (34.4–45.1)
Current	12.2 (10.6–14.0)	15.2 (12.9–17.7)	24.7 (21.7–27.9)	29.0 (25.8–32.4)	–
Smoking					
Never	–	–	–	65.2 (60.1–70.6)	59.0 (53.5–65.7)
Former	–	–	–	56.4 (51.2–62.1)	54.6 (48.2–61.8)
Current	–	–	–	42.8 (40.7–45.2)	49.4 (45.2–54.6)
Presentation at enrolment*					
CD4$^+$ cell count <200 cells/μl	3.2 (2.9–3.6)	6.5 (5.5–7.6)	35.1 (30.2–40.3)	46.7 (42.6–51.2)	47.6 (41.9–54.3)
200–349 cells/μl	11.2 (9.9–12.5)	26.0 (21.7–30.6)	48.0 (40.3–55.6)	50.2 (45.0–55.9)	54.0 (47.0–63.0)
≥350 cells/μl	25.2 (23.5–27.1)	44.5 (40.2–48.6)	59.9 (52.8–66.0)	53.0 (48.7–57.9)	63.9 (54.8–76.0)
Late presentation	6.1 (5.6–6.7)	12.1 (10.6–13.6)	41.1 (36.8–45.5)	48.7 (45.5–52.3)	53.2 (48.2–59.5)
Presentation with advanced HIV disease	3.5 (3.2–3.9)	7.3 (6.2–8.4)	36.0 (31.2–41.0)	46.5 (42.7–50.8)	49.1 (43.5–55.5)
Presentation with AIDS	2.4 (2.2–2.7)	4.4 (3.7–5.2)	29.1 (23.4–35.3)	42.1 (37.1–47.7)	46.3 (38.4–54.9)

Results from Gompertz parametric survival regression models. Univariate analyses based on 16 532 patients. CI, confidence interval. –, not estimated due to large amount of missing data or small number of patients and deaths.

Table from Gueler A., et al . AIDS 2017, 31:427-436. The life expectancy at the age of 20 years during 2006-2013 in the general population was 63 years.

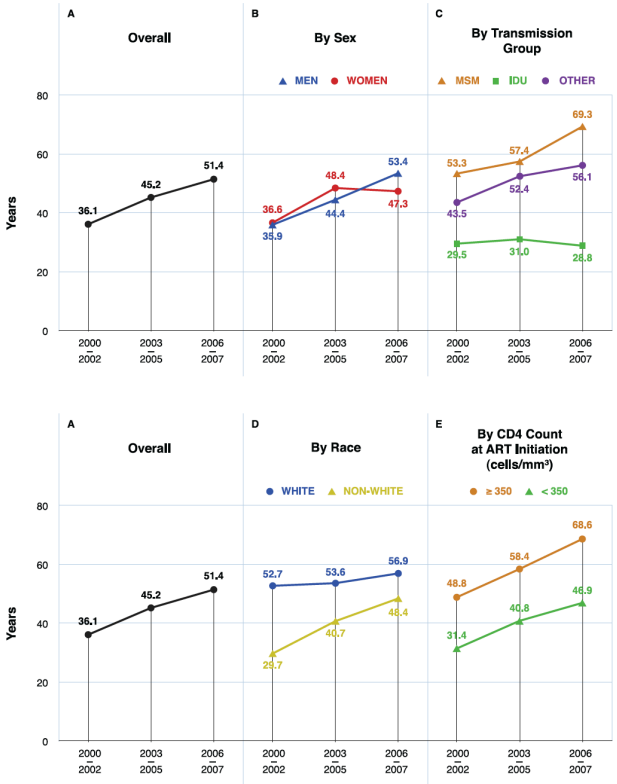


Figure 2. Mid-point life expectancy estimates at age 20 years in three calendar periods, overall and by sociodemographic characteristics, 2000–2007. Panel A: Life expectancy at age 20 years, overall. Panel B: Life expectancy at age 20 years, by sex. Panel C: Life expectancy at age 20 years, by transmission group. Panel D: Life expectancy at age 20 years, by race. Panel E: Life expectancy at age 20 years, by CD4 cell count (cells/mm^3) at ART initiation.
doi:10.1371/journal.pone.0081355.g002

Figure from Samji H, et al. Plos One.2013, 8,(12):e81355.

The association between low CD4 cell count at ART initiation and increased mortality tends to fade over the years

Therefore, CD4 cell count at ART initiation might not accurately predict long-term survival after this high-risk period. The available evidence shows that the association between increased long-term mortality and low CD4 cell count at ART initiation wanes over the years as the patients survive. However, determinants of long-term survival after this high-risk period are less explored. Furthermore, many of the studies evaluating long-term mortality in LP are from before the year 2012 and thus with exposure to older ART drugs.

In the COHERE study described above, the risk in LP was still increased by approximately 50% compared to non-LP in the second year with no statistically significant differences from the third year onwards. Interestingly, this mortality disparity was driven mainly by LP with advanced disease.[9] In the CoRIS study, during the 2-4 years after diagnosis, those with a prior AIDS diagnosis remained with a 3-fold increased risk of mortality. LP without ADE appeared to have a 50% increased risk of death compared to non-LP, though this difference did not reach statistical significance. From the 5th year onwards, there was no difference in survival in those with an initial CD4 cell count 200-350 cells/µL without ADE and non-LP, but the gap remained among those with a history of ADE (aHR 1.5 [0.5-4.4]) and in those with initial CD4 cell count < 200 cells/µL without ADE (1.9 [0.9-4.0]).[15]

Similarly, an ART-CC cohort study including a population of PLWH initiating ART between 1996-2001 with a median CD4 cell count of 221 cells/µL, showed that after 5 years, there were no survival differences among those starting ART with a CD4 cell count 200-350 cells/µL or those with CD4 ≥500 cells/µL.[74]

Table 3. Number and percentage of AIDS/deaths and adjusted incidence rate ratios of AIDS/death after HIV diagnosis in COHERE 2000–2011; late presenters versus non late presenters stratified by European region of care and time since presentation.

European Region of Care	*Years since Diagnosis*	*n* (%) AIDS/	Univariate		Multivariate	
		Deaths[a]	IRR (95% CI)	*p*-Value	aIRR (95% CI)	*p*-Value
Late presenters versus non late presenters						
South	<1	376 (95.2)	15.83 (9.97–25.10)	<0.0001	13.02 (8.19–20.70)	<0.0001
Central	<1	2,228 (93.5)	12.87 (10.94–15.14)	<0.0001	11.24 (9.54–13.25)	<0.0001
North	<1	1,528 (91.9)	10.70 (8.96–12.75)	<0.0001	9.30 (7.79–11.11)	<0.0001
East	<1	75 (86.2)	7.55 (4.10–13.88)	<0.0001	6.64 (3.55–12.43)	<0.0001
South	1–2	56 (70.0)	1.93 (1.20–3.11)	0.0070	1.55 (0.94–2.53)	0.081
Central	1–2	374 (69.5)	2.03 (1.69–2.43)	<0.0001	1.78 (1.48–2.15)	<0.0001
North	1–2	280 (64.7)	1.72 (1.42–2.10)	<0.0001	1.57 (1.29–1.93)	<0.0001
East	1–2	16 (45.7)	1.02 (0.53–1.99)	0.95	0.94 (0.47–1.91)	0.87
South	>2	112 (64.4)	1.47 (1.08–2.01)	0.015	1.21 (0.88–1.67)	0.24
Central	>2	726 (55.7)	1.04 (0.94–1.16)	0.45	0.95 (0.85–1.06)	0.35
North	>2	535 (52.5)	0.96 (0.84–1.08)	0.47	0.88 (0.78–1.00)	0.049
East	>2	35 (45.5)	0.90 (0.57–1.41)	0.64	0.80 (0.50–1.29)	0.36

Table from Mocroft A, et al. Plos Medicine. 2013, 10 (9), e1001510.

Table 2 Impact of late presentation and clinical status at HIV diagnosis on mortality. Overall mortality rates. Results of survival analysis and Cox regression model for follow-up periods.

	Overall results			Results for follow-up periods								
				First year since diagnosis			1–4 years Since diagnosis			>4 years since diagnosis		
	Deaths	p-y	Rate (95%CI)	N at risk	Deaths	aHR(*) (95%CI)	N at risk	Deaths	aHR(*) (95CI%)	N at risk	Deaths	aHR(*) (95CI%)
Total	240	23,820	1.01 (0.89–1.14)	6956	132		5511	72		2840	36	
Late presentation												
nLP	37	11,921	0.31 (0.22–0.43)	3666	8	1	2923	19	1	1378	10	1
LP	203	11,900	1.71 (1.49–1.96)	3290	124	10.3 (5.5–19.3)	2588	53	1.9 (1.2–3.0)	1462	26	1.5 (0.7–3.1)
LPAD	176	7564	2.33 (2.00–2.70)	2010	114	14.5 (**) (7.4–28.3)	1578	41	2.2 (**) (1.4–3.5)	952	21	1.7 (**) (0.8–3.8)
Clinical status at HIV diagnosis												
AIDS	132	3817	3.46 (2.92–4.10)	999	94	22.6 (11.5–44.6)	769	28	3.0 (1.8–5.0)	476	10	1.5 (0.5–4.4)
CD4 < 200 – AIDS-free	44	3747	1.17 (0.87–1.58)	1011	20	5.6 (2.7–11.9)	809	13	1.5 (0.9–2.4)	476	11	1.9 (0.9–4.0)
200 ≤ CD4 < 350 – AIDS-free	27	4335	0.62 (0.43–0.91)	1280	10	2.8 (1.5–5.4)	1010	12	1.4 (0.7–2.8)	510	5	1.1 (0.4–2.7)
CD4 ≥ 350 – AIDS-free	37	11,921	0.31 (0.22–0.43)	3666	8	1	2923	19	1	1378	10	1

(*) aHR (CI 95%): adjusted Hazard Ratios by category of transmission-sex and age at HIV diagnosis and confidence intervals 95%.
(**) Reference category: Subjects not LPAD (CD4 ≥ 200 and AIDS-free).

Table from Sobrino-Vegas P, et al. Journal of Infection. 2016;72:587-596.

Other risk factors for increased mortality

Additional factors other than low CD4 cell count and ADE may also contribute to this disparity in long-term mortality risk in LP compared to non-LP and may include the following:

- Prolonged and advanced immune damage and chronic immune activation, and chronic inflammation.
- Differences in access and adherence to care.
- Residual confounders associated with greater morbidity and mortality:
    - Behavioural or lifestyle factors such as substance abuse, alcohol consumption, and smoking.
    - Comorbidities such as age-related illnesses, and hepatitis C.
    - Socio-economic factors such as educational level or income level.
- Adverse events and interactions with ART and other drugs.

Early immune response might better predict long-term survival

Several studies have shown that early immune recovery is associated with improved long-term survival in some subpopulations, even resulting in a life span close to the background population. A UK CHIC study evaluating a population of mainly LP initiating ART during 2000-2010 with a median CD4 cell count of 220 cells/μL, showed that those LP achieving an optimal early immune response with CD4 cell count ≥350 cells/μL after 1 year (only observed in 16% of the study population) had a life expectancy similar to the background population.[32] In an ART-CC study including patients from 2001-2007 (75% of them were LP), Egger et al also showed that MSM achieving an early optimal immune and virologic response, defined as CD4 cell count ≥350 cells/μL 6 months after ART initiation, who initiated ART without ADE, had similar standardized mortality ratio as the background population (1.05 [0.82-1.35]).[34] In a study from Denmark including a population of PLWH from 1998-2009 (2/3 starting ART before the year 2005), Obel et al showed that individuals achieving virologic suppression (HIV viral load <200 c/ml) and with one-year CD4 cell count >200 cells/μL after ART initiation, and without associated comorbidities (including hepatitis C), drug and alcohol abuse had a life expectancy close to an age- and gender-

matched sample from the general population with no comorbidities or drug/alcohol abuse. [75] However, this subset of patients had a high CD4 cell count at ART initiation, with a median CD4 cell count of 360 cells/μL (240-513), and thus many of them were not LP. Interestingly, factors not directly related to HIV infection, particularly behavioural and lifestyle factors such as drug/alcohol abuse and, to a lesser extent, comorbidities, had the greatest impact on survival. Other studies have also shown that smoking has a large impact on survival, with approximately ten-year decrease in life expectancy among smokers.[2,76] A recent meta-analysis has shown that PLWH has 64% higher odds of being current tobacco smokers than people without HIV (OR = 1.64; [95% CI, 1.45-1.85]).[77]

Current CD4 cell count (time-updated) is strongly correlated with long-term survival

Current CD4 cell count (time-updated) has proven to be a strong predictor of long-term survival.[35,37,78,79] Some studies have shown that PLWH attaining CD4 cell counts ≥500 cells/μL without a previous history of IDU or ADE, have a life span close to the general population. In a study from Australia including PLWH from 1992-2012, Bijker et al showed that in 5-year survivors, those remaining with 5-year CD4 cell count <200 cells/μL had increased mortality compared to those with CD4 count ≥500 cells/μL.[36] There were no differences in survival between those PLWH with 5-year CD4 cell count ≥500 cells/μL and those with 350-499 cells/μL. In another study from Australia including 2,675 PLWH initiating ART between 1999-2011, McManus et al showed that individuals reaching current CD4 cell count ≥500 cells/μL achieved a life expectancy close to the general population (SMR of 1.5 (1.1-2.0)).[37] Similarly, in an Italian study including 9,671 PLWH between 1985-2011, Guaraldi et al showed an increasing proportion of individuals attaining CD4 cell count ≥ 500 cells/μL and, like that, approaching life expectancies of the general population.[78]

In an earlier European collaborative cohort, Lewden et al compared mortality rates in 80,642 PLWH who initiated ART to the general background population. The patients were included from 1998-2008 and approximately 60% started ART before the year 2003. This population was mainly LP with a median CD4 cell count of 225 cells/μL (IQR 107-357) and 44% had a CD4 cell count ≤200 cells/μL at ART initiation. The study period elapsed before the introduction of INSTI into the standard of care for HIV infection. The number of patients attaining CD4 ≥500 cells/μL was not directly stated in the article, but 29% of the risk time in the study was spent with CD4 ≥500 cells/μL. Men with no previous history of IDU who achieved CD4 ≥500 cells/μL and women with no history of IDU and after 3 years above this threshold had the same mortality rates as the general population (SMR 0.9 [95%CI: 0.7-1.3] and 1.1 [95%CI: 0.7-1.7], respectively).[35] In the same way, from the SMART and ESPRIT studies, Rodger A et al described standardized mortality rates similar to the general population for patients without a history of IDU and attaining viral suppression and CD4 cell counts >500 cells/μL (SMR 1.0 [95%CI: 0.69-1.40].[33]

Study III. Two-year immune response as an early predictor for long-term survival

In our study, we aimed to explore the performance of an early predictor for long-term survival after ART initiation once the patient has survived the initial high-risk two-year period after ART initiation. We found that the long-term mortality was not different in LP achieving a two-year CD4 cell count >500 cells/μL compared to a group of non-LP with an optimal immune response (two-year CD4 cell counts > 500 cells/μL with a median 2-year CD4 837 [IQR: 697-1030]), regardless of their initial CD4 cell count. In fact, nearly half of LP surviving the first two years after ART start attained this threshold, closing the survival gap between late and non-LP. However, the mortality

risk remained increased in PLWH with two-year CD4 cell counts below this threshold. Those individuals attaining two-year CD4 cell counts between 200-500 cells/μL exhibited an approximately 2-fold increased mortality risk and those with <200 cells/μL an approximately five times greater compared to non-LP. In our study, the percentage of immunological non-responders (i.e., LP remaining with a two-year CD4 cell count <200 cells/μL) was low (8.7%), and one-quarter of these patients had detectable two-year viremia (defined as HIV viral load >200 copies/mL). This fact indicates that the increased mortality in these patients could partly be explained by ART non-adherence and probably non-adherence to care, aside from their low baseline CD4 count.

Our findings align with the existing body of evidence that ART-associated immune recovery is a better prognostic tool for assessing long-term mortality in LP compared to the initial CD4 cell count and that immune recovery two years after ART initiation is a useful early predictor of long-term mortality, which could be used in routine practice. Patients presenting late to care achieving a good early immunological response can reach survival rates similar to non-late presenters and, extrapolating the results from the studies mentioned above, probably to the survival of the general population in the absence of a history of drug/alcohol abuse and ADE.

In our study, risk factors for not attaining this threshold included older age at ART initiation and lower CD4 at baseline, and a higher burden of comorbidities. These facts emphasize the importance of an early HIV diagnosis and prompt ART initiation before severe immunodeficiency occurs. This is particularly important in older patients, as they had more difficulties in reaching this threshold when initiating ART with low CD4 cell count

Study III. Immunological non-responders

Our results showing a particularly elevated mortality risk in immunological non-responders are in accordance with findings from other studies. In a study with data from ART-CC and COHERE including 5,550 individuals on ART for at least three years and being virologically suppressed, Engsig et al showed that those individuals remaining with CD4 count <200 cells/μL (n=835) had an increased long-term mortality risk (aHR 2.60) compared to those above this threshold. Deaths were mostly related to non-AIDS conditions, mainly non-AIDS cancers and liver events.[80] Similarly, the Italian MASTER, the Dutch ATHENA, and the TAHOD cohorts have described an increased risk of mortality and non-AIDS-defining events in immunological non-responders.[13,36,45]

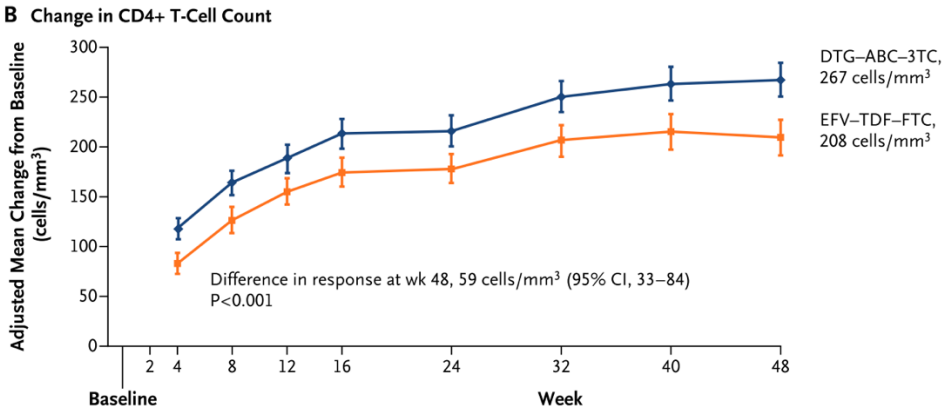
Study III. Lower risk for long-term mortality and incomplete immune reconstitution when initiating ART with INSTI-based regimens

We found that exposure to an INSTI-based regimen in the first two years was associated with a 46% decrease in long-term mortality from any cause and a 30% lower risk of incomplete immune recovery at two years, defined as two-years CD4 cell count <500 cells/μL. We chose this CD4 cell count threshold as it was associated with increased mortality in our study but choosing a higher threshold >700 cells/μL yielded similar results.
A study from COHERE showed that the incidence of AIDS-defining events, especially malignant AIDS-defining events, was lower in those PLWH attaining CD4 cell counts > 750 cells/μL, which might indicate that complete immune restoration is achieved above this threshold.[81]

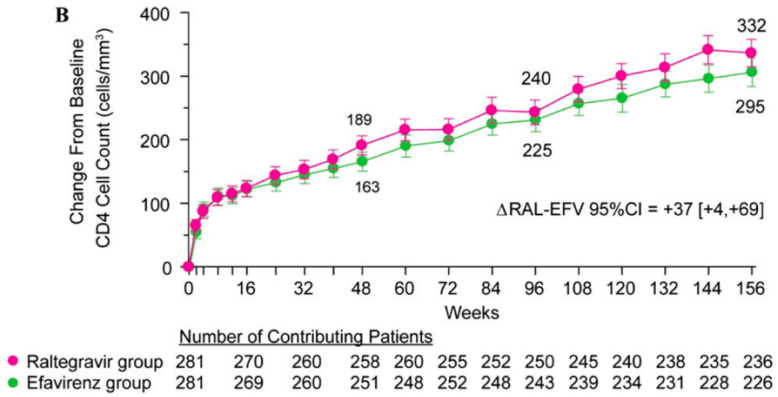
Previously, three cohort studies and two randomized control trials (RCT) have suggested that INSTI-based regimens could be associated with an improved immune recovery compared to other regimens, however no studies found an association with better survival[49–55]. In the SINGLE and STARTMRK phase III RCT, initiation with an INSTI-based regimen was associated with a higher

increase in CD4 cell count compared to efavirenz[50,51,55]. However, no differences were observed when comparing Dolutegravir with Darunavir in the FLAMINGO and SYMTRI RCT.[82,83]

In a RESPOND cohort study including 13,703 PLWH starting or switching ART, Neesgaard et al found that, after 12 months, PLWH receiving PI- or NNRTI-based regimens had a lower probability of a favourable immune response, defined as a 25% increase in CD4 cell count or as attaining CD4 counts >750 cells/µL, compared to INSTI-based regimens (aOR 0.87 [0.76-0.99] and 0.80 [0.71-0.91]).[53] However, the study included both treatment-naïve and treatment-experienced, did not assess the impact in the subgroup of late presenters and the existence of confounder by indication could not be ruled out. Similarly, a recent Spanish cohort study showed a greater CD4/CD8 gain in the first year of ART and higher CD4/CD8 normalization rates when an INSTI-based regimen was used. However, the rates of participants with low CD4 counts at ART initiation were generally low. None of the studies assessed the potential impact of treatments neither on mortality (due to their limited long-term follow-up) nor the specific effect on late presenters.[49]



Single study. Figure from Walmsley S et al. NEJM.2013.369;19.
The difference continued in week 96 and week 144 (W96: +325 cells/mm3 vs. +281 cells/mm3; difference and 95% CI: 44.0 [14.3 to 73.6 cells/mm3 (P = 0.004)]; and W144: +378 cells/mm3 vs. +332 cells/mm3; difference and 95% CI: 46.9 [15.6 to 78.2 cells/mm3 (P = 0.003)]}.



STARTMRK study. Figure from Rockstroh J et al. CID. 2011;53(8):807-816. Mean changes in CD4 cell count from baseline to week 156 were 332 [95%CI: 309, 354] cells/µL for the raltegravir group and 295 [95%CI: 271, 319] cells/µL for the efavirenz group (Difference 37 [95%CI:4, 69] cells/µL)

Our study contributes to the growing body of evidence that INSTI-based regimens are associated with a more favourable immune response. To the best of our knowledge, our study is the first that identified the exposure to an INSTI-based regimen as first-line ART with a higher probability of favourable immune response in the subpopulation of LP and the first that finds a beneficial impact on long-term survival. These findings indicate that INSTI-based regimens should be positioned as the preferred first-line therapy in this specific scenario.

Nonetheless, the strongest independent risk factor for unfavourable immune recovery was a lower initial CD4 cell count at ART initiation, reinforcing the need to improve HIV screening strategies and timely HIV diagnosis.

Earlier diagnosis

Aside from late diagnosis, undiagnosed HIV infection poses a challenge in the HIV field, contributing to feeding the hidden HIV epidemic, as individuals with undiagnosed HIV infection may unknowingly contribute to the virus's spread in the population.[84] It is estimated that 40% of PLWH globally, and 15% in Europe, are unaware of their HIV serostatus, and the median time in Europe from infection to diagnosis is estimated to 2.9 years.[85–87] Furthermore, according to a previous study, more than one-third of new HIV transmissions in the United States occur among people who are unaware of their HIV serostatus.[84] Importantly, knowledge of HIV serostatus has been associated with reducing risk behaviour, potentially lowering the risk of onward transmission.[88]

Guidelines

Therefore, the significance of early HIV detection cannot be emphasized enough. To adequately target patients for HIV testing, different guidelines have been developed, and broadly speaking, they differ between routine or target HIV testing approaches. In the US, since 2006, the CDC has recommended routine HIV testing in all pregnant women and in all individuals between 13-64 years, regardless of risk, at least once in their life as part of routine care in all healthcare settings (opt-out testing). For persons presumed to be at risk for HIV acquisition, CDC recommends HIV testing at least once a year.[89] However, the most optimal strategy for retesting people that do not have a priori high risk of HIV is unclear. The rationale for universal testing was that targeted screening strategies that had focused on people with epidemiological or clinical risk factors for HIV had failed to detect a significant proportion of undiagnosed PLWH.

On the other side, European testing guidelines recommend routine HIV screening during pregnancy and targeted HIV testing in high-risk groups for HIV acquisition (MSM, people with a history of IDU, sex workers, and people coming from high HIV endemic countries, and their partners) and in the presence of an indicator condition. Indicator conditions include AIDS-defining illnesses and conditions associated with an undiagnosed HIV prevalence of more than 0.1%, which has been shown to be cost-effective.[57,58] In fact, a prevalence of undiagnosed HIV as low as 0.05% has been found cost-effective.[58] HIV testing is also recommended in the presence of some specific conditions where not identifying HIV may have negative consequences for the management of the disease (Table S3 appendix). Altogether, it consists of a list of 64 conditions, mainly caused by infections that share the route of transmission with HIV or conditions related to immune deficiency.[90–94] The UK BHIVA guidelines also recommend routine/universal testing for all individuals seeking health care in areas with high HIV prevalence (defined as >0.2%).[95,96]

Missed opportunities

In many cases, this targeted approach fails to detect people early because physicians fail to identify high-risk individuals based on these epidemiological or clinical risk factors, and patients fail to disclose behavioural risk factors. Several studies have shown that in the years preceding HIV diagnosis, PLWH visited health care services frequently, including primary care, often belonging to risk groups or presenting with indicator conditions, resulting thus in missed opportunities for earlier HIV diagnosis. Missed opportunities refer to an individual presenting with an indicator condition to a healthcare service but not offered an HIV test. Joore et al found that 60% of newly diagnosed PLWH had had at least one indicator condition in the five years before HIV diagnosis compared to 7.4% in the controls.[97] Missed opportunities have been reported with a wide range of frequency, between 14-82% of PLWH presenting with indicator conditions in primary care, emergency rooms, hospital, or outpatient settings, which probably relates to different settings, populations, and definitions of missed opportunities. [59,98–122]

Several studies have described a higher risk of having missed opportunities for HIV testing among individuals without epidemiological indicators of HIV infection, such as non-migrants, women, heterosexual men, and older patients.[101,123] These patients were also more prone to neglect HIV-related symptoms, most likely due to a lack of self-awareness of HIV risk.[123] Having at least one missed opportunity for HIV diagnosis was associated with a lower CD4 cell count at diagnosis and a higher risk of advanced disease.[107,123] On the other side, presenting with an indicator condition and being tested for HIV was associated with a 50% lower risk of late diagnosis in an Italian study (OR 0.5 (95%CI: 0.4-0.7)), reassuring the effectiveness of the indicator condition-guided testing when performed.[105]

Study I. Primary care is an appropriate setting for promoting earlier HIV testing.

In our study I, we found that despite overall efforts to implement HIV testing, late and very late HIV diagnoses remained common in Denmark in the most recent calendar period (2010-2016), with 17% late presentation and 31% very late presentation (e.g., CD4 counts <200 cells/μL or presenting with an ADE). With such a high rate of late presentation, it is clear that improvements can be made. Individuals not belonging to classical risk groups for HIV like heterosexual men, females, and older patients were at greater risk for late presentation. This indicates that these people are not perceived to be at risk, either by their physicians or by themselves. As a result, traditional targeted screening guidelines are not able to adequately capture them. Furthermore, individuals of non-Danish origin were also at higher risk of late or very late diagnosis. This is paradoxical as many of these patients came from high endemic areas, and any healthcare contact should have prompted HIV testing.[124] However, an increasing amount of data suggests that migrants from high HIV prevalence regions do not necessarily arrive in Europe with HIV infection but acquire the infection some months or years after arrival through assortative sexual mixing.[122,125] Our findings are in line with results from other studies describing the same demographic characteristics of late presenters.[8,12,126]

Besides these factors, lower education has also been linked to late HIV diagnosis as found in our study III or in other studies. In a pooled analysis of European HIV cohorts, the authors found that advanced HIV disease was more common among those with lower education.[28] However, Gueler et al from the Swiss cohort found educational inequalities in life expectancy between PLWH and the general population but not in late presenters compared to non-late presenters.[2] Similarly, Legarth et al from the Danish HIV Cohort did not find an association between lower education and late presentation.[127] These differences may reflect socioeconomic disparities across countries.

We found that PLWH in Denmark had very frequent contact with primary care three years preceding HIV diagnosis. Only 4% and 13% of Danish-born and non-Danish-born men and 3% and 26% of Danish-born and non-Danish-born women had had no contact with primary care in the previous three years for HIV diagnosis. In addition, we observed that the number of visits of ≥ 1 was associated with subsequent risk of HIV diagnosis in men regardless of ethnicity, and in non-Danish women. This association was higher with a higher number of visits and in non-Danish individuals. Testing all individuals attending primary care at least once in one year would capture a large proportion of undiagnosed HIV-infected patients (80% of Danish undiagnosed individuals and 70% of non-Danish born HIV-infected individuals). However, further studies are necessary to assess if this intervention would be cost-effective in a low-incidence country such as Denmark, with an estimated undiagnosed HIV prevalence of 0.01%. [128]

Danish women had a U-shaped association between the number of visits to primary care and the risk of subsequent HIV diagnosis. Even though this is a subpopulation with a priori low risk of HIV, they were more difficult to capture based on the number of visits to primary care. Only those who had no visits (3%) and those who had many visits (>12, 23.2%) to primary care had an increased risk of subsequent HIV diagnosis. The reason for this is unknown, but it is possible that women who had no visits were less aware of their risk behaviour and had a less favourable health-seeking behaviour than their matched controls.

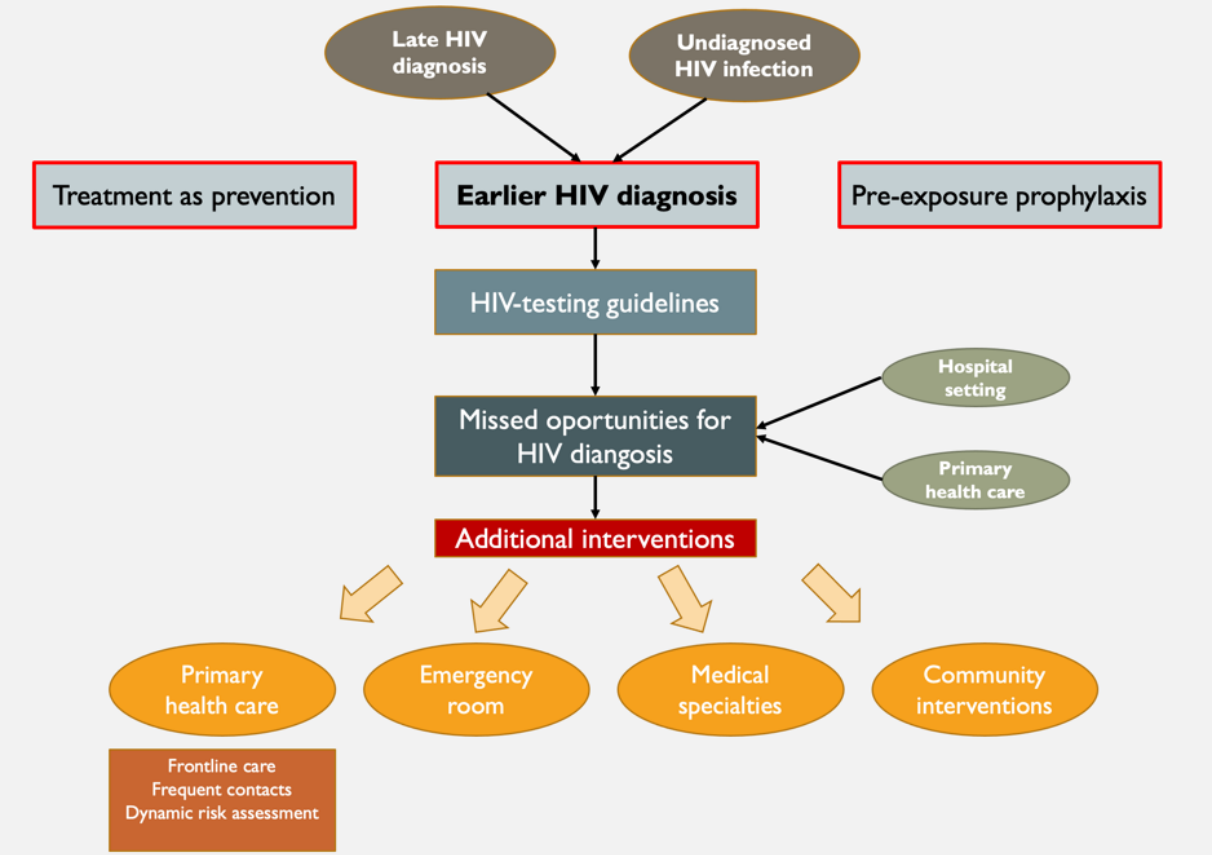
A higher percentage of non-Danish did not attend primary care in the 3-year period. This pattern may reflect differences in ethnic minorities' health-seeking behaviour, lack of knowledge of the health-care system, and/or cultural/language barriers, and highlights the need for alternative health interventions to reach these patients.

In general, the different medical procedures performed in primary care could not be used to guide who should be offered an HIV test. Only a few medical procedures were found to be strongly associated with a subsequent increased risk of HIV diagnosis, particularly anoscopy, sending biological samples to reference laboratories, and counselling for abortion and/or sterilization in women. These interventions could represent proxies for indicator conditions such as STI and anal cancer/dysplasia or an indicator for risk behaviour in the case of counselling for abortion and/or sterilization in women. Individuals undergoing these procedures should be offered an HIV test, but due to the low prevalence of such procedures, HIV testing in these scenarios would not capture a large fraction of the undiagnosed population.
In a study from the UK, Chadwick et al evaluated a risk-based electronic alert to prompt HIV testing when patients underwent routine blood tests in an area of high HIV prevalence. The prompt consisted of a pop-up message suggesting that an HIV test was added, stating that the test request indicated that the patient could be at higher risk of HIV infection. This strategy was found feasible and acceptable by primary care physicians.[129] This intervention could be applied to our findings in the case of "sending of biological samples excl. blood to laboratory", which was associated with an increased risk of subsequent HIV diagnosis, and a similar intervention could be considered.

Involuntary psychiatric hospitalization was 5 times more common in cases than controls in the last year before HIV diagnosis, whereas it was 21-38% higher in the other two years. Similarly, in an Australian retrospective cohort study, evaluating missed opportunities for earlier HIV diagnosis in newly diagnosed PLWH, Mallitt et al found that hospital admission related to mental health

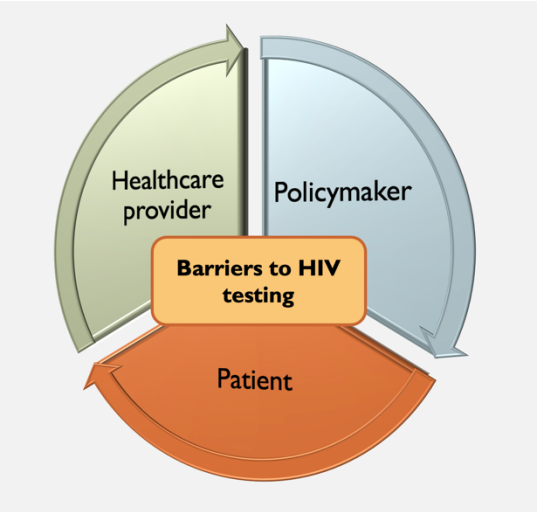
disorders and drug abuse was common among undiagnosed PLWH. The number needed to test for HIV to diagnose one HIV case in this situation was 2,803.[103]

Overall, our results indicate that primary care should provide numerous opportunities for HIV testing and play an essential role in the early identification of people living with undiagnosed HIV infection and thus be a pivotal point to curb the epidemic further. Our findings are in line with the available literature, that indicates that primary care should have a more active role in provider-initiated HIV testing.



Barriers to HIV testing

To scale-up timely HIV diagnosis, we need to understand the circumstances around missed opportunities and identify potential barriers to HIV testing. It is important to normalize testing, minimize stigma, increase timely test uptake, develop strategies to improve guideline implementation in primary care and develop complementary diagnostic strategies to the existing mandatory recommendations.



Barriers to HIV testing can be divided into policy-, healthcare provider- and patient-related. When a healthcare provider offers an HIV test, the test uptake approaches 100%, indicating that the main barrier is related to the healthcare provider.[90] However, testing barriers may differ significantly in different settings and populations.

Healthcare provider barrier

Primary care is where the majority of patients are seen, but where they are also missed. The following barriers to provider-initiated testing in primary care have been identified:[130]

- Lack of awareness of current HIV epidemiology trends and HIV testing recommendations.
- Lack of specific recommendations in primary care guidelines.
- Operational barriers: lack of time, uncertainties about practical issues about delivering HIV testing.
- Lack of reporting sexual history or information about risk factors.[131]
- Misconceptions about risks.
- Lack of experience/training.
- Concerns about offering an HIV test that is unrelated to the medical complaint that brought the individual to the consultation.
- Lack of indicator condition guidelines tailored to primary care: the existing list is long, and not easily applicable to primary care.

To increase awareness among primary care physicians, educational training programs in primary care should be prioritized. However, some studies have shown that educational interventions in primary care may not be enough to significantly affect healthcare provider testing behaviour, requiring more innovative approaches.[132]

The list of indicator conditions could be adapted to primary care to be more concise and focus on diagnoses seen frequently in primary care. The most common indicator conditions reported in primary care are STI, hepatitis B or C, seborrheic dermatitis, mononucleosis-like disease, herpes zoster, unexplained thrombocytopenia, recurrent pneumonia, weight loss, lymphadenopathy, unexplained fever, diarrhoea, psoriasis, and cervical dysplasia.[97,120,123,133]

Individuals presenting with STIs must be offered an HIV test as these infections share the same transmission route and they are part of the indicator conditions list. Studies from primary care have shown that general practitioners tend to test more selectively, usually limited to 1 or 2 STIs, even in high-risk groups, such as MSM.[59] In a retrospective cohort study from Australia, including newly diagnosed PLWH between 1993-2012, Mallitt et al described that individuals presenting with an STI (particularly gonorrhoea and syphilis) in the years before HIV diagnosis were frequently not offered an HIV test and the time from notifiable conditions (80% STI or viral hepatitis) to HIV diagnosis was 13.3 months.[103] In another study from New York, Kapadia et al showed that same-day testing for HIV in patients tested for gonorrhoea and Chlamydia was low, even though it increased from 59% in 2010 to 70% in 2015 in men and from 41% to 51% in women. Emergency rooms and inpatient locations were negatively associated with the receipt of HIV testing.[104] In a study from London including 63,599 people diagnosed with HIV, Croxford et al showed that the proportion of people diagnosed outside sexual health clinics increased from 2005 to 2014 and 6.4% were diagnosed in primary care. Importantly, patients diagnosed in primary care had lower CD4 cell counts compared to those diagnosed in sexual health clinics, reflecting another population and potential missed opportunities.[114] Therefore, better implementation of HIV testing in people diagnosed with STI and viral hepatitis in primary care and in other settings is crucial.

Barriers related to the patients.

Some studies have shown that MSM were also at risk of being missed in healthcare settings, mainly related to non-disclosure of risk behaviour by the patient, as an HIV test is mostly offered to those perceived to be at risk. [118,123] In a Spanish study, Espinel et al showed that HIV testing increased when healthcare providers were aware of MSM behaviour, and the missed opportunities decreased from 70% to 40%. [134]

Another patient-related barrier is the lack of awareness of HIV-related symptoms and neglect of symptoms.

Barriers related to policymakers

Policymakers should facilitate access to HIV testing and allocate resources in this regard by securing the availability of rapid point of care testing, resources to facilitate targeting difficult-to-capture populations, vulnerable populations (undocumented migrants, sex workers), and strategies focussing on community-level testing tailored to the specific populations at increased risk (including HIV self-sampling and self-testing), and increased test offer at immigration[135].

Study II. Reducing the need for risk assessment

Avoiding or reducing the need for risk assessment in primary care could help to scale up HIV testing. In our study II we observed that PLWH had a higher prescription of antimicrobials compared to controls in the 3 years before HIV diagnosis. The antimicrobial drug prescription of any of the antimicrobial drug classes included in the study was statistically significantly associated with the risk of subsequent HIV diagnosis. This association was higher with higher levels of antimicrobial consumption.

We did not have information on the disease indication for the prescriptions, but we assume that the use of antimicrobial drugs was in many cases a surrogate marker for missed HIV indicator conditions such as community-acquired pneumonia, STIs, herpes zoster, or candidiasis.

Proactive HIV screening based on antimicrobial prescription could therefore be useful in primary care. Increased prescription of one of these antimicrobial drugs should alert the physicians of a potential proxy for indicator conditions and occult HIV infection. Besides, this strategy could help

detect groups of individuals not belonging to classical risk groups where the clinical suspicion of occult HIV is low and often result in a late HIV diagnosis because are difficult to capture through traditional risk assessments, such as older and heterosexual men and women.

Implementing this testing strategy in primary care would be easy by using electronic health record alerts. It could be a simple and supportive tool for general practitioners to proactively offer HIV testing to populations at increased risk, diminishing the counselling time and contributing to normalizing HIV testing and decreasing HIV-related stigma. It would also allow a more dynamic risk assessment throughout the lifespan of sexually active adults, as the risk can vary over time and one single test may not capture the individual when at risk.

Further studies are needed to confirm if this approach is cost-effective. It seems however reasonable to offer an HIV test after a prescription of acyclovir, azoles, nystatin, doxycycline, quinolones, and macrolides. Furthermore, recurrent beta-lactam use, e.g., > two prescriptions in a 1-year period, may also be used as an indicator to perform an HIV test.
The antimicrobial consumption in these situations was associated with a high risk of HIV with an OR > 2 (even higher in macrolides with OR=3, in quinolones and doxycycline with OR=4, in acyclovir with OR=7, and nystatin with OR=10). With a presumed undiagnosed HIV prevalence of 0.01-0.02% in the general Danish population, HIV prevalence in these subgroups could be estimated above >0.04-0.05%, which has been reported as a cost-effective strategy.[58] Even more so, taking into consideration the difficulties in reaching the population of late presenters earlier through the classical intervention.

Studies using electronic clinical reminders or computer prompts have shown a positive impact on HIV test rates.[121,129,136–138] In a prospective interventional study, Agustí et al evaluated the efficacy of an electronic alert implemented in primary care centers in Barcelona between 2018-2019. An electronic reminder prompted up in the electronic record if patients between 16-65 years were diagnosed/coded with an indicator condition. Overall, 13,000 patients were diagnosed with ≥1 indicator condition. However, only 18% in the control group and 21% in the alert group were offered an HIV test. Five individuals (0.08%) and 10 (0.17%) were diagnosed with HIV in the control or the intervention group, respectively. The electronic reminder had a discrete positive impact on HIV testing in the subpopulation of individuals >50 years (OR 1.77 (95%CI 1.33-2.38) and in case of IC other than AIDS-defining conditions or STI (mononucleosis, herpes zoster, hepatitis B and C, OR 1.51 (95%CI 1.20-1.92).

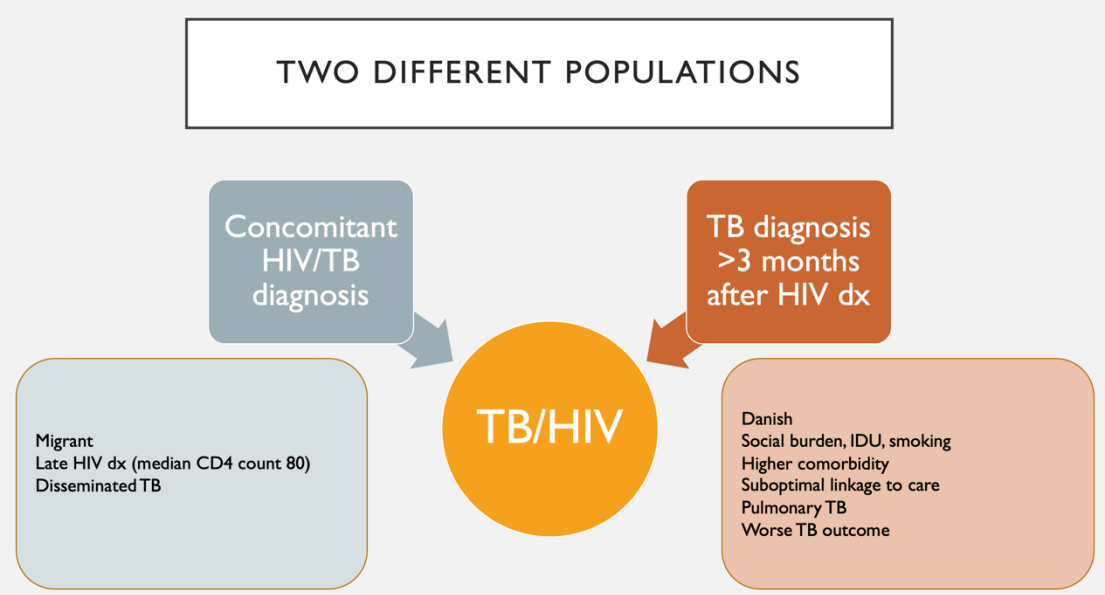
Opportunistic infections. Tuberculosis

Despite the use of ART, TB continues to be a primary cause of morbidity and mortality in PLWH globally with 214,000 deaths reported in 2020, representing one-third of all HIV-related deaths[139,140] In low TB incidence countries, reactivation of untreated latent TB infection (LTBI) is the main cause of new active TB cases and onward transmission. The World Health Organization's (WHO) "End TB" strategy aims to reduce TB incidence rates by 90% (<100 cases per million population) and TB mortality by 95% by 2035 compared to 2015 and TB elimination by 2050, the latter, particularly in low TB incidence countries.[141] The identification of risk factors for the development of TB, treatment failure, and death in PLWH is pivotal in order to design effective preventive public health strategies, improved management and achieve the TB elimination target.[142]

Denmark has a low TB incidence with 49 cases per million in 2019, with 72% of the cases in persons of non-Danish origin (immigrants or descendants of immigrants).[143] Elimination of TB as a public health issue is defined as <1 per million population per year, and thus, further efforts need to be done.[142]

Two distinct populations

In our study, we identified two distinct populations of HIV/TB coinfected individuals with different presentation patterns: those presenting to care with concomitant HIV/TB infection (more frequently migrants, late presenters with a higher risk of disseminated TB) and those diagnosed with TB >3 months from HIV diagnosis (median time from HIV to TB diagnosis 5.1 years (IQR 2.0-9.6)), more frequently Danish-born individuals, with a higher social burden, higher rates of liver and non-TB AIDS-related comorbidities, a higher percentage of pulmonary TB and with a higher risk of suboptimal linkage to care judged from low rates of ART, viral suppression and high prevalence of other non-TB ADE at the moment of TB diagnosis. The latter had a worse TB outcome, with higher rates of treatment discontinuation and death, highlighting the need for individualized interventions to improve prevention and linkage to care of this subpopulation.



Trends in TB incidence over time and TB risk factors

The incidence of concomitant HIV/TB coinfection remained high and unchanged over the study period. This fact relates to the high and persistent rates of late diagnosis in PLWH, with the risk of ADE, and reinforces the urgent need for more proactive testing and earlier diagnosis of HIV and TB. Three-quarters of these patients were migrants, most of whom came from countries with high HIV and TB incidences, highlighting the importance of addressing the specific needs of this vulnerable population which is at high risk of late HIV presentation and advanced disease. These findings point to inadequate screening upon arrival in the country, and as previously stated in the discussion, likely missed opportunities for earlier diagnosis in relation to healthcare contacts. Increased screening at immigration is pivotal,[135] even though it is acknowledged that some degree of transmission occurs post-migration because of assertive social mixing.[122] Primary care should also play an important role in LTBI and HIV case detection as we described in study I that these patients frequently attend primary care. However, 26% and 13% of the non-Danish-born women

and men with undiagnosed HIV infection did not attend primary care services in the three years preceding HIV diagnosis, indicating the need for alternative interventions to reach this difficult-to-capture population and facilitate access to care. Furthermore, it is necessary to develop surveillance systems to assess the degree of TB and HIV spread post-migration.[122]

The incidence of TB among those with known HIV infection (TB diagnosed > 3 months after HIV diagnosis), declined over time, probably related to better ART coverage, better ART options, and earlier ART initiation.
The risk of TB also declined with time from HIV diagnosis and with CD4 immune recovery.

Incidence of TB stratified by years from HIV diagnosis			
Period	Incidence rate (per 1000 person-years)	Person-years at risk, PYR	Number of patients diagnosed with TB (n)
0-3 months	58.1 (47.7-70.8)	1703.6	99
>3 -12 months	5.1 (3.5-7.6)	4881.5	25
>1-5 years	2.0 (1.5-2.7)	22107.6	44
>5-10 years	1.4 (1.0-2.0)	20658.5	29
>10 years	0.8 (0.5-1.3)	24244.6	20
IR of TB stratified by CD4 cell count			
<=100	76.1 (57.5-100.7)	643.8	49
>100-200	16.0 (10.7-23.9)	1500.2	24
>200-300	12.2 (8.7-17.1)	2777.5	34
>300-500	5.9 (4.5-7.8)	8440.0	50
>500	1.1 (0.9-1.4)	52528.6	58

The risk factors for developing TB in the HIV-infected population identified in our study are in line with the current evidence and include migration, vulnerable groups (history of IDU, harmful alcohol use, homelessness, stay in a shelter or prison facilities, and/or prostitution), PLWH not receiving ART and HIV-induced immune suppression.[144,145] There is frequently some overlap regarding these risk factors.[146,147] In a recent Danish retrospective cohort study evaluating 142,314 migrants moving to Denmark between 1993 and 2015 and a matched Danish-born population, the incidence rate of TB among migrants was found 30 times higher than in the background population. The HIV prevalence was higher in migrants than in the Danish-born population (0.4% vs 0.1%) but no differences were observed among TB cases (2.3% versus 2.0%, respectively (p=0.72)).[148] The incidence of active TB among migrants from high TB incidence is greatest within the first 5 years after country arrival, probably driven by socioeconomic and health-related determinants.[149–151]

Our findings emphasize the importance of promoting TB/HIV screening in migrants and vulnerable populations and the importance of early HIV diagnosis and ART initiation.[152] Studies have shown that immediate ART initiation significantly reduces the risk of progression to TB.[31,153] These populations can be difficult to reach, and it is important to understand barriers to healthcare access and therapy adherence, address underlying social risk factors, and provide social support.[142] Even when everyone is guaranteed officially access to care, these groups frequently face access barriers due to cultural differences, language/communication difficulties, stigma, discrimination, and/or marginalization.[142] Systematic screenings programs for LTBI for immigrants from countries with high TB incidence have been implemented by some low-incidence European countries with good results and acceptability.[135,154] In Norway, screening for LTBI and active TB is mandatory upon

country arrival, including symptom screening, IGRA test, and chest X-ray and the strategy is widely implemented and well-accepted.[135] In a recent cohort study in England, they evaluated the effectiveness of a nationwide PHC-based LTBI screening and treatment program including migrants aged 16-35 years and born in high-TB-incidence countries. Of the individuals with a positive IGRA test (n=6,640), 26% (n=1,740) received treatment for LTBI, and of these ones, only 7 developed incident TB compared to 128 in the untreated group (86% reduction of TB risk, HR 0.14 (95%CI 0.06-0.32).[154] A recent systematic review and meta-analysis of LTBI treatment with data on 31,598 migrants from 2000-2020 in 13 low-incidence countries, reported an initiation treatment rate of 69% in those with positive testing and only 52% of treatment initiation and completion whilst another systematic review and meta-analysis reported much lower rates of initiation and completion in these patients (14%).[151,155,156]

Trends in mortality rates over time and prognostic factors

The mortality rate in TB/HIV co-infected patients was high and remained mostly unchanged throughout the study period, whilst the mortality in the mono-infected group decreased over time. The main prognostic factors included immune suppression, disseminated TB, and social burden. These mortality figures are in line with current knowledge and emphasize the need for earlier HIV and TB diagnosis as well as the need for interventions tailored to these vulnerable populations with a high social burden to improve prevention and linkage to HIV/TB care.[157]

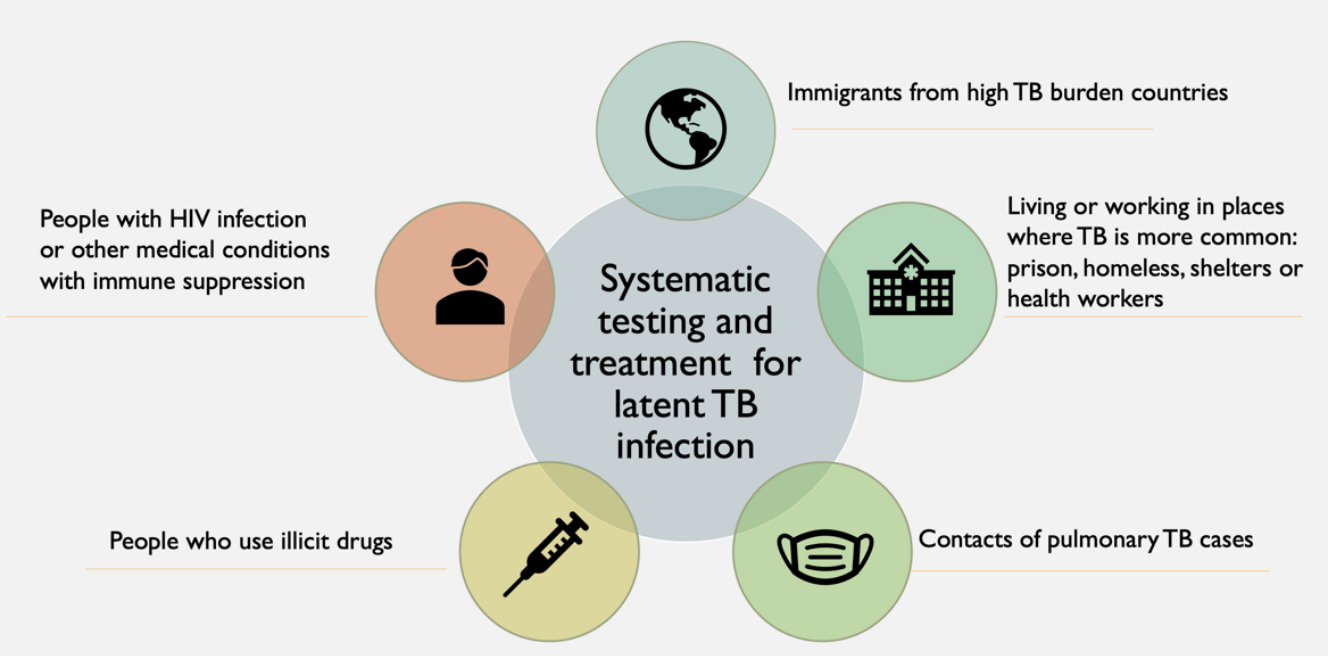
Individuals with social burden experienced particularly high mortality, especially those with known HIV infection (60% versus 27% in those with concomitant TB/HIV). Thirty-eight percent of the deaths were TB-HIV-related. The high all-cause and TB mortality rates reported in this subset of patients could be explained by an interaction of high social burden, a high index of comorbidities, a lack of adherence to treatment and altogether care, and in many cases harmful use of drugs and/or alcohol, given the high proportion of individuals with a history of IDU (25% among those with known HIV *versus* 8% among those diagnosed with HIV/TB concomitantly). Other studies have also reported similar lower mortality risk in the migrant compared to national coinfected patients.[158]

Current guidelines for screening and treatment for latent tuberculosis infection.

LTBI refers to the persistent immune response to a previously acquired infection with *Mycobacterium tuberculosis* without clinical evidence of active TB.[159] Mathematical models have estimated that one-quarter of the global population is latently infected with *Mycobacterium tuberculosis*.[160] From a public health perspective, it is pivotal to reduce the LTBI reservoir through preventive therapy in those with a higher risk of reactivation, along with active TB case detection and treatment in order to achieve the goals of the End TB Strategy.[141,161,162]

The lifetime risk of reactivation in otherwise healthy people with documented LTBI is estimated to be between 5-15%.[163] However, a variety of comorbidities and risk factors have been linked to an increased risk of progressing to active TB. PLWH with LTBI have a higher lifelong risk of progression to active TB and the WHO recommends systematic screening and treatment of all PLWH for LTBI once active TB has been ruled out in order to avert the risk of TB reactivation.[159,164,165] Treatment for LTBI reduces the overall risk of developing active TB by up to 64-86%% in those individuals with positive TB screening tests.[154,165,166] Other international guidelines in Western countries recommend testing for LTBI in all PLWH at the time of HIV diagnosis. [167–170] However, while the evidence for these recommendations is high for middle and

high-TB-burden countries,[171–174] is less so for low-incidence countries with good access to ART.[175–180]



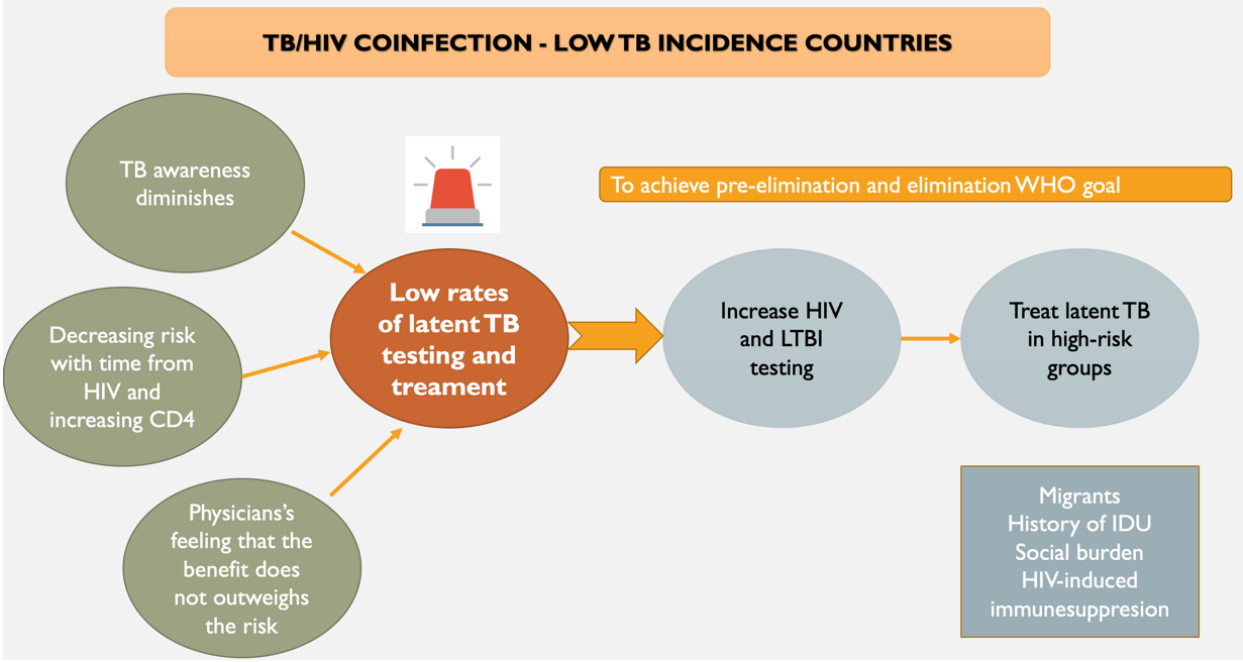
Who should be tested for latent tuberculosis infection?

Recommended treatment regimens for LTBI include 6-9 months isoniazid; 3 months rifapentine plus isoniazid; 3-4 months rifampicin plus isoniazid; or 3-4 months rifampicin alone. A recent study showed that even 1-month rifapentine plus isoniazid was non-inferior to 9 months of isoniazid in preventing HIV-related TB[181]. Shorter treatment durations are associated with increased adherence and fewer side effects.[182–185]

In low TB-burden countries, because of the overall low TB incidence, immediate ART initiation, and decreasing TB risk along with immune recovery, the potential benefit of treating LTBI is perceived by physicians as less crucial than the potential harm of drug-related adverse events. This, combined with overall low awareness of TB risk in PLWH among physicians, has resulted in poor implementation of these guidelines in Denmark and many other western European countries with low TB incidence.[177,186–189] This is reflected in the low rates of LTBI screening and the lack of LTBI treatment in our study group, despite positive screening tests. Physicians' lack of a priori TB risk perception in PLWH was more pronounced in patients of Danish origin, as evidenced by lower rates of LTBI screening among Danish-born individuals.

Despite a substantial decrease in risk of TB/HIV coinfection over calendar time, and an overall low TB incidence in Denmark, the TB rates among PLWH are 16 times higher than in the general Danish population (0.8/1000 person-years in the PLWH with known HIV infection in the period 2015-2017 and 0.05/1000 person-years in the background population). Thus, although the absolute risk is low, the relative risk compared to the background population is high. In a recent study from the Netherlands, a country with a similar TB incidence compared to Denmark, they found that the number of patients with no previous risk factors needed to test in order to find one patient with

LTBI was only 40.[190] Our results highlight the ongoing need for screening for LTBI in all PLWH at HIV diagnosis and treatment for LTBI, most importantly in those at the highest risk for developing TB, mainly migrants, people with a history of IDU, people with social burden, and HIV-induced immunosuppression. There is a need of improvement in the LTBI care cascade both in the HIV-infected population and in the migrant population. This is especially important if we want to achieve the WHO TB elimination goal by 2050 and to improve the prognosis in these patients.[191]



Conclusions

Late HIV presentation and the need for earlier diagnosis (studies I and II)

Late HIV presentation to care remains a challenge. The existing evidence and the findings presented in this Ph.D, support the need for additional interventions to improve the implementation of existing testing guidelines, as well as novel targeted strategies to identify people with undiagnosed HIV infection in the general population in order to improve timely diagnosis, early ART initiation and avoid poor clinical outcomes and new onward transmissions. Promoting the normalization of HIV testing and reducing stigma are important tools in the fight against late HIV diagnosis.

Our findings support the evidence that primary care can be an ideal setting for diagnosing these patients, as PLWH attended primary care frequently in the years preceding HIV diagnosis. Only 4% and 3% of Danish-born men and women and 13% and 26% of non-Danish men and women, respectively, had no contact with primary care in the three years preceding HIV diagnosis.

Undiagnosed PLWH have higher antimicrobial use compared to controls in the years preceding HIV diagnosis, and it was associated with a higher risk of later HIV diagnosis. The consumption of antimicrobials represents likely a proxy for HIV indicator conditions in many cases and like that missed opportunities for earlier HIV diagnosis. Antimicrobial consumption in primary care could be used as a targeted intervention to guide HIV testing and avoid or reduce the need for risk assessment. Antimicrobial consumption could be used as a marker for occult HIV and could represent a simple tool that could simplify the HIV testing process and help to normalize HIV testing and reduce HIV-related stigma. It could be implemented as an electronic alert and would allow for a dynamic risk assessment, as a single HIV test may miss the individual when they are at risk. It would also aid in capturing groups, such as heterosexual men, women, and older patients, who do not belong to traditional risk groups but who are at relatively higher risk of late diagnosis and are difficult to capture through traditional risk assessment. Further studies are needed to assess whether this intervention would be cost-effective.

Late presenters and predictors of long-term survival and immune recovery (study III)

In late presenters, the two-year CD4 cell count can be used as an early predictor of long-term survival in routine practice. Late presenters with satisfactory immune recovery two years after treatment start (CD4 cell count > 500 cells/μL) had the same long-term survival as a group of non-late presenters with optimal immune recovery, regardless of their initial CD4 cell count. Despite being diagnosed at a late stage with severe immune damage, LP has the potential to have a similar prognosis as non-LP if optimal immune recovery occurs early, closing the survival gap between late and non-late presenters. Initiating ART with an INSTI-based regimen was associated with significantly better immune recovery at two years and significantly better long-term survival. Thus, INSTI-based regimens should be positioned as the preferred first-line therapy in this population.

Finally, there are some factors in which physicians can intervene to improve survival in late presenters:

- Earlier HIV diagnosis
- Better linkage to care in vulnerable populations with a social burden
- Identify interventions associated with improved immune recovery (e.g., INSTI-based regimens)

- Prevention (harmful use of drugs and alcohol, smoking cessation programs, weight control, lipid screening)
- Early identification of comorbidities (cancer screening)
- Better management of chronic comorbidities

Tuberculosis (Studies IV)

Further efforts are necessary for low-TB-incidence countries to reach the TB pre-elimination and elimination goals set by WHO. We identified two distinct populations of HIV/TB co-infected patients, each with a different presentation pattern and approach needs. Late HIV diagnosis with concomitant TB, consisting mainly of a migrant population, has remained a challenge with high rates over time, underlining the need for intensified strategies to ensure the earlier diagnosis of both diseases. On the other side, rates of TB in PLWH with known HIV infection declined over calendar time, which was also inversely correlated to time from HIV diagnosis and immune recovery. This emphasizes the importance of early and successful ART.

Migration, IDU, social burden, and HIV-induced immune suppression were identified as important risk factors for TB. Proactive LTBI screening and treatment in these high-risk groups in low-TB-incidence countries are pivotal to averting the risk of TB reactivation.

Mortality of coinfected patients remained high and unchanged over time. Late diagnosis of TB and HIV, and social burden were important independent predictors of survival in these patients. Earlier TB and HIV diagnosis, treatment of LTBI in high-risk groups, and strategies to address the underlying social determinants that improve adherence to treatment and care would all improve survival.

Future perspectives

Efforts should be made to prevent late HIV presentation and diagnose and treat LTBI based on our findings and the current body of evidence:

- To increase awareness among PHC physicians: develop educational interventions tailored to PHC workers regarding HIV and TB risk, and the need for HIV testing in case of STIs or other indicator conditions.
- To develop electronic alert systems in PHC regarding indicator conditions and antimicrobial consumption to prompt proactive HIV testing, avoiding the need for risk assessment.
- To consider the automatic addition of an HIV test with a prior electronic alert in case of an indicator condition (opt-out screening).
- To adapt the existing indicator condition list to PHC, prioritizing diagnoses that are often seen in PHC.
- To implement additional testing interventions for LTBI and occult HIV targeted vulnerable populations: migrants, undocumented migrants, prisoners, people living in shelters, homeless people, people with illicit drug use, and sex workers.
- To improve access to care for migrants.
- To build up a health care access check-up for all immigrants coming to Denmark including infection screening and vaccination policies (hepatitis A and B, human papillomavirus, etc).
- To improve political commitment to avoid co-payments for undocumented migrants regarding TB and HIV treatment.
- To investigate if there are areas with high HIV prevalence in Denmark (>0.2%) and to consider implementing routine HIV and TB testing in these areas.
- To systematically screen all PLWH for LTBI and treat, particularly those patients with the highest risk for developing active TB.
- To implement additional target intervention in high-risk populations: MSM (community testing).
- To improve testing implementation in other settings: e.g., in the emergency room department.
- To improve disclosure of sex orientation in PHC consultations.

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Appendix

Supplementary Table S1. Description of ICD-10 codes and additional clinical, lab and drug-related parameters, used to define the comorbidities assess in the study (based on the Swedish National Study of Aging and Care in Kungsholmen (SNAC-K) coding).

CEREBROVASCULAR DISEASE	
Included ICD-10 codes and labels	
G45	Transient cerebral ischaemic attacks and related syndromes
G46	Vascular syndromes of brain in cerebrovascular diseases
I60	Subarachnoid haemorrhage
I61	Intracerebral haemorrhage
I62	Other nontraumatic intracranial haemorrhage
I63	Cerebral infarction
I64	Stroke, not specified as haemorrhage or infarction
I67	Other cerebrovascular diseases
I69	Sequelae of cerebrovascular disease
CHRONIC KIDNEY DISEASES	
Included ICD-10 codes and labels	
I120	Hypertensive renal disease with renal failure
I130	Hypertensive heart and renal disease with (congestive) heart failure
I131	Hypertensive heart and renal disease with renal failure
I132	Hypertensive heart and renal disease with both (congestive) heart failure and renal failure
I139	Hypertensive heart and renal disease, unspecified
N01	Rapidly progressive nephritic syndrome
N03	Chronic nephritic syndrome
N04	Nephrotic syndrome
N05	Unspecified nephritic syndrome
N07	Hereditary nephropathy, not elsewhere classified
N08	Glomerular disorders in diseases classified elsewhere
N11	Chronic tubulo-interstitial nephritis
N183	Chronic kidney disease, stage 3
N184	Chronic kidney disease, stage 4
N185	Chronic kidney disease, stage 5
N189	Chronic kidney disease, unspecified
Q60	Renal agenesis and other reduction defects of kidney
Q611	Polycystic kidney, autosomal recessive
Q612	Polycystic kidney, autosomal dominant
Q613	Polycystic kidney, unspecified
Q614	Renal dysplasia

Q615	Medullary cystic kidney
Q618	Other cystic kidney diseases
Q619	Cystic kidney disease, unspecified
Z905	Acquired absence of kidney
Z940	Kidney transplant status
Additional parameters	Glomerular filtration rate <60 ml/min/1.73m2 (assessed using the CKD-EPI equation)[2]
CHRONIC LIVER DISEASES	
Included ICD-10 codes and labels	
B18	Chronic viral hepatitis
K70	Alcoholic liver disease
K713	Toxic liver disease with chronic persistent hepatitis
K714	Toxic liver disease with chronic lobular hepatitis
K715	Toxic liver disease with chronic active hepatitis
K717	Toxic liver disease with fibrosis and cirrhosis of liver
K721	Chronic hepatic failure
K73	Chronic hepatitis, not elsewhere classified
K74	Fibrosis and cirrhosis of liver
K753	Granulomatous hepatitis, not elsewhere classified
K754	Autoimmune hepatitis
K758	Other specified inflammatory liver diseases
K761	Chronic passive congestion of liver
K766	Portal hypertension
K767	Hepatorenal syndrome
K778	Liver disorders in other diseases classified elsewhere
Q446	Cystic disease of liver
Z944	Liver transplant status
Excluded ICD-10 codes and labels	
K700	Alcoholic fatty liver
K701	Alcoholic hepatitis
COPD, EMPHYSEMA, CHRONIC BRONCHITIS	
Included ICD-10 codes and labels	
J41	Simple and mucopurulent chronic bronchitis
J42	Unspecified chronic bronchitis
J43	Emphysema
J44	Other chronic obstructive pulmonary disease
J47	Bronchiectasis
Additional parameters	Use of anticholinergics (R03BB)
DEMENTIA	
Included ICD-10 codes and labels	
F00	Dementia in Alzheimer disease

F01	Vascular dementia
F02	Dementia in other diseases classified elsewhere
F03	Unspecified dementia
F051	Delirium superimposed on dementia
G30	Alzheimer disease
G31	Other degenerative diseases of nervous system, not elsewhere classified
Additional parameters	Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revised (assessed by two different physicians, and a third one in case of disagreement) Use of anticholinesterases (N06DA) or memantine (N06DX01)
DEPRESSION AND MOOD DISEASES	
Included ICD-10 codes and labels	
F30	Manic episode
F31	Bipolar affective disorder
F32	Depressive episode
F33	Recurrent depressive disorder
F34	Persistent mood [affective] disorders
F38	Other mood [affective] disorders
F39	Unspecified mood [affective] disorder
F412	Mixed anxiety and depressive disorder
DIABETES	
Included ICD-10 codes and labels	
E10	Insulin-dependent diabetes mellitus
E11	Non-insulin-dependent diabetes mellitus
E13	Other specified diabetes mellitus
E14	Unspecified diabetes mellitus
E891	Postprocedural hypoinsulinaemia
Additional parameters	Glycated hemoglobin (A1C) ≥6.5% Use of antidiabetics (A10)
DYSLIPIDEMIA	
Included ICD-10 codes and labels	
E78	Disorders of lipoprotein metabolism and other lipidaemias
HEART FAILURE	
Included ICD-10 codes and labels	
I110	Hypertensive heart disease with (congestive) heart failure
I130	Hypertensive heart and renal disease with (congestive) heart failure
I132	Hypertensive heart and renal disease with both (congestive) heart failure and renal failure
I27	Other pulmonary heart diseases
I280	Arteriovenous fistula of pulmonary vessels
I42	Cardiomyopathy
I43	Cardiomyopathy in diseases classified elsewhere
I50	Heart failure
I515	Myocardial degeneration

I517	Cardiomegaly
I528	Other heart disorders in other diseases classified elsewhere
Z941	Heart transplant status
Z943	Heart and lungs transplant status
HEMATOLOGICAL NEOPLASMS	
Included ICD-10 codes and labels	
C81	Hodgkin lymphoma
C82	Follicular lymphoma
C83	Non-follicular lymphoma
C84	Mature T/NK-cell lymphomas
C85	Other and unspecified types of non-Hodgkin lymphoma
C86	Other specified types of T/NK-cell lymphoma
C88	Malignant immunoproliferative diseases
C90	Multiple myeloma and malignant plasma cell neoplasms
C91	Lymphoid leukaemia
C92	Myeloid leukaemia
C93	Monocytic leukaemia
C94	Other leukaemias of specified cell type
C95	Leukaemia of unspecified cell type
C96	Other and unspecified malignant neoplasms of lymphoid, haematopoietic and related tissue
HYPERTENSION	
Included ICD-10 codes and labels	
I10	Essential (primary) hypertension
I11	Hypertensive heart disease
I12	Hypertensive renal disease
I13	Hypertensive heart and renal disease
I15	Secondary hypertension
Additional parameters	Blood pressure ≥140/90 mmHg
ISCHEMIC HEART DISEASE	
Included ICD-10 codes and labels	
I20	Angina pectoris
I21	Acute myocardial infarction
I22	Subsequent myocardial infarction
I24	Other acute ischaemic heart diseases
I25	Chronic ischaemic heart disease
Z951	Presence of aortocoronary bypass graft
Z955	Presence of coronary angioplasty implant and graft
Additional parameters	Use of organic nitrates (C01DA) or ranolazine (C01EB18)
PERIPHERAL VASCULAR DISEASE	
Included ICD-10 codes and labels	

I702	Atherosclerosis of arteries of extremities
I73	Other peripheral vascular diseases
I792	Peripheral angiopathy in diseases classified elsewhere
I798	Other disorders of arteries, arterioles and capillaries in diseases classified elsewhere
Additional parameters	Use of cilostazol (B01AC23)
Excluded ICD-10 codes and labels	
I731	Thromboangiitis obliterans [Buerger]
I738	Other specified peripheral vascular diseases
SOLID NEOPLASMS	
Included ICD-10 codes and labels	
C	Malignant neoplasms
D00	Carcinoma in situ of oral cavity, oesophagus and stomach
D01	Carcinoma in situ of other and unspecified digestive organs
D02	Carcinoma in situ of middle ear and respiratory system
D03	Melanoma in situ
D04	Carcinoma in situ of skin
D05	Carcinoma in situ of breast
D06	Carcinoma in situ of cervix uteri
D07	Carcinoma in situ of other and unspecified genital organs
D09	Carcinoma in situ of other and unspecified sites
D320	Benign neoplasm: Cerebral meninges
D321	Benign neoplasm: Spinal meninges
D329	Benign neoplasm: Meninges, unspecified
D330	Benign neoplasm: Brain, supratentorial
D331	Benign neoplasm: Brain, infratentorial
D332	Benign neoplasm: Brain, unspecified
D333	Benign neoplasm: Cranial nerves
D334	Benign neoplasm: Spinal cord
Q85	Phakomatoses, not elsewhere classified
Excluded ICD-10 codes and labels	
C81	Hodgkin lymphoma
C82	Follicular lymphoma
C83	Non-follicular lymphoma
C84	Mature T/NK-cell lymphomas
C85	Other and unspecified types of non-Hodgkin lymphoma
C86	Other specified types of T/NK-cell lymphoma
C88	Malignant immunoproliferative diseases
C90	Multiple myeloma and malignant plasma cell neoplasms
C91	Lymphoid leukaemia
C92	Myeloid leukaemia

C93	Monocytic leukaemia
C94	Other leukaemias of specified cell type
C95	Leukaemia of unspecified cell type
C96	Other and unspecified malignant neoplasms of lymphoid, haematopoietic and related tissue

Table S2. Additional clinical and drug-related parameters used in SNAC-K to define specific chronic conditions.

Condition	Clinical and drug-related parameters
Chronic kidney diseases	Glomerular filtration rate <60 ml/min/1.73m2 (assessed using the CKD-EPI equation)[1]
COPD, Emphysema, Chronic Bronchitis	Use of anticholinergics (R03BB)
Dementia	Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revised[2] (assessed by two different physicians, and a third one in case of disagreement) Use of anticholinesterases (N06DA) or memantine (N06DX01)
Diabetes	Glycated hemoglobin (A1C) ≥6.5%[3] Use of antidiabetics (A10)
Hypercholesterolemia	Serum total cholesterol ≥6.22 mmol/L[4]
Hypertension	Blood pressure ≥140/90 mmHg[5]
Ischemic heart disease	Use of organic nitrates (C01DA) or ranolazine (C01EB18)
Peripheral vascular disease	Use of cilostazol (B01AC23)

*The ATC codes corresponding to each drug are shown in brackets. Only those drugs that can be unequivocally linked to chronic conditions were considered. The selection of ATC codes was based on a literature review and the clinical judgment of physicians.
NOTE: The criteria presented in this table were used in addition to the diagnoses assigned in SNAC-K

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Table S3. AIDS-defining and Indicator conditions

AIDS-defining illnesses in PLWH	**Conditions, associated with a prevalence of undiagnosed HIV >0,1%**	**Risk factors**
Cervical cancer	Sexually transmitted infections	Men who have sex with men
Non-Hodgkin's lymphoma	Non-Hodgkin's lymphoma	History of injection drug use
Kaposi sarcoma	Anal cancer/dysplasia	Partners to PLWH
Mycobacterium tuberculosis, pulmonary or extrapulmonary	Cervical dysplasia	Heterosexual with > 1 partner the last 12 months or with risk behaviour
Mycobacterium avium-complex (MAC) or Mycobacterium kansasii, disseminated or extrapulmonary	Herpes zoster	Sex workers
Mycobacterium, other species or unidentified species, disseminated or extrapulmonary	Hepatitis B or C	**Other situations**
Pneumonia, recurrent (>1 episode in 12 months)	Mononucleosis-like illness	Pregnancy
Salmonella septicaemia, recurrent	Leukopenia/thrombocytopenia > 4 months	Persons from high endemic countries and their partners
Cytomegalovirus retinitis	Seborrheic dermatitis/exanthema	
Cytomegalovirus, other (except liver, spleen, glands)	Invasive pneumococcal disease	
Herpes simplex, ulcer(s) for > 1 month/bronchitis/pneumonitis	Fever of unknown origin	
Progressive multifocal leukoencephalopathy	Candidaemia	
Cerebral toxoplasmosis	Visceral leishmaniasis	
Cryptosporidium diarrhoea > 1 month	**Other conditions likely to have a prevalence of undiagnosed HIV > 0,1%**	
Isosporiasis, > 1 month	Primary lung cancer	
Atypical disseminated leishmaniasis	Lymphocytic meningitis	
Reactivation of American trypanosomiasis	Oral hairy leukoplakia	
Pneumocystis jirovecii pneumonia	Severe or atypical psoriasis	
Candidiasis, oesophageal	Guillain-Barré syndrome	
Candidiasis, bronchial/tracheal/lungs	Mononeuritis	
Cryptococcosis, extrapulmonary	Subcortical dementia	
Histoplasmosis, disseminated/extrapulmonary	Multiple sclerosis-like disease	
Coccidioidomycosis, disseminated/extrapulmonary	Peripheral neuropathy	
Penicilliosis, disseminated	Unexplained weight loss	
	Unexplained lymphadenopathy	
	Unexplained oral candidiasis	
	Unexplained chronic diarrhea	
	Chronic kidney insufficiency	
	Hepatitis A	
	Community-acquired pneumonia	
	Candidiasis	
	Conditions, where to identifying and HIV infection may have negative consequences for the treatment and the prognosis of the disease.	
	Cancer	
	Transplantation	
	Auto-immune disease requiring immunosuppressive therapy	
	Primary space-occupying lesion of the brain	
	Idiopathic/thrombotic thrombocytopenic purpura	

ICD-10 diagnosis codes used for the study IV (from 1st January 1994)

DA15 – Respiratory tuberculosis
DA16 – Respiratory tuberculosis, not confirmed bacteriologically or histologically.
DA17 – Tuberculosis of nervous system
DA18 – Tuberculosis of other organs
DA19 – Milliary tuberculosis
DM49.0 – Tuberculous spondylodiscitis
DN33.0 – Tuberculous cystitis
DN74.0 – Tuberculous infection of cervix uteri
DN74.1– Female tuberculous pelvic inflammatory disease
DK23.0 – Tuberculous oesophagitis
DK67.3 – Tuberculous peritonitis
DB200A – HIV with tuberculosis
DB90 – Sequelae of tuberculosis

Table S4. Charlson comorbidity score used in Study IV

Comorbid conditions	Score	Number of patients (%) N=217	Number of deaths (%) N=55
Myocardial infarction	1	2	1
Congestive heart failure	1	0	0
Peripheral vascular disease	1	0	0
Cerebrovascular disease	1	0	0
Dementia	1	0	0
Chronic pulmonary disease	1	4	2
Connective tissue disease	1	2	0
Peptic ulcer disease	1	1	1
Diabetes without chronic complications	1	3	0
Mild liver disease	1	39	11
Hemiplegia or paraplegia	2	2	2
Diabetes with chronic complications	2	0	0
Moderate or severe renal disease	2	1	0
Solid organ malignancy	2	4	1
Leukaemia	2	0	0
Malignant lymphoma	2	3	0
End organ diseases	2	0	0
Moderate to severe liver disease	3	4	4
Metastatic solid tumour	6	1	1
Presence of other AIDS-defining diseases	6	33	10

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ORIGINAL RESEARCH

Primary health care: an opportunity for early identification of people living with undiagnosed HIV infection

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Objectives

We aimed to determine the fraction of HIV-diagnosed individuals who had primary health care (PHC) contacts 3 years prior to HIV diagnosis and whether the risk of HIV diagnosis and degree of immunodeficiency were associated with the frequency of visits or procedures performed.

Methods

We used data from national registries to conduct a population-based nested case–control study. Cases were individuals diagnosed with HIV infection in Denmark from 1998 to 2016. Population controls were extracted from the general population matched 13:1 on gender and age. We used conditional logistic regression. As there was a statistically significant interaction, analyses were further stratified by gender and Danish/non-Danish origin.

Results

We identified 2784 cases and 36 192 controls. Ninety-three per cent of cases and 88% of controls attended PHC at least once in the 3 years prior to diagnosis, with a higher median number of visits to PHC (NVPC) for cases. We found a statistically significant positive association between NVPC and risk of subsequent HIV diagnosis in men and non-Danish women. A U-shaped association between NVPC and risk of HIV diagnosis among Danish women. No substantial association between NVPC and degree of immunodeficiency was found. Risk of HIV diagnosis and degree of immunodeficiency were weakly associated with type of procedures performed.

Conclusions

For most HIV-infected individuals, there seem to be many opportunities for earlier diagnosis in PHC. In men and non-Danish women, the risk of HIV diagnosis but not the degree of immunodeficiency was related to NVPC. The results suggest that the type of medical procedure performed cannot not be used as a guide by the primary physician to indicate which patients to test.

Keywords: HIV, HIV diagnosis, late HIV presentation, primary health care

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Introduction

Despite the enormous advances achieved in the management of HIV infection, it is estimated that approximately 40% of people living with HIV (PLHIV) world-wide [1] and 15% in Europe [2,3] are unaware of their HIV status. An American study reported that more than one-third of the new HIV transmissions in the USA occurred from individuals unaware of their HIV diagnosis [4]. Recent data from the European Centre for Disease Prevention and Control [5] and other surveillance studies [6–9] indicate that around half of the newly diagnosed PLHIV are at late stages of the disease at the time of the diagnosis (CD4 cell count < 350 cells/μL). Thus, late HIV diagnosis remains a major concern globally with high costs both for the individual and for public health. It is associated with increased morbidity and mortality for the individual [7,10,11], jeopardized CD4 cell recovery, poorer response to antiretroviral treatment (ART) [12,13], increased health

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care costs [14] and increased risk of transmission of HIV in the community [15–19]. Furthermore, knowledge of HIV status is associated with risk behaviour reduction, with the potential of reducing onward transmission [19].

Targeted HIV testing is recommended in conditions where HIV infection is seen with an increased frequency, so-called indicator conditions (ICs) [20–22], in certain groups with a high risk of HIV infection [people who inject drugs (PWID), men who have sex with men (MSM) and populations originating from countries with a high HIV prevalence], and in pregnant women. Despite these recommendations, several studies have shown that PLHIV have had frequent contacts with health care services in the years prior to their HIV diagnosis, often presenting as risk groups or with ICs, representing missed opportunities for earlier HIV diagnosis [7,23–28]. Therefore, efforts to identify potential barriers to HIV testing and to improve the rate of earlier HIV diagnosis are crucial [29–31]. HIV risk factors pertaining to primary care are poorly described. Therefore, we aimed to assess the fraction of individuals diagnosed with HIV infection who had primary health care (PHC) contacts in the 3-year period prior to HIV diagnosis and investigated whether the risk of HIV diagnosis and degree of immunodeficiency were associated with the frequency of visits or the type of procedures performed in PHC.

Methods

Setting

As of 31 December 2017, Denmark had a population of 5.7 million with an estimated HIV prevalence among the adult population of 0.1% [32]. People are offered HIV testing under an opt-in policy. The Danish Health Authority recommends that people with a high risk of HIV infection are routinely offered HIV testing when in contact with the health care system [33]. The Danish health care system is universally tax-funded and all Danish residents have free and direct access to PHC.

Data sources

We used the unique 10-digit personal identification number assigned to all individuals in Denmark at birth or upon immigration to track individuals in the following national health care registries:

The Danish HIV Cohort Study (DHCS)
The DHCS is an ongoing nationwide, prospective, population-based cohort study of all PLHIV treated at Danish hospitals since 1 January 1995, which has been described in detail elsewhere [34]. The DHCS consecutively enrols patients newly diagnosed with HIV infection and immigrants with HIV infection. Data are updated yearly.

The Danish Civil Registration System (DCRS)
The DCRS, established in 1968, is a national registry that stores information on vital status, residency and migration for all residents in Denmark [35].

The Danish National Health Service Register (DNHSR)
The DNHSR is a national register collecting data from health contractors in PHC and contains data on all citizens, health providers and health care services reimbursed by the health authorities. Data are complete from 1990 [36].

Design

The study was designed as a case–control study nested in the DHCS.

Study population

Cases
From the DHCS, we identified all Danish PLHIV ≥ 18 years old, diagnosed between 1 January 1998 and 31 December 2016, who had been registered as citizens in Denmark in the 3-year period prior to HIV diagnosis. All cases were stratified into PLHIV with (1) very late HIV diagnosis (VLHIV) (CD4 count < 200 cells/μL or presentation with an AIDS-defining event within 6 months of the HIV diagnosis, regardless of the CD4 cell count), (2) late HIV diagnosis (LHIV) (CD4 count between 200 and 350 cells/μL), and (3) early HIV diagnosis (EHIV) (CD4 count ≥ 350 cells/μL or documented seroconversion within the last 12 months regardless of CD4 cell count). Study inclusion was date of HIV diagnosis.

Population controls
For every individual diagnosed with HIV infection, we identified 13 population controls from the DCRS who were not diagnosed with HIV infection in the years before 31 December 2016. All controls had to be ≥ 18 years old, alive and living in Denmark in the 3 years prior to HIV diagnosis for the matched case. All controls were individually matched on gender and age at study inclusion. The date of study inclusion was the date of HIV diagnosis of the matched case.

Exposure

In the 3 years prior to baseline, we assessed the following factors: (1) PHC contact patterns assessed using the total number of visits to PHC (NVPC) within a year, analysed as a categorical variable based on different cut-offs [(a) 1–2, 3–6, 7–12 and > 12 (13–24 and > 24) versus 0, and (b) ≥ 1, ≥ 2, ≥ 3, ≥ 4 and ≥ 5 versus < cut-off], and (2) specific medical procedures (analysed as binary variables). These procedures were chosen based on a prevalence of ≥ 1% and/or clinical knowledge (selecting those perceived to have a potential association with HIV infection) and were as follows: blood tests, rapid throat antigen Group A *Streptococcus* tests, urinalysis for urinary tract infections, anoscopy, lung function tests, sending of biological samples other than blood to reference laboratories (including microbiological tests and tests for urine microalbuminuria), biopsy, conversation therapy, self-measured blood pressure monitoring, involuntary psychiatric hospitalizations and counselling for pregnancy prevention, abortion or sterilization.

Statistics

At study inclusion, we assessed the following baseline characters: age, gender, mode of infection (MSM, heterosexual transmission, PWID or other/unknown), country of origin (Danish born versus non-Danish), calendar year of diagnosis, coinfection with hepatitis B and C viruses, AIDS diagnosis, first CD4 cell count and first HIV plasma viral load (within 180 days of baseline).

To identify exposures (or risk factors) associated with an HIV diagnosis and with severe immunosuppression, we used logistic regression analysis to compare exposure to (i) different NVPC cut-offs (as described above), and (ii) different medical procedures (performed versus not performed) in the last 3 years before diagnosis. These exposures were estimated for (1) PLHIV with matched controls and (2) HIV subgroups (EHIV, LHIV and VLHIV) with matched controls. We used conditional logistic regression in order to compute the unadjusted odds ratio (OR). As there was a clinical and statistically significant interaction as determined by the likelihood ratio test ($P < 0.0001$), we further stratified the analyses for gender and Danish/non-Danish origin. In a sensitivity analysis, further stratification was performed according to age intervals (18–29, 30–39, 40–49, 50–59 and ≥ 60 years).

Based on the rare disease assumption, these ORs were used as estimates of the relative risk of acquiring HIV and presenting with advanced immunosuppression (LHIV or VLHIV infection).

Finally, based on the assumption that we would test all exposed individuals (based on certain cut-offs for NVPC or type of procedures performed), we used the fraction of exposed controls and cases in the study to estimate the percentage of people being tested for HIV, the percentage being diagnosed and the effectiveness factor. The effectiveness factor was calculated by dividing the fraction of cases that would be diagnosed by the fraction of controls that would be tested and provided an estimation of how effective the different interventions would be compared to testing at random if the population had the same demographical pattern as that of our sample. Only a factor > 1 was considered effective.

STATA software (version 14, StataCorp, College Station, TX, USA) was used for data analysis.

The study was approved by the Danish Data Protection Agency (journal no. 2008-41-1781).

Ethical approval and individual consent

Ethics approval and individual consent are not required by Danish legislation governing this type of research in HIV-infected individuals.

Results

From the DHCS and DCRS, we identified 2784 cases and 36192 controls that fulfilled the inclusion criteria. Eighty per cent were male and the median age at diagnosis was 39 years [interquartile range (IQR) 32–48 years]. Eighty-six per cent of the controls were of Danish origin compared with 81% of male cases and 49% of female cases. The main mode of transmission was heterosexual for women (80%) and MSM for men (61%). Non-Danish origin, older age and heterosexual mode of transmission were more common among the VLHIV and LHIV groups. Furthermore, late and very late presentation remained common in the recent calendar period. Additional baseline characteristics are provided in Table 1.

During the entire 3-year period prior to HIV diagnosis, 92.6% of cases (Danish men: 96.1%; non-Danish men: 86.9%; Danish women: 97%; non-Danish women: 74.3%) and 87.8% of controls (Danish men: 92.2%; non-Danish men: 53.7%; Danish women: 98.5%; non-Danish women: 53%) had at least one contact with PHC (Table 2). The median NVPC was higher for cases than for controls (men of Danish origin: cases: 14; controls: 8; men of non-Danish origin: cases: 10; controls: 2; women of Danish origin: cases: 22; controls: 17; women of non-Danish origin: cases: 13; controls: 3). The median NVPCs per year are shown in Table 2 and Figure 1a–d.

Table 1 Baseline characteristics of the study population

	HIV-infected patients (n = 2784)	Controls (n = 36 192)	HIV-infected patients with NVPC ≥ 1 (n = 2578)	HIV-infected subgroups		
				VLHIV (n = 954)	LHIV (n = 481)	EHIV (n = 1349)
Male sex [n (%)]	2237 (80.4)	29 081 (80.4)	2111 (81.9)	744 (78.0)	372 (77.3)	1121 (83.1)
Age at HIV diagnosis (years) [median (IQR)]	39 (32–48)	39 (32–48)	40 (33–48)	43 (36–52)	39 (32–47)	36 (30–44)
Age at study inclusion [n (%)]						
18–39 years	1478 (53.1)	19 231 (53.1)	1337 (51.9)	370 (38.8)	255 (53.0)	853 (63.2)
40–49 years	727 (26.1)	9431 (26.1)	685 (26.6)	299 (31.3)	128 (26.6)	300 (22.2)
50–59 years	381 (13.7)	4962 (13.7)	366 (14.2)	183 (19.2)	63 (13.1)	135 (10.0)
> 60 years	198 (7.1)	2568 (7.1)	190 (7.4)	102 (10.7)	35 (7.3)	61 (4.5)
Danish origin [n (%)]						
Yes	2075 (74.5)	31 064 (85.8)	1997 (77.5)	671 (70.3)	340 (70.7)	1064 (78.9)
No	709 (25.5)	5128 (14.2)	581 (22.5)	283 (29.7)	141 (29.3)	285 (21.1)
Infection mode [n (%)]						
MSM	1365 (49.0)	–	1305 (50.6)	366 (38.4)	230 (47.8)	769 (57.0)
Heterosexually infected	1029 (37.0)	–	924 (35.8)	442 (46.3)	178 (37.0)	409 (30.3)
PWID	191 (6.9)	–	166 (6.4)	41 (4.3)	31 (6.4)	119 (8.8)
Other/unknown	199 (7.2)	–	183 (7.1)	105 (11.0)	42 (8.7)	52 (3.9)
HCV infection [n (%)]	267 (9.6)	–	234 (9.1)	77 (8.1)	38 (7.9)	152 (11.3)
HBV core antibody positive [n (%)]	397 (14.3)	–	352 (13.7)	163 (17.1)	79 (16.4)	155 (11.5)
CD4 count at study inclusion (cells/μL) [median (IQR)]	330 (130–540)	–	331 (130–540)	73 (30–140)	276 (236–310)	541 (430–690)
VL at study inclusion (log_{10} copies/mL) [median (IQR)]	4.8 (4.1–5.4)	–	4.8 (4.2–5.4)	5.2 (4.8–5.9)	4.7 (4.2–5.2)	4.5 (3.7–5.0)
Calendar year of diagnosis [n (%)]						
1998–2003	825 (29.6)		756 (29.3)	349 (42.3)	135 (16.4)	341 (41.3)
2004–2009	1168 (42.0)		1093 (42.4)	362 (31.0)	212 (18.2)	594 (52.3)
2010–2016	791 (28.4)		729 (28.3)	243 (30.7)	134 (16.9)	414 (50.6)

EHIV, early HIV diagnosis; HBV, hepatitis B virus; HCV, hepatitis C virus; IQR, interquartile range; LHIV, late HIV diagnosis; MSM, men who have sex with men; NVPC, number of visits to primary health care; PC, primary care; PWID, people who inject drugs; VL, viral load; VLHIV, very late HIV diagnosis.

We observed an association between NVPC ≥ 1 compared with an NVPC of 0 and a subsequent HIV diagnosis. The association between NVPC and risk of HIV diagnosis increased with increasing NVPC and with shorter time to HIV diagnosis. When stratifying results according to immune status at HIV diagnosis (VLHIV, LHIV and EHIV), we found no substantial differences in OR between HIV subgroups when compared to controls (Table 2).

As there was a clinical and statistically significant interaction between the effects of gender and country of origin on risk of HIV diagnosis, we further stratified the results according to gender and Danish/non-Danish origin. We found similar results for men and non-Danish women to those described above; however, we found a U-shaped association with NVPC for Danish women in which an NVPC of both 0 and > 12 was associated with an increased OR of subsequent HIV diagnosis compared with an NVPC of 1–2 (Fig. 1).

Interestingly, a high percentage of non-Danish individuals did not attend PHC in the 3-year period (female cases: 25.7%; male cases: 13%; female controls: 47.0%; male controls: 46.3%), whereas only a few individuals of Danish origin did not attend PHC (female cases: 3.0%; male cases: 3.9%; female controls: 1.5%; male controls: 7.8%).

Finally, in a sensitivity analysis, results were stratified according to age group (results not shown). Although the association between NVPC and subsequent HIV diagnosis in men and non-Danish women was observed for all age groups, the association was more pronounced in non-Danish men < 30 years old [OR 6.16; 95% confidence interval (CI) 3.23–11.75 for results from year 3]. Among Danish women, no difference in results was observed when they were stratified according to age. However, especially for women, data were limited by small numbers.

Further stratification of the results according to immune status at HIV diagnosis by gender and country of origin did not alter the results substantially (data not shown).

Table 2 Association between the total annual number of contacts with primary health care (PHC) in the 3 years before HIV diagnosis and being diagnosed with HIV infection (HIV-infected patients versus matched controls; HIV subgroups versus controls)

	HIV-infected patients versus age- and gender-matched controls					
	Total annual number of contacts with PHC in the 3 years before HIV diagnosis					
Contacts with PHC	HIV-infected patients (n = 2784)	Controls (n = 36 192)	HIV versus controls OR (95% CI)	VLHIV versus controls OR (95% CI)	LHIV versus controls OR (95% CI)	EHIV versus controls OR (95% CI)
Cumulative number of contacts in the 3 years before HIV diagnosis [n (%)]						
Median number of visits (IQR)	14 (6–25)	8 (3–16)		15 (6–27)	14 (6–24)	13 (6–24)
Total number of contacts with PHC						
0	206 (7.4)	4418 (12.2)	Ref (1)	Ref (1)	Ref (1)	Ref (1)
1–2	135 (4.9)	4044 (11.2)	0.71 (0.57–0.89)	0.51 (0.35–0.75)	0.59 (0.35–0.99)	0.96 (0.70–1.34)
3–6	373 (13.4)	7645 (21.1)	1.06 (0.89–1.26)	0.75 (0.56–1.01)	0.77 (0.51–1.14)	1.51 (1.16–1.96)
7–12	559 (20.1)	7734 (21.4)	1.62 (1.38–1.92)	1.09 (0.83–1.44)	1.08 (0.75–1.57)	2.47 (1.92–3.17)
13–24	782 (28.1)	7350 (20.3)	2.53 (2.16–2.97)	1.77 (1.36–2.31)	2.12 (1.50–3.01)	3.47 (2.71–4.45)
> 24	729 (26.2)	5001 (13.8)	3.72 (3.16–4.40)	2.61 (1.99–3.42)	2.28 (1.57–3.30)	5.79 (4.48–7.47)
In the year before HIV diagnosis [n (%)]						
Median number of visits (IQR)	6 (2–11)	2 (0–6)		7 (3–13)	6 (2–11)	5 (2–10)
Total number of contacts with PHC						
0	346 (12.4)	10 136 (28.0)	Ref (1)	Ref (1)	Ref (1)	Ref (1)
1–2	411 (14.8)	8897 (24.6)	1.44 (1.24–1.67)	1.16 (0.89–1.53)	1.43 (1.01–2.02)	1.64 (1.34–2.01)
3–6	750 (26.9)	9405 (26.0)	2.65 (2.32–3.03)	2.35 (1.85–2.99)	2.46 (1.80–3.37)	2.92 (2.42–3.53)
7–12	701 (25.2)	4986 (13.8)	5.04 (4.38–5.79)	4.90 (3.85–6.25)	3.65 (2.61–5.10)	5.69 (4.67–6.93)
> 12	576 (20.7)	2768 (7.7)	7.93 (6.83–9.20)	8.68 (6.74–11.17)	6.87 (4.85–9.73)	7.48 (6.00–9.32)
In the 2nd year before HIV diagnosis [n (%)]						
Median number of visits (IQR)	3 (1–8)	2 (0–6)		3 (1–8)	4 (1–7)	4 (1–8)
Total number of contacts with PHC						
0	639 (23.0)	10 366 (28.6)	Ref (1)	Ref (1)	Ref (1)	Ref (1)
1–2	552 (19.8)	8923 (24.7)	1.02 (0.91–1.15)	0.82 (0.67–1.01)	0.85 (0.63–1.13)	1.27 (1.07–1.50)
3–6	741 (26.6)	9355 (25.9)	1.35 (1.21–1.51)	1.23 (1.02–1.49)	1.11 (0.85–1.45)	1.54 (1.31–1.81)
7–12	512 (18.4)	4855 (13.4)	1.85 (1.63–2.09)	1.39 (1.12–1.72)	1.71 (1.27–2.30)	2.32 (1.93–2.78)
> 12	340 (12.2)	2693 (7.4)	2.27 (1.97–2.63)	1.61 (1.26–2.06)	2.02 (1.43–2.85)	3.09 (2.50–3.82)
In the 3rd year before HIV diagnosis [n (%)]						
Median number of visits (IQR)	3 (1–7)	2 (0–5)		3 (0–7)	3 (1–7)	3 (1–7)
Total number of contacts with PHC						
0	657 (23.6)	10 317 (28.5)	Ref (1)	Ref (1)	Ref (1)	Ref (1)
1–2	603 (21.7)	9211 (25.5)	1.05 (0.93–1.17)	0.78 (0.64–0.95)	0.91 (0.69–1.20)	1.35 (1.14–1.59)
3–6	760 (27.3)	9329 (25.8)	1.33 (1.19–1.48)	1.04 (0.86–1.25)	1.25 (0.96–1.63)	1.62 (1.37–1.91)
7–12	469 (16.9)	4710 (13.0)	1.66 (1.46–1.89)	1.14 (0.92–1.41)	1.49 (1.10–2.02)	2.29 (1.90–2.76)
> 12	295 (10.6)	(7.3)2625	1.92 (1.65–2.23)	1.13 (0.88–1.46)	1.41 (0.97–2.05)	3.18 (2.56–3.95)

CI, confidence interval; EHIV, early HIV diagnosis; IQR, interquartile range; LHIV, late HIV diagnosis; OR, odds ratio calculated by conditional logistic regression; PHC, primary health care; VLHIV, very late HIV diagnosis.

In Figure 2, we present the risk of subsequent HIV diagnosis for NVPC above different cut-offs (versus below the cut-offs), as these numbers may be easier to relate to in daily clinical practice. Furthermore, we estimated the percentage of the population that would be tested for HIV, the percentage that would be diagnosed and the effectiveness factor of such interventions based on our results (Fig. 2). For both men and women, we found a straight linear association between the number who were tested and the number who were diagnosed. As an example, by testing all Danish adult men attending PHC at least once during a year (~ 72.4% of the Danish male adults in our study population, based on calculations from year 3 before inclusion), we would identify 79% of the undiagnosed Danish male HIV-infected population, with an effectiveness factor of such an intervention of 1.09. In contrast, the effectiveness factor was higher for the non-Danish population (1.58 for non-Danish men and 1.38 for non-Danish women). As a result of the more frequent Danish female attendance at PHC and the overall lower prevalence of HIV infection among women of Danish origin, such interventions would have a lower effectiveness in identifying HIV-infected Danish women; 91% of Danish women would need to be tested to identify 84% of the undiagnosed women, with an estimated effectiveness factor < 1 (Fig. 2).

When analysing differences in procedures performed in PHC between PLHIV and controls, we observed that most of the assessed procedures performed in the last year

(a) Contacts with primary health care in the 3 years before HIV diagnosis

NVPC	HIV	Controls	OR (95%CI)
Danish men	*N* = 1808	*N* = 25 050	
Median (IQR)	14 (7–25)	7 (3–14)	
0	70 (3.9)	1947 (7.8)	Ref (1)
1–2	85 (4.7)	3554 (14.2)	0.66 (0.48–0.92)
3–6	271 (15.0)	6412 (25.6)	1.16 (0.89–1.52)
7–12	402 (22.2)	5808 (23.2)	1.95 (1.50–2.53)
13–24	512 (28.3)	4656 (18.6)	3.17 (2.45–4.10)
>24	468 (25.9)	2673 (10.7)	5.37 (4.13–6.98)
Non-Danish men	*N* = 429	*N* = 4031	
Median (IQR)	10 (4–19)	2 (0–10)	
0	56 (13.1)	1865 (46.3)	Ref (1)
1–2	34 (7.9)	272 (6.8)	4.26 (2.39–7.61)
3–6	59 (13.8)	548 (13.6)	3.81 (2.28–6.36)
7–12	89 (20.8)	525 (13.0)	5.61 (3.46–9.11)
13–24	120 (28.0)	507 (12.6)	6.89 (4.37–10.85)
>24	71 (16.6)	314 (7.8)	5.52 (3.27–9.30)
Danish women	*N* = 267	*N* = 6014	
Median (IQR)	22 (12–41)	17 (9–27)	
0	8 (3.0)	90 (1.5)	3.49 (1.52–8.01)
1–2	10 (3.8)	196 (3.3)	1.79 (0.85–3.77)
3–6	21 (7.9)	624 (10.4)	1.27 (0.72–2.24)
7–12	32 (12.0)	1282 (21.3)	Ref (1)
13–24	76 (28.5)	1995 (33.2)	1.55 (1.01–2.37)
>24	120 (44.9)	1827 (30.4)	2.52 (1.68–3.77)
Non-Danish women	*N* = 280	*N* = 1097	
Median (IQR)	13 (0–25)	3 (0–18)	
0	72 (25.7)	516 (47.0)	Ref (1)
1–2	6 (2.1)	22 (2.01)	1.50 (0.53–4.26)
3–6	22 (7.9)	61 (5.6)	2.42 (1.13–5.19)
7–12	36 (12.9)	119 (10.9)	1.87 (1.09–3.20)
13–24	74 (26.4)	192 (17.5)	2.64 (1.70–4.12)
>24	70 (25.0)	187 (17.1)	2.83 (1.79–4.48)

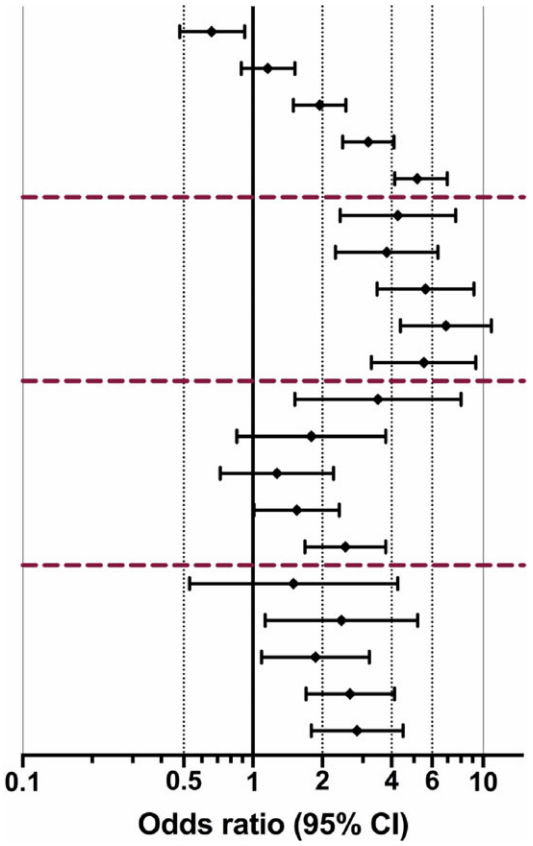

Fig. 1 Association between the total number of annual contacts with primary care in the 3 years before HIV diagnosis and being diagnosed with HIV infection in men and women stratified by Danish or non-Danish origin (HIV-infected individuals versus matched controls). (a) Men; (b) women. CI, confidence interval; IQR, interquartile range; OR, odds ratio; PHC, primary health care.

before HIV diagnosis were strongly associated with subsequent HIV infection. However, in the 2nd and 3rd years, these associations were much less pronounced and only the following procedures remained statistically significant: anoscopy (OR 2.87; 95% CI 1.67–4.95), sending biological samples to reference laboratories (OR 2.41; 95% CI 2.13–2.72), and counselling for abortion and/or sterilization in women (OR 2.13; 95% CI 1.22–3.70), and to a lesser extent urinalysis for urinary tract infection, throat antigen Group A *Streptococcus* tests, blood tests for infection/inflammation and other blood tests (Table 3). In a sensitivity analysis, in which the rate of performance of these interventions was estimated according to gender and age interval, the association with anoscopy was only statistically significant in men < 50 years old (OR 4.15; 95% CI 2.22–7.77; results from year 3) (results not shown).

Discussion

This study shows that PLHIV had frequent contacts with PHC in the 3 years preceding HIV diagnosis, with a median NVPC of 14 and 10 for men of Danish and non-Danish origin and 22 and 13 for women of Danish and non-Danish origin, respectively. We found a statistically significant association between an NVPC ≥ 1 and a greater risk of subsequent HIV diagnosis in men and non-Danish women, incrementing with increasing NVPC, with the strongest association in the most recent year before an HIV diagnosis. In contrast, a U-shaped association of NVPC with risk of subsequent HIV diagnosis was found among women of Danish origin. No substantial association between NVPC and degree of immunodeficiency was found. Based on our estimations from the 3rd year before inclusion, approximately 80% of the

(b) Contacts with primary health care in the last year before HIV diagnosis

NVPC	HIV	Controls	OR (95%CI)
Danish men			
Median (IQR)	3 (6–11)	2 (0–5)	
0	165 (9.1)	6750 (27.0)	Ref (1)
1–2	285 (15.8)	7070 (28.2)	1.75 (1.44–2.14)
3–6	521 (28.8)	6594 (26.3)	3.62 (3.01–4.36)
7–12	476 (26.3)	3057 (12.2)	7.48 (6.17–9.05)
>12	361 (20.0)	1579 (6.3)	11.71 (9.54–14.38)
Non-Danish men			
Median (IQR)	5 (1–9)	0 (0–3)	
0	80 (18.7)	2264 (56.2)	Ref (1)
1–2	75 (17.5)	607 (15.1)	4.09 (2.62–6.38)
3–6	112 (26.1)	669 (16.6)	4.02 (2.68–6.05)
7–12	101 (23.5)	314 (7.8)	9.06 (5.62–14.61)
>12	61 (14.2)	177 (4.4)	6.11 (3.57–10.48)
Danish women			
Median (IQR)	9 (4–18)	5 (2–9)	
0	21 (7.9)	560 (9.3)	1.25 (0.70– 2.24)
1–2	31 (11.6)	1121 (18.6)	Ref (1)
3–6	53 (19.9)	1956 (32.5)	0.97 (0.61–1.53)
7–12	63 (23.6)	1464 (24.3)	1.63 (1.04–2.55)
>12	99 (37.1)	913 (15.2)	3.67 (2.39–5.62)
Non-Danish women			
Median (IQR)	5 (0–10)	0 (0–6)	
0	80 (28.6)	562 (51.2)	Ref (1)
1–2	20 (7.1)	99 (9.0)	1.23 (0.65–2.32)
3–6	64 (22.9)	186 (17.0)	2.25 (1.41–3.59)
7–12	61 (21.8)	151 (13.8)	3.33 (2.05–5.41)
>12	55 (19.6)	99 (9.0)	4.36 (2.62–7.24)

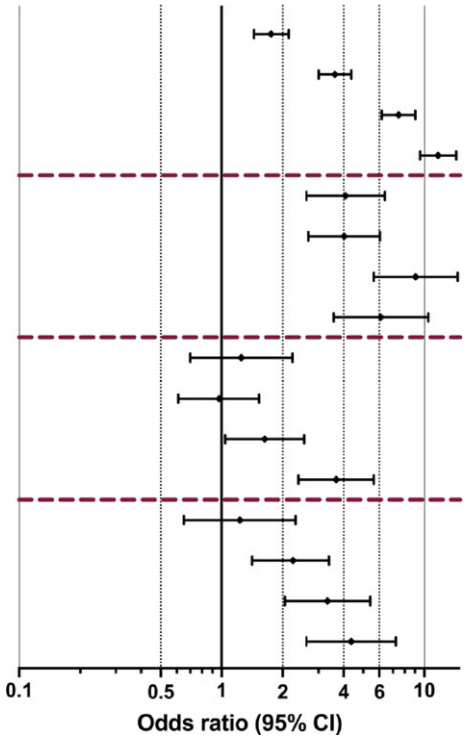

Fig. 1 Continued

undiagnosed Danish-born HIV-infected population and 70% of the non-Danish-born HIV-infected population in Denmark could be identified by testing all adult individuals attending PHC at least once during a year. Lastly, although some procedures were associated with an increased risk of a later HIV diagnosis, such interventions would not capture a large fraction of the undiagnosed HIV-infected population because of the low procedure prevalence.

The strengths of our study include the nesting of the case–control study in a well-established nationwide population-based HIV-infected cohort, the ability to use a well-matched control cohort and the possibility to look 3 years back in time from the established HIV diagnosis. We had full access to Danish registries of a high quality in order to identify PHC contact patterns.

Our study has some limitations. First, no validation studies of DNHSR have been performed. However, as most health care services in PHC are reimbursed by the health care authorities and thus require registration, the completeness and degree of accuracy of this registry are considered high [36]. The registry mainly contains administrative data, has no diagnostic codes and in general only has minimal clinical data and information about the presumptive diagnosis. Therefore, we had to rely on the NVPC and the health care services received in the 3 years preceding HIV diagnosis in order to describe contact patterns. Importantly, we used the same data source to ascertain this information for both cases and controls and assume that the accuracy of the data did not vary by either HIV or exposure status, which minimizes differential misclassification. Furthermore, HIV-infected individuals and controls may differ according to country of origin, socioeconomic status, education level, sexual orientation, drug abuse and overall health care-seeking behaviour. Despite matching for age and gender and further stratification of data, the possibility of residual or unmeasured confounding cannot be excluded. Finally, the effectiveness factor was used as an estimate of how effective the different interventions would be compared to testing at random, with the assumption that the general population had the same demographic pattern as that of our study sample, which is the best approximation that our data allow us.

(c) Contacts with primary health care in the 2nd year before HIV diagnosis

NVPC	HIV	Controls	OR (95%CI)
Danish men			
Median (IQR)	3 (1–7)	2 (0–5)	
0	359 (19.9)	6965 (27.8)	Ref (1)
1–2	403 (22.3)	7148 (28.5)	1.11 (0.96–1.29)
3–6	510 (28.2)	6541 (26.1)	1.57 (1.36–1.81)
7–12	336 (18.6)	2912 (11.6)	2.38 (2.02–2.79)
>12	200 (11.1)	1484 (5.9)	2.78 (2.30–3.36)
Non-Danish men			
Median (IQR)	2 (0–6)	0 (0–3)	
0	139 (32.4)	2298 (57.0)	Ref (1)
1–2	86 (20.1)	602 (14.9)	1.95 (1.35–2.82)
3–6	103 (24.0)	625 (15.5)	2.51 (1.72–3.66)
7–12	67 (15.6)	339 (8.4)	2.57 (1.68–3.93)
>12	34 (7.9)	167 (4.1)	1.98 (1.13–3.47)
Danish women			
Median (IQR)	6 (2–13)	5 (2–10)	
0	41 (15.4)	533 (8.9)	2.56 (1.58–4.15)
1–2	34 (12.7)	1068 (17.8)	Ref (1)
3–6	61 (22.9)	2006 (33.4)	0.96 (0.62–1.48)
7–12	58 (21.7)	1465 (24.4)	1.24 (0.80–1.92)
>12	73 (27.3)	942 (15.7)	2.29 (1.49–3.51)
Non-Danish women			
Median (IQR)	3 (0–8)	0 (0–6)	
0	100 (35.7)	570 (52.0)	Ref (1)
1–2	29 (10.4)	105 (9.6)	1.60 (0.91–2.82)
3–6	67 (23.9)	183 (16.7)	1.87 (1.23–2.83)
7–12	51 (18.2)	139 (12.7)	2.28 (1.43–3.64)
>12	33 (11.8)	100 (9.1)	1.87 (1.07–3.25)

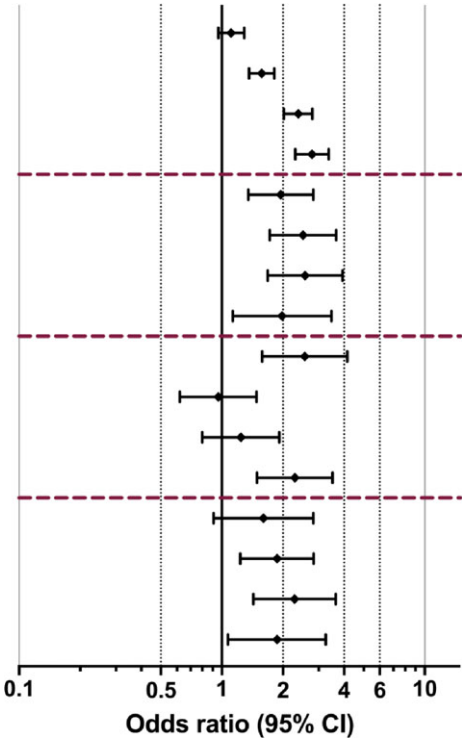

Fig. 1 Continued

Despite major efforts to implement HIV testing for earlier diagnosis, we observed that in Denmark approximately half of the subjects with newly diagnosed HIV infection were still diagnosed in late or very late stages of HIV disease. We found that non-Danish origin was associated with not only a higher risk of HIV diagnosis but also 57% higher odds of being diagnosed with VLHIV compared with EHIV (data not shown). The latter may seem surprising, as targeted HIV testing is recommended for people from countries with a high HIV prevalence, and because it has previously been shown that most people of non-Danish origin living with HIV in Denmark originate from such countries [37]. We also found that LHIV and VLHIV presenters were more likely to be older and heterosexuals, which seems to indicate that these individuals are not perceived at risk of HIV infection either by themselves or by their PHC physicians, and are thereby not being adequately reached by the traditional targeted HIV screening programmes.

We found a strong correlation between the NVPC and risk of a later HIV diagnosis in men and in non-Danish women when comparing cases with age- and gender-matched controls. These associations were independent of age, although a stronger association for non-Danish men < 30 years old was observed (data not shown). Sixty-one per cent of male cases were MSM and, although no information on sexual orientation was available in the control group, we expect the percentage of MSM to have been somewhat lower. However, restricting the analysis to heterosexual men did not alter our results significantly (data not shown).

Among Danish women, we found a higher risk for later HIV diagnosis among those with no contacts with PHC and in those with a high NVPC (> 12). There is no clear explanation for this U-shaped association, but we believe that this subgroup of individuals lacked awareness of risk behaviour and generally had less favourable health-seeking behaviour than their age- and gender-matched controls. Although cases and controls of non-Danish origin may differ substantially, it seems interesting that a high percentage of these individuals did not attended PHC in the 3-year period. This pattern may reflect differences in health care-seeking behaviour in ethnic minorities, lack of knowledge of the health care system and/or language barriers, and highlights the need for alternative health interventions to reach these patients.

(d) Contacts with primary health care in the 3rd year before HIV diagnosis

NVPC	HIV	Controls	OR (95%CI)
Danish men			
Median (IQR)	3 (1–7)	2 (0–5)	
0	385 (21.3)	6908 (27.6)	Ref (1)
1–2	428 (23.7)	7364 (29.4)	1.06 (0.92–1.22)
3–6	500 (27.7)	6609 (26.4)	1.39 (1.21–1.60)
7–12	318 (17.6)	2766 (11.0)	2.11 (1.80–2.48)
>12	177 (9.8)	1403 (5.6)	2.38 (1.96–2.89)
Non-Danish men			
Median (IQR)	2 (0–5)	0 (0–3)	
0	134 (31.2)	2279 (56.5)	Ref (1)
1–2	103 (24.0)	661 (16.4)	2.51 (1.73–3.63)
3–6	115 (26.8)	610 (15.1)	2.60 (1.81–3.73)
7–12	48 (11.2)	314 (7.8)	2.24 (1.38–3.64)
>12	29 (6.8)	167 (4.1)	1.69 (0.94–3.04)
Danish women			
Median (IQR)	6 (2–12)	5 (2–10)	
0	42 (15.7)	554 (9.2)	2.46 (1.52–3.97)
1–2	34 (12.7)	1086 (18.1)	Ref (1)
3–6	72 (27.0)	1926 (32.0)	1.18 (0.77–1.80)
7–12	57 (21.4)	1502 (25.0)	1.22 (0.79–1.89)
>12	62 (23.2)	946 (15.7)	2.07 (1.34–3.22)
Non-Danish women			
Median (IQR)	3 (0–7)	0 (0–6)	
0	96 (34.3)	576 (52.5)	Ref (1)
1–2	38 (13.6)	100 (9.1)	2.23 (1.30–3.82)
3–6	73 (26.1)	184 (16.8)	2.48 (1.62–3.81)
7–12	46 (16.4)	128 (11.7)	2.24 (1.36–3.70)
>12	27 (9.6)	109 (9.9)	1.52 (0.85–2.73)

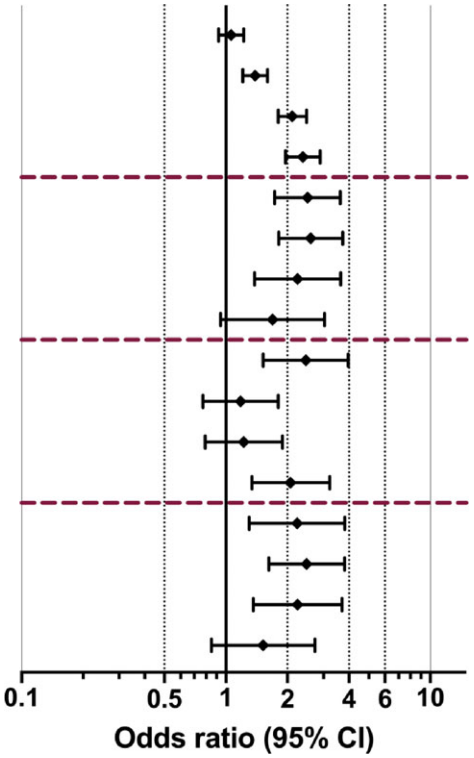

Fig. 1 Continued

In the last year before HIV diagnosis, we observed an increase in NVPC and procedures performed for all cases, which could reflect a deterioration of health status. This prompted a reaction in the PHC physician that led to HIV testing. However, given that half of the patients were diagnosed at advanced stages of the disease, earlier HIV testing and diagnosis should be the goal.

Since 2006, the Centers for Disease Control and Prevention (CDC) has recommended voluntary routine HIV screening at least once for all patients aged 13–64 years presenting to any health care facilities where the community HIV prevalence is > 0.1% without regard to risk factors [38]. A lower threshold of HIV prevalence of 0.05% was found to be cost-effective in a modelling study in the USA [39]. Furthermore, UK national guidelines recommend routine HIV screening when the local HIV prevalence is > 0.2% [40,41].

As of today, targeted HIV testing is recommended in most European countries [42,43] and should be offered in high-risk groups, in patients with AIDS-defining diseases, in pregnant women and in patients with ICs, where HIV infection is seen with a prevalence of ≥ 0.1%[20–22,44]. However, several European studies have revealed a lack of recognition among health care providers of the risk factors included in the targeted HIV guidelines, and this represents an important barrier to earlier diagnosis [20,23–27,45].

The cumulated PHC data show that approximately 96% of the Danish HIV-infected population, and 87% and 74% of non-Danish HIV-infected men and women, respectively, attended PHC in the 3-year period before their HIV diagnosis. This makes PHC an excellent platform for earlier identification of undiagnosed HIV-infected individuals. Our results suggest that it could be effective to offer universal testing to all non-Danish men and non-Danish women attending PHC at least once a year [men: OR 2.41; 95% CI 1.80–3.21; women: OR 2.19; 95% CI 1.55–3.09] and to Danish men attending PHC at least five times a year (OR 1.80; 95% CI 1.62–1.99). In contrast, Danish women at risk of HIV infection seem more difficult to reach, as only those that did not attend PHC (15.7%) or those attending > 12 times a year (23.2%) had a higher risk of HIV infection compared with their matched controls. Although Danish women are clearly the group with the lowest risk, this means that around 10% will be difficult to reach in PHC.

NVPC	Cases	Controls	OR (95% CI)	EF
Danish men	*N* = 1808	*N* = 25 050		
≥1 visits (*vs.* 0)	1423 (78·7)	18142 (72·4)	1·42 (1·26–1·60)	1·09
≥2 visits (*vs.* <2)	1212 (67·0)	14002 (55·9)	1·62 (1·46–1·80)	1·20
≥3 visits (*vs.* <3)	995 (55·0)	10778 (43·0)	1·64 (1·49–1·81)	1·28
≥4 visits (*vs.* <4)	837 (46·3)	8442 (33·7)	1·72 (1·56–1·90)	1·37
≥5 visits (*vs.* <5)	700 (38·7)	6618 (26·4)	1·80 (1·62–1·99)	1·47
Non-Danish men	*N* = 429	*N* = 4031		
≥1 visits (*vs.* 0)	295 (68·8)	1752 (43·5)	2·41 (1·80–3·21)	1·58
≥2 visits (*vs.* <2)	230 (53·6)	1382 (34·3)	1·73 (1·33–2·26)	1·56
≥3 visits (*vs.* <3)	192 (44·8)	1091 (27·1)	1·67 (1·28–2·19)	1·65
≥4 visits (*vs.* <4)	153 (35·7)	863 (21·4)	1·78 (1·32–2·39)	1·67
≥5 visits (*vs.* <5)	120 (28·0)	702 (17·4)	1·44 (1·05–1·97)	1·61
Danish women	*N* = 267	*N* = 6014		
≥1 visits (*vs.* <1)	225 (84·3)	5460 (90·8)	0·52 (0·37–0·76)	0·93
≥2 visits (*vs.* <2)	210 (78·7)	4923 (81·9)	0·86 (0·62–1·17)	0·96
≥3 visits (*vs.* <3)	191 (71·5)	4374 (72·7)	0·94 (0·71–1·25)	0·98
≥4 visits (*vs.* <4)	172 (64·4)	3861 (64·2)	1·02 (0·78–1·33)	1·00
≥5 visits (*vs.*<5)	160 (59·9)	3351 (55·7)	1·20 (0·93–1·57)	1·08
Non-Danish women	*N* = 280	*N* = 1097		
≥1 visits (*vs.* 0)	184 (65·7)	521 (47·5)	2·19 (1·55–3·09)	1·38
≥2 visits (*vs.* <2)	163 (58·2)	473 (43·1)	1·90 (1·36–2·65)	1·35
≥3 visits (*vs.* <3)	146 (52·1)	421 (38·4)	1·78 (1·29–2·46)	1·36
≥4 visits (*vs.* <4)	127 (45·4)	369 (33·6)	1·57 (1·14–2·17)	1·35
≥5 visits (*vs.* <5)	111 (39·6)	319 (29·1)	1·51 (1·09–2·09)	1·36

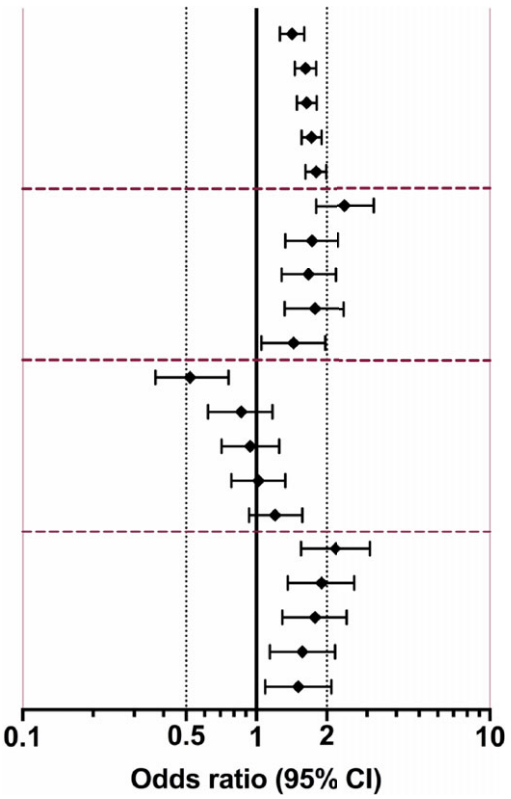

Fig. 2 Estimated percentage of HIV-infected patients being diagnosed, percentage of patients in the community being tested for HIV and the effectiveness factor according to the number of visits to primary care for men and women stratified by non-Danish/Danish origin in the third year before diagnosis.

Finally, we observed an association between the performance of certain procedures in PHC and the risk of subsequent HIV diagnosis, especially in the last year. It is not clear if this association represents a real risk indicator for HIV infection, or if it perhaps reflects the physician's tendency to perform different procedures when patients attend the clinic frequently, thereby representing confounding by indication. Certain procedures were highly associated with being subsequently diagnosed with HIV infection, such as counselling for abortion and/or sterilization in women, performance of anoscopy in men aged < 50 years, and repeated sending of biological material other than blood to reference laboratories. Although we do not have clinical information concerning the last two procedures, we presume that they act as proxies for indicator conditions such as sexually transmitted infections and anal cancer/dysplasia. Furthermore, counselling for abortion and/or sterilization in women could act as an indicator for risk behaviour. Testing individuals undergoing these procedures seems effective and should be recommended as the ORs of subsequent diagnosis of HIV infection are high; however, such interventions would not capture a large fraction of the undiagnosed HIV-infected population because of the low prevalence of the procedures among subjects with occult HIV infection.

The most important finding in our study was that people attended PHC frequently in the years before an HIV diagnosis. Only 4% and 13% of Danish-born and non-Danish-born men and 3% and 26% of Danish-born and non-Danish-born women, respectively, had no contact with PHC in the 3-year period preceding an HIV diagnosis. Thus, PHC ought to provide many opportunities for HIV testing, and should play a major role in early identification of people living with undiagnosed HIV infection. However, despite the many opportunities, the type of medical procedure performed could not be used as a guide by the primary physician to indicate which patients to test. And, although an NVPC ≥ 1 was associated with subsequent HIV diagnosis in men and non-Danish women and universal testing in PHC would capture a large proportion of undiagnosed HIV-infected patients, further

Table 3 Association between interventions performed in primary health care (PHC) in the 3 years before HIV diagnosis and being diagnosed with HIV infection (HIV-infected patients versus age- and gender-matched controls)

Performance of specific interventions in PHC	Cases (n = 2784)	Controls (n = 36 192)	OR (95% CI)	Per cent of HIV-infected patients diagnosed	Per cent of controls tested	Effectiveness factor
In the year before HIV diagnosis [n (%)]						
Conversation therapy	127 (4.6)	639 (1.8)	2.69 (2.21–3.27)	4.6	1.8	2.58
Involuntary psychiatric hospitalization*	14 (0.5)	34 (0.1)	5.35 (2.87–9.97)	0.5	0.1	5.35
Anoscopy	23 (0.8)	82 (0.2)	3.67 (2.31–5.84)	0.8	0.23	3.65
Urinalysis for urinary tract infections†	362 (13.0)	2527 (7.0)	2.01 (1.83–2.34)	13.0	7.0	1.86
Rapid strep A test	359 (12.9)	2218 (6.1)	2.31 (2.04–2.60)	12.9	6.1	2.10
Blood tests	1128 (40.5)	6069 (16.8)	3.68 (3.38–4.00)	40.5	16.8	2.42
Blood tests for inflammation/infections‡	610 (21.9)	3369 (9.3)	2.85 (2.58–3.15)	21.9	9.3	2.35
Measurement of haemoglobin	335 (12.0)	2294 (6.3)	2.07 (1.82–2.34)	12.0	6.3	1.90
Measurement of blood sugar	305 (11.0)	2345 (6.5)	1.82 (1.60–2.07)	11.0	6.5	1.69
Sending of biological samples excl. blood to laboratory§	626 (22.5)	2340 (6.5)	4.30 (3.88–4.76)	22.5	6.5	3.48
Biopsy	48 (1.7)	590 (1.6)	1.06 (0.79–1.43)	1.7	1.6	1.06
Lung function tests	140 (5.0)	877 (2.4)	2.14 (1.78–2.57)	5.0	2.4	2.08
Self-measured blood pressure monitoring	33 (1.2)	449 (1.2)	0.95 (0.67–1.37)	1.2	1.2	0.96
Gynaecological exam with cytology¶	62 (11.3)	1076 (15.1)	0.71 (0.54–0.94)	11.3	15.1	0.75
Pregnancy prevention counselling¶	69 (12.6)	1170 (16.5)	0.72 (0.55–0.94)	12.6	16.5	0.76
Counselling for abortion and/or sterilization¶	12 (2.2)	79 (1.1)	2.02 (1.09–3.75)	2.2	1.1	2.00
In the 2nd year before HIV diagnosis [n (%)]						
Conversation therapy	64 (2.3)	566 (1.6)	1.49 (1.14–1.93)	2.3	1.6	1.47
Involuntary psychiatric hospitalization*	5 (0.2)	47 (0.1)	1.38 (0.55–3.48)	0.2	0.1	1.38
Anoscopy	12 (0.4)	76 (0.2)	2.07 (1.12–3.81)	0.4	0.2	2.05
Urinalysis for urinary tract infections†	246 (8.8)	2496 (6.9)	1.32 (1.15–1.53)	8.8	6.9	1.28
Rapid strep A test	269 (9.7)	2253 (6.2)	1.63 (1.42–1.86)	9.7	6.2	1.55
Blood tests	590 (21.2)	5711 (15.8)	1.47 (1.33–1.62)	21.2	15.8	1.34
Blood tests for inflammation/infections‡	308 (11.1)	3161 (8.7)	1.31 (1.16–1.49)	11.1	8.7	1.27
Measurement of haemoglobin	178 (6.4)	2279 (6.3)	1.02 (0.87–1.19)	6.4	6.3	1.02
Measurement of blood sugar	180 (6.5)	2271 (6.3)	1.03 (0.88–1.21)	6.5	6.3	1.03
Sending of biological samples excl. blood to laboratory§	390 (14.0)	2352 (6.5)	2.45 (2.17–2.76)	14.0	6.5	2.16
Biopsy	44 (1.6)	579 (1.6)	0.99 (0.73–1.35)	1.6	1.6	0.99
Lung function tests	86 (3.1)	835 (2.3)	1.35 (1.08–1.69)	3.1	2.3	1.34
Self-measured blood pressure monitoring	24 (0.9)	362 (1.0)	0.86 (0.56–1.30)	0.86	1.0	0.86
Gynaecological exam with cytology¶	54 (9.9)	1101 (15.5)	0.59 (0.44–0.79)	9.9	15.5	0.64
Pregnancy prevention counselling¶	63 (11.5)	1279 (18.0)	0.58 (0.44–0.76)	11.5	18.0	0.64
Counselling for abortion and/or sterilization¶	15 (2.7)	88 (1.2)	2.25 (1.29–3.93)	2.7	1.2	2.22
In the 3rd year before HIV diagnosis [n (%)]						
Conversation therapy	57 (2.1)	561 (1.6)	1.33 (1.01–1.75)	2.0	1.6	1.32
Involuntary psychiatric hospitalization*	4 (0.1)	43 (0.1)	1.21 (0.43–3.38)	0.14	0.12	1.21
Anoscopy	16 (0.6)	73 (0.2)	2.87 (1.67–4.95)	0.57	0.20	2.85
Urinalysis for urinary tract infections†	218 (7.8)	2467 (6.8)	1.17 (1.01–1.36)	7.8	6.8	1.15
Rapid strep A test	244 (8.8)	2279 (6.3)	1.44 (1.25–1.65)	8.8	6.3	1.39
Blood tests	501 (18.0)	5280 (14.6)	1.31 (1.18–1.45)	18.0	14.6	1.23
Blood tests for inflammation/infections‡	247 (8.9)	2891 (8.0)	1.13 (0.98–1.29)	8.9	8.0	1.11
Measurement of haemoglobin	138 (5.0)	2035 (5.6)	0.87 (0.73–1.04)	5.0	5.6	0.88
Measurement of blood sugar	152 (5.5)	2086 (5.8)	0.94 (0.79–1.12)	5.5	5.8	0.95
Sending of biological samples excl. blood to laboratory§	371 (13.3)	2258 (6.2)	2.41 (2.13–2.72)	13.3	6.2	2.14
Biopsy	44 (1.6)	541 (1.5)	1.06 (0.78–1.44)	1.6	1.5	1.06
Lung function tests	65 (2.3)	847 (2.3)	1.00 (0.77–1.29)	2.3	2.3	1.00
Self-measured blood pressure monitoring	20 (0.7)	289 (0.8)	0.90 (0.57–1.42)	0.72	0.80	0.90
Gynaecological exam with cytology¶	58 (10.6)	1013 (14.3)	0.71 (0.54–0.94)	10.6	14.3	0.74
Pregnancy prevention counselling¶	62 (11.3)	1317 (18.5)	0.54 (0.41–0.72)	11.3	18.5	0.61
Counselling for abortion and/or sterilization¶	15 (2.7)	93 (1.3)	2.13 (1.22–3.70)	2.7	1.3	2.08

CI, confidence interval; excl., excluding; OR, unadjusted odds ratio calculated by conditional logistic regression; strep, *Streptococcus*.
*Based on physician's certificate for involuntary hospitalization in psychiatric department.
†Performance of urine stix testing, urine microscopy, urine culture and/or resistance.
‡Measuring C-reactive protein sedimentation rate and/or leucocytes.
§Includes urine microalbuminuria test and microbiology tests.
¶Women only.

studies are needed to investigate whether this intervention would be cost effective.

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Data sharing statement

Data can only be accessed from Statistics Denmark by authorized research persons. Our research group have access to the data during a 2-year period with the possibility of prolonging it by applying again to Statistics Denmark.

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Prescription of antimicrobials in primary health care as a marker to identify people living with undiagnosed HIV infection, Denmark, 1998 to 2016

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Background: Development of additional diagnostic strategies for earlier HIV diagnosis are needed as approximately 50% of newly diagnosed HIV-infected individuals continue to present late for HIV care. **Aim:** We aimed to analyse antimicrobial consumption in the 3 years preceding HIV diagnosis, assess whether there was a higher consumption in those diagnosed with HIV compared with matched controls and whether the level of consumption was associated with the risk of HIV infection. **Methods:** We conducted a nested case–control study, identifying all individuals (n = 2,784 cases) diagnosed with HIV in Denmark from 1998 to 2016 and 13 age-and sex-matched population controls per case (n = 36,192 controls) from national registers. Antimicrobial drug consumption was estimated as defined daily doses per person-year. We used conditional logistic regression to compute odds ratios and 95% confidence intervals. **Results:** In the 3 years preceding an HIV diagnosis, we observed more frequent and higher consumption of antimicrobial drugs in cases compared with controls, with 72.4% vs 46.3% having had at least one prescription ($p < 0.001$). For all antimicrobial classes, the association between consumption and risk of subsequent HIV diagnosis was statistically significant ($p < 0.01$). The association was stronger with higher consumption and with shorter time to HIV diagnosis. **Conclusion:** HIV-infected individuals have a significantly higher use of antimicrobial drugs in the 3 years preceding HIV diagnosis than controls. Prescription of antimicrobial drugs in primary healthcare could be an opportunity to consider proactive HIV testing. Further studies need to identify optimal prescription cut-offs that could endorse its inclusion in public health policies.

Introduction

Despite extensive efforts in healthcare policies, ca 50% of new HIV cases in Europe continue to be diagnosed at late stages of the disease ($CD4^+$ T-cell count < 350 cells/µL) and ca 30% present with very late HIV infection ($CD4^+$ T-cell count < 200 cells/µL or presenting with an AIDS-defining illness (ADI)) [1-3].

Late presentation is associated with higher rates of morbidity and mortality, poorer response to antiretroviral therapy (ART), incomplete immune recovery, increased healthcare costs and increased risk of ongoing HIV transmission [4-11]. Therefore, late diagnosis has negative consequences for both the individual and society as a whole, some of which are irreversible. In the Collaboration of Observational HIV Epidemiological Research Europe (COHERE) study, late HIV diagnosis was associated with a 6–13-fold increased risk of ADI and/or death in the first year after HIV diagnosis, depending on the area in Europe [12]. Furthermore, the Strategic Timing of Antiretroviral Treatment (START) study showed that early diagnosis and ART initiation (at $CD4^+$ T-cell count > 500 cells/µL) was associated with lower rates of serious ADI and of non-ADI including death [7]. Initiation of ART in individuals with very high $CD4^+$ T-cell counts (> 750 cells/µL) has also been associated with a reduction in rates of ADI, especially malignant ADI [4]. Taken together, there is a large body of evidence supporting the benefits of identifying HIV infection at earlier stages.

A number of medical conditions (so-called indicator conditions) have been associated with an undiagnosed HIV prevalence > 0.1% and data from the United States (US) and France have shown that HIV testing in

Table 1

Baseline characteristics of the study population, Denmark, 1998–2016 (n = 38,976)

Characteristics	HIV n = 2,784		Controls n = 36,192		HIV subgroups					
					VLHIV n = 954		LHIV n = 481		EHIV n = 1,349	
	n	%	n	%	n	%	n	%	n	%
Male	2,237	80.4	29,081	80.4	744	78.0	372	77.3	1,121	83.1
Age at HIV diagnosis, median years (IQR)	39 (32–48)		39 (32–48)		43 (36–52)		39 (32–47)		36 (30–44)	
Age at study inclusion (years)										
18–39	1,478	53.1	19,231	53.1	370	38.8	255	53.0	853	63.2
40–49	727	26.1	9,431	26.1	299	31.3	128	26.6	300	22.2
50–59	381	13.7	4,962	13.7	183	19.2	63	13.1	135	10.0
≥ 60 years	198	7.1	2,568	7.1	102	10.7	35	7.3	61	4.5
Danish origin[a]										
Yes	2,075	74.5	31,064	85.8	671	70.3	340	70.7	1,064	78.9
No	709	25.5	5,128	14.2	283	29.7	141	29.3	285	21.1
Infection mode										
MSM	1,365	49.0	Not recorded		366	38.4	230	47.8	769	57.0
Heterosexually infected	1,029	37.0	Not recorded		442	46.3	178	37.0	409	30.3
PWID	191	6.9	Not recorded		41	4.3	31	6.4	119	8.8
Other/unknown	199	7.2	Not recorded		105	11.0	42	8.7	52	3.9
HCV	267	9.6	Not recorded		77	8.1	38	7.9	152	11.3
Hepatitis B core antibody-positive	397	14.3	Not recorded		163	17.1	79	16.4	155	11.5
$CD4^+$ T-cell count at study, median cells/μL (IQR)	330 (130–540)		Not recorded		73 (30–140)		276 (236–310)		541 (430–690)	
Viral load at study inclusion, median log10 copies/mL (IQR)	4.8 (4.1–5.4)		Not applicable		5.2 (4.8–5.9)		4.7 (4.2–5.2)		4.5 (3.7–5.0)	
Calendar year of diagnosis[b]										
1998–2003	825	29.6	Not applicable		349	42.3	135	16.4	341	41.3
2004–2009	1,168	42.0	Not applicable		362	31.0	212	18.2	594	50.9
2010–2016	791	28.4	Not applicable		243	30.7	134	16.9	414	52.3
Antimicrobial prescription in the study period[c]	**2,015**	**72.4**	**16,750**	**46.3**	**743**	**77.9**	**324**	**67.4**	**948**	**70.3**
Antibiotics	1,895	68.1	15,973	44.1	688	72.1	304	63.2	903	66.9
Antiviral	353	12.7	736	2.0	172	18.0	55	11.4	126	9.3
Antifungals	382	13.7	1,563	4.3	223	23.4	45	9.4	114	8.5

EHIV: early HIV diagnosis; HCV: hepatitis C virus; HIV: human immunodeficiency virus; IQR: interquartile range; LHIV: late HIV diagnosis; MSM: men who have sex with men; PWID: people who inject drugs; VLHIV: very late HIV diagnosis.

[a] $p < 0.01$, Pearson's chi-squared test.

[b] Presented as row percentages.

[c] One or more prescriptions of at least one of the antimicrobials included in the study.

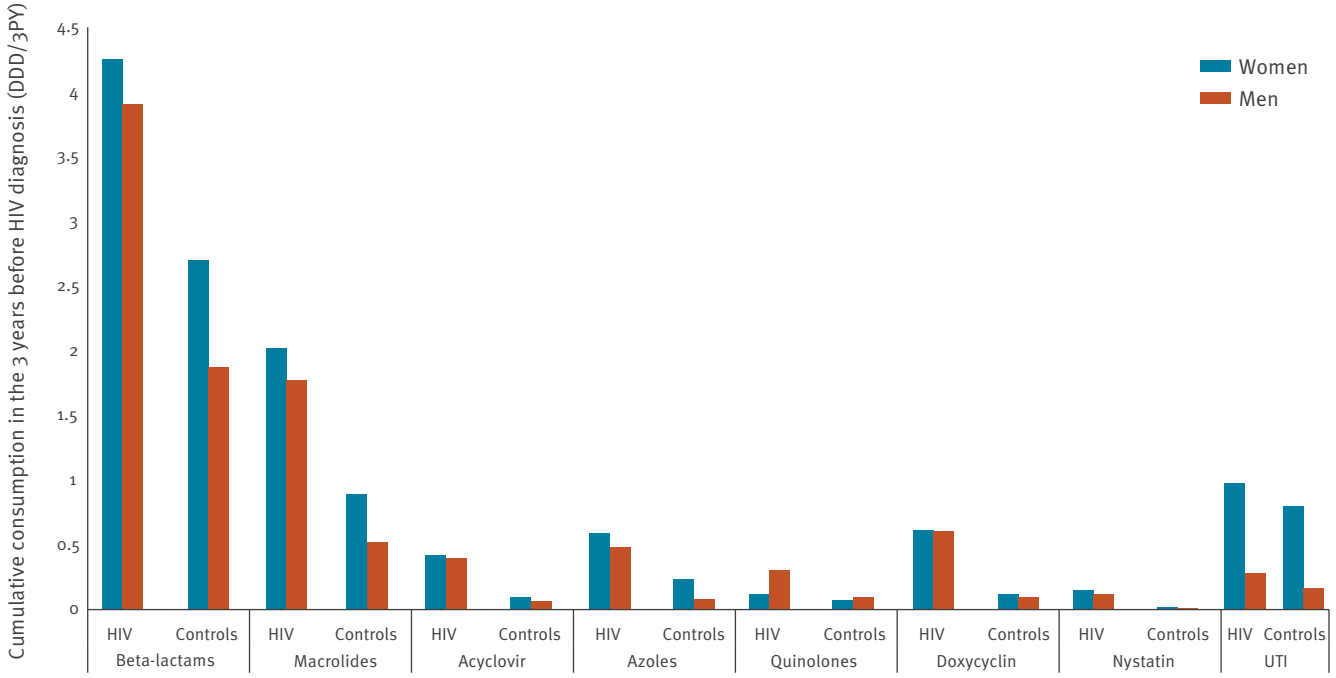
conditions above this threshold is cost-effective [13-15]. European guidelines recommend routine HIV testing in individuals presenting with indicator conditions or an ADI, in high-risk groups or in conditions where not identifying HIV would adversely affect the management of the disease [16-19]. Furthermore, guidelines in the United Kingdom (UK) recommend universal HIV testing for all adults presenting for care in any healthcare setting in geographical areas with high HIV prevalence, defined as >0.2% [20]. Despite these recommendations, many missed opportunities for earlier HIV diagnosis occur in both primary and secondary healthcare [21-23].

Denmark has achieved the target from the Joint United Nations Programme on HIV/AIDS (UNAIDS) of 90% diagnosis, treatment and undetectability [24]. However, ca half of the infected individuals are still diagnosed at late stages of the HIV infection and therefore, new strategies to deal with this problem are needed.

With data from the Danish HIV Cohort Study, we recently found that HIV-infected individuals have frequent contacts with primary healthcare (PHC) in the 3 years preceding their HIV diagnosis. Consequently, PHC could play a major role in earlier detection of occult HIV infection [25].

Figure 1

Antimicrobial drug prescription in the 3 years before HIV diagnosis, stratified by sex, Denmark, 1998–2016 (n = 38,976)



DDD/3PY: defined daily dose per person in the 3-year study period; HIV: human immunodeficiency virus; UTI: urinary tract infection.

Antimicrobial prescription in PHC is related to the treatment of some of the infectious ADI or indicator conditions such as bacterial infections (recurrent pneumonia, sexually transmitted infections (STI)), viral infections (herpes simplex, herpes zoster), and fungal infections (candidiasis), and its prescription could be regarded as a proxy for these conditions. Therefore, the aim of the present study was (i) to assess antimicrobial drug prescription (i.e. antibiotics, antivirals, and antifungals) in the 3 years preceding the HIV diagnosis compared with age and sex-matched controls, and (ii) to assess whether consumption of antimicrobials was associated with an increased risk of subsequent HIV diagnosis.

Methods

Setting

On 31 December 2017, Denmark had a population of 5.7 million with an estimated HIV prevalence among the adult population of 0.1% [26].

The Danish healthcare system is universally tax-funded, guaranteeing free access to healthcare for all citizens in Denmark. Antimicrobials can only be obtained with a prescription from a physician in primary or secondary care or administered during hospitalisation.

Study period

The study was performed from 1 January 1998 to 31 December 2016.

Data sources

We used the Danish personal identification number, a unique 10-digit personal identifier assigned to all Danish citizens at birth or upon immigration, to link individuals in the following national healthcare registries:

The Danish HIV Cohort Study

The Danish HIV Cohort Study (DHCS) is an ongoing nationwide, prospective, population-based cohort study of all HIV-infected patients treated at Danish hospitals since 1 January 1995. The cohort has been described in detail elsewhere [27]. The DHCS consecutively enrols patients newly diagnosed with HIV and immigrants with HIV infection. Data are updated yearly.

The Danish Civil Registration System

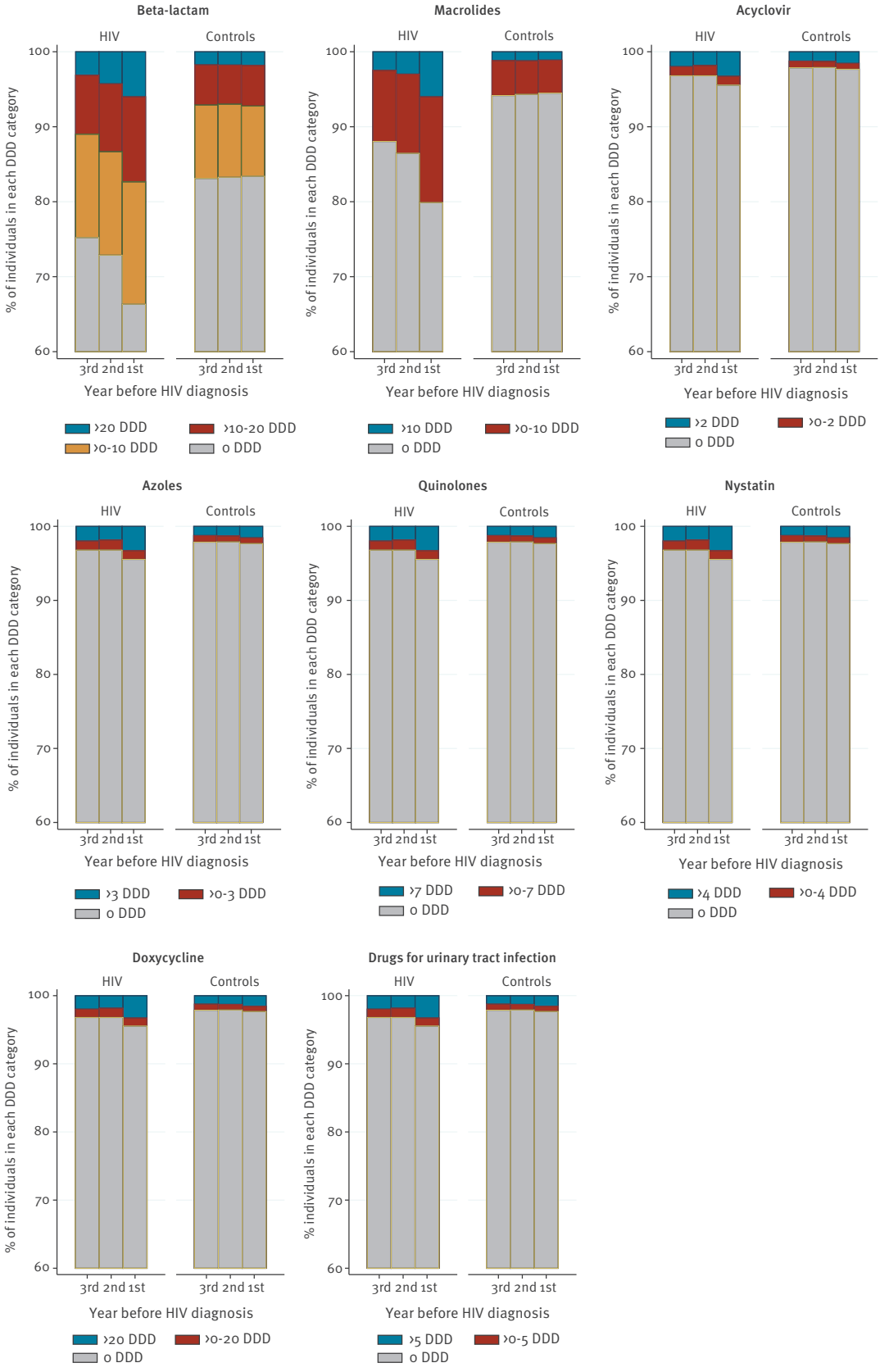
The Danish Civil Registration System (DCRS), established in 1968, is a national registry, which stores information on vital status, residency and migration for all Danish residents. It is updated daily (Monday through Friday). All immigrants registered as living in Denmark are included. While undocumented migrants have access to healthcare through a temporary registration number, they cannot be followed over time through the administrative registries [28].

The Danish National Prescription Register

The Danish National Prescription Register (DNPR) collects individual-level data on all medical prescriptions

Figure 2

Prescription of antimicrobials for cases and controls, by year before HIV diagnosis of the matched case, Denmark, 1998–2016 (n = 38,976)



DDD: defined daily dose; HIV: human immunodeficiency virus; UTI: urinary tract infection.

For each antimicrobial class, we categorised the consumption based on different DDD cut-offs. The specific DDD cut-offs used for each antimicrobial drug class illustrate the average estimated number of DDD for one standard treatment for the most common infections.

redeemed at Danish community pharmacies since 1 January 1995. The registry includes variables related to the patient, prescriber, pharmacy and dispensed drug. The latter includes date of dispensing, name, drug code, anatomical therapeutic chemical classification (ATC) code (available to 5th ATC level), number of packages, package size, strength, number of defined daily doses (DDD) per package, and drug formulation. The registry does not contain data on drugs used during hospital admission [29].

Design

The study was designed as a case–control study nested in the DHCS.

Study population

Cases

From the DHCS, we identified all adults (≥18 years) in Denmark diagnosed with HIV infection between 1 January 1998 and 31 December 2016 who had been living in Denmark in the 3 years before their HIV diagnosis. All cases were stratified as (i) very late HIV diagnosis (VLHIV), i.e. $CD4^+$ T-cell count<200 cells/µL or presentation with an ADI within 6 months of the HIV diagnosis, regardless of the $CD4^+$ T-cell count, (ii) late HIV diagnosis (LHIV), i.e. $CD4^+$ T-cell count 200–349 cells/µL, and (iii) early HIV diagnosis (EHIV), i.e. $CD4^+$ T-count≥350 cells/µL or documented seroconversion within the last 12 months, regardless of $CD4^+$ T-cell count. Study inclusion was the date of HIV diagnosis.

Population controls

For every HIV-infected individual, we identified 13 age and sex-matched population controls from the DCRS. We chose this number of controls to secure the statistical power of the analysis. None of the controls had been diagnosed with HIV before 31 December 2016. All controls had to be alive and living in Denmark in the 3 years before HIV diagnosis for the matched case. Study inclusion was the date of HIV diagnosis for the matched case.

Exposure

Using data from the DNPR, we assessed antimicrobial consumption based on all redeemed prescriptions of oral antimicrobial drugs in the 3 years before study inclusion. The consumption was further expressed as DDD per person-year (PY) (for names and ATC codes see Supplementary Table S1). Only those drug classes prescribed to>1% of the HIV-infected individuals were included for further analysis (beta-lactams, macrolides, fluoroquinolones, tetracycline, antibiotics only used to treat urinary tract infections (UTI drugs), antivirals, azoles and nystatin).

For each antimicrobial drug class, we categorised the consumption based on different DDD cut-offs. These cut-offs were chosen based on clinical criteria as the average estimated number of DDD for one standard treatment for the most common infections (Supplementary Table S1).

Consumption of each antimicrobial class was assessed according to (i) the total DDD/3 PY, in the whole 3-year study period before HIV diagnosis and (ii) the percentage of patients in each DDD/PY category both per year and in the whole 3-year period.

Statistics

For all antimicrobial drug classes, we assessed the fraction of cases and controls in each drug consumption group (i.e. category of DDD/PY) as defined above. To identify exposures associated with risk of subsequent HIV diagnosis, we used conditional logistic regression analysis to compare exposure to different levels of antimicrobial drug consumption between cases and controls, providing an odds ratio (OR) and a 95% confidence interval (CI). These exposures were estimated both as categorical (DDD categories defined above vs no consumption) and as binary variables (consumption above different DDD/PY cut-offs vs consumption at or under this level). Based on the rare disease assumption, these OR were used as estimates of the relative risk (RR) of acquiring HIV.

To analyse the trend of increase in consumption of antimicrobial drugs in the different years before HIV diagnosis, we used the Cochran–Armitage test.

As there was a clinical and statistically significant interaction between the person's sex and the risk of HIV diagnosis (likelihood ratio test; $p<0.01$), we further stratified the analyses by sex. In a sensitivity analysis, we further stratified the data by HIV subgroup (EHIV, LHIV and VLHIV), mode of infection, Danish origin and age group (18–39, 40–59 and ≥60 years).

Finally, based on the assumption that we would test for HIV all exposed individuals within certain cut-offs for antimicrobial consumption, we used the fraction of exposed controls and cases in the study to estimate the percentage of people being tested, the percentage being diagnosed and the effectiveness factor [25]. The effectiveness factor was calculated by dividing the fraction of cases that would be diagnosed by the fraction of controls that would be tested and provided an estimation of how effective the interventions at different cut-offs would be compared with testing at random in a population with the same demographical pattern as our sample. Only a factor>1 was considered effective.

STATA software (version 14) was used for data analysis.

Ethical statement

The study was approved by the Danish Data Protection Agency (journal no 2008–41–1781). Ethics approval and individual consent are not required by Danish legislation governing this type of research on HIV-infected individuals.

Table 2

Association between antimicrobial prescription in the 3 years before HIV diagnosis and risk of HIV diagnosis, Denmark, 1998–2016 (n = 38,976)

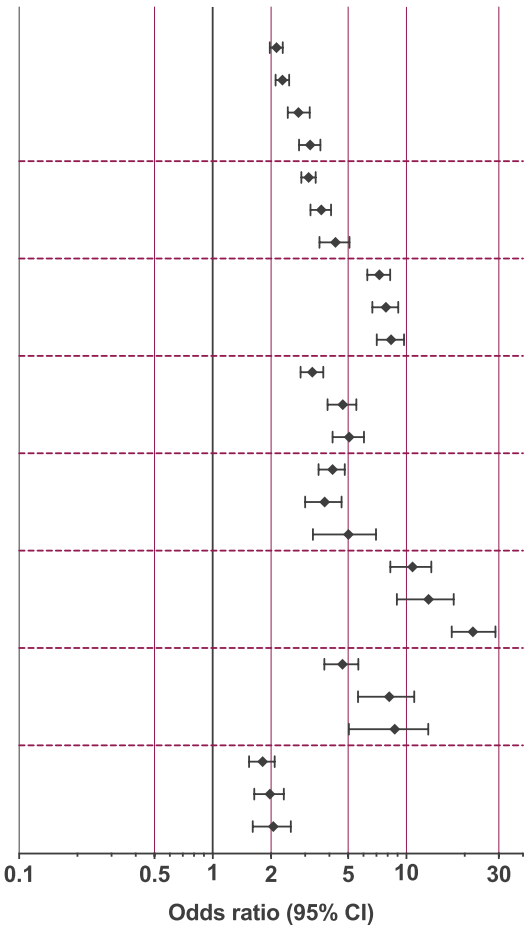
	DDD/1 PY	First year before cases' HIV diagnosis					Second year before cases' HIV diagnosis					Third year before cases' HIV diagnosis				
		HIV n = 2,784		Controls n = 36,192		OR (95% CI)	HIV n = 2,784		Controls n = 36,192		OR (95% CI)	HIV n = 2,784		Controls n = 36,192		OR (95% CI)
		n	%	n	%		n	%	n	%		n	%	n	%	
Beta-lactams	0	1,847	66.3	30,186	83.4	Ref (1)	2,029	72.9	30,137	83.3	Ref (1)	2,094	75.2	30,078	83.1	Ref (1)
	>0–10	454	16.3	3,386	9.4	2.21 (1.98–2.46)	384	13.8	3,526	9.7	1.63 (1.45–1.83)	384	13.8	3,545	9.8	1.56 (1.39–1.75)
	>10–20	316	11.4	1,963	5.4	2.64 (2.33–3.01)	252	9.1	1,888	5.2	2.00 (1.74–2.31)	219	7.9	1,939	5.4	1.63 (1.41–1.89)
	>20	167	6.0	657	1.8	4.23 (3.54–5.05)	119	4.3	641	1.8	2.77 (2.27–3.39)	87	3.1	630	1.7	2.00 (1.59–2.52)
Macrolides	0	2,224	79.9	34,197	94.5	Ref (1)	2,407	86.5	34,136	94.3	Ref (1)	2,451	88.0	34,081	94.2	Ref (1)
	>0–10	393	14.1	1,593	4.4	3.80 (3.38–4.28)	294	10.6	1,623	4.5	2.57 (2.25–2.93)	264	9.5	1,686	4.7	2.19 (1.91–2.50)
	>10	167	6.0	402	1.1	6.45 (5.35–7.78)	83	3.0	433	1.2	2.73 (2.15–3.46)	69	2.5	425	1.2	2.26 (1.75–2.92)
Acyclovir	0	2,564	92.1	35,832	99.0	Ref (1)	2,656	95.4	35,834	99.0	Ref (1)	2,682	96.3	35,852	99.1	Ref (1)
	>0–2	54	1.9	171	0.5	6.37(4.60–8.83)	36	1.3	125	0.4	3.98 (2.74–5.80)	30	1.1	111	0.3	3.68 (2.45–5.51)
	>2	166	6.0	189	0.5	9.64 (7.88–11.79)	92	3.3	233	0.6	5.42 (4.24–6.94)	72	2.6	229	0.6	4.31 (3.29–5.64)
Azoles	0	2,579	92.6	35,578	98.3	Ref (1)	2,681	96.3	35,556	98.2	Ref (1)	2,707	97.2	35,561	98.3	Ref (1)
	>0–3	78	2.8	410	1.1	2.79 (2.17–3.60)	49	1.8	426	1.2	1.57 (1.16–2.13)	36	1.3	412	1.1	1.16 (0.82–1.64)
	>3	127	4.6	204	0.6	8.74 (6.97–10.96)	54	1.9	210	0.6	3.42 (2.53–4.63)	41	1.5	219	0.6	2.48 (1.77–3.47)
Quinolones	0	2,650	95.2	35,867	99.1	Ref (1)	2,704	97.1	35,871	99.1	Ref (1)	2,716	97.6	35,872	99.1	Ref (1)
	>0–7	66	2.4	167	0.5	5.38 (4.04–7.18)	51	1.8	176	0.5	3.85 (2.81–5.28)	44	1.6	190	0.5	3.05 (2.20–4.25)
	>7	68	2.4	158	0.4	5.92 (4.43–7.90)	29	1.0	145	0.4	2.67 (1.78–3.98)	24	0.9	130	0.4	2.47 (1.59–3.83)
Nystatin	0	2,690	96.6	36,118	99.8	Ref (1)	2,755	99.0	36,139	99.9	Ref (1)	2,773	99.6	36,141	99.9	Ref (1)
	>0–4	39	1.4	41	0.1	13.44 (8.59–21.04)	14	0.5	29	0.1	6.47 (3.39–12.37)	6	0.2	30	0.1	2.62 (1.08–6.31)
	>4	55	2.0	33	0.1	23.45 (15.08–36.46)	15	0.5	24	0.1	8.13 (4.26–15.49)	5	0.2	21	0.1	3.13 (1.17–8.36)
Doxycycline	0	2,719	97.7	36,057	99.6	Ref (1)	2,743	98.5	36,055	99.6	Ref (1)	2,746	98.6	36,052	99.6	Ref (1)
	>0–20	41	1.5	101	0.3	5.37 (3.77–7.74)	23	0.8	97	0.3	3.12 (1.98–4.92)	23	0.8	116	0.3	2.63 (1.68–4.14)
	>20	24	0.9	34	0.1	9.27 (5.49–15.63)	18	0.7	40	0.1	5.89 (3.38–10.28)	15	0.5	24	0.1	8.22 (4.31–15.67)
UTI drugs	0	2,660	95.6	35,350	97.7	Ref (1)	2,695	96.8	35,433	97.9	Ref (1)	2,695	96.8	35,422	97.9	Ref (1)
	>0–5	33	1.2	283	0.8	1.59 (1.10–2.29)	38	1.4	296	0.8	1.73 (1.22–2.44)	34	1.2	321	0.9	1.42 (0.99–2.04)
	>5	91	3.3	559	1.5	2.25 (1.79–2.84)	51	1.8	463	1.3	1.48 (1.10–1.99)	55	2.0	449	1.2	1.65 (1.23–2.20)

CI: confidence interval; DDD/1 PY: defined daily dose per person-year rate; HIV: human immunodeficiency virus; OR: unadjusted odds ratio; Ref: reference value; UTI: urinary tract infection.

Figure 3

Association between different antimicrobial prescriptions in the 3 years before an HIV diagnosis and the risk of being subsequently diagnosed with HIV infection, Denmark, 1998–2016 (n = 38,976)

DDD/3 PYR	HIV n	HIV %	Controls n	Controls %	OR (95% CI)	EF
Beta-lactams						
>0 vs 0	1,533	55.1	13,315	36.8	2.13 (1.97–2.30)	1.50
>10 vs ≥ 10	1,032	37.1	7,485	20.7	2.28 (2.11–2.48)	1.79
>20 vs ≤ 20	557	20.0	3,104	8.6	2.70 (2.44–2.98)	2.33
>30 vs ≤ 30	333	12.0	1,508	4.2	3.17 (2.79-3.60)	2.86
Macrolides						
>0 (vs 0)	932	33.5	5,037	13.9	3.12 (2.87-3.40)	2.41
>10 (vs ≤ 10)	373	13.4	1,494	4.1	3.62 (3.20-4.08)	3.27
> 20 (vs ≤ 20)	170	6.1	551	1.5	4.26 (3.56-5.08)	4.07
Acyclovir						
>0 (vs 0)	353	12.7	736	2.0	7.20 (6.29–8.25)	6.35
>2 (vs ≤ 2)	279	10.0	525	1.4	7.78 (6.67–9.08)	7.14
>4 (vs ≤ 4)	255	9.2	444	1.2	8.27 (7.03–9.72)	7.67
Azoles						
>0 (vs 0)	310	11.1	1,448	4.0	3.25 (2.84–3.72)	2.78
>3 (vs ≤ 3)	190	6.8	574	1.6	4.65 (3.92–5.52)	4.25
>6 (vs ≤ 6)	160	5.8	440	1.2	5.01 (4.16–6.04)	4.83
Quinolones						
>0 (vs 0)	252	9.1	869	2.4	4.14 (3.52–4.81)	3.79
>7 (vs ≤ 7)	110	4.0	403	1.1	3.72 (3.00–4.63)	3.64
>14 (vs ≤ 14)	38	1.4	106	0.3	4.79 (3.29–6.97)	4.67
Nystatin						
>0 (vs 0)	122	4.4	164	0.5	10.55 (8.27–13.46)	8.80
>4 (vs ≤ 4)	67	2.4	72	0.2	12.53 (8.94–17.57)	12.0
>8 (vs ≤ 8)	33	1.2	25	0.1	17.16 (10.21-28.85)	12.0
Doxycycline						
>0 (vs 0)	132	4.7	386	1.1	4.61 (3.77–5.65)	4.27
>20 (vs ≤ 20)	56	2.0	95	0.3	7.87 (5.63–10.99)	6.67
>40 (vs ≤ 40)	28	1.0	45	0.1	8.09 (5.05–12.97)	10.0
Drugs for urinary tract infection						
>0 (vs 0)	230	8.3	1,834	5.1	1.80 (1.54–2.09)	1.63
> 5 (vs ≤ 5)	165	5.9	1,190	3.3	1.95 (1.64–2.33)	1.79
>10 (vs ≤ 10)	94	3.4	635	1.8	2.02 (1.61–2.53)	1.89



CI: confidence interval; DDD/3PYR: defined daily dose per person in the 3-year study period rate; EF: effectiveness factor; HIV: human immunodeficiency virus; OR: odds ratio.

Percentages refer to the totals of all cases/all controls in this study.

Results

Baseline characteristics

We identified 2,784 cases and 36,192 controls that fulfilled the inclusion criteria in the period from 1998 to 2016. Eighty per cent of cases and controls were male and the median age at diagnosis was 39 years (interquartile range (IQR): 32–48). Sexual transmission was the main infection route among cases, heterosexual for women (80%) and homosexual for men (61%). The majority of cases and controls were of Danish origin (male cases: 81%, male controls 86%, female controls 85%), except female cases of whom only 49% were of Danish origin. Non-Danish origin, older age and heterosexual transmission route were more common among those presenting with LHIV and VLHIV compared with EHIV. Although a slight decrease in the fraction of VLHIV was observed in recent calendar years, the fraction of LHIV and VLHIV remained high during the period 2010 to 2016 (16.9% and 30.7%, respectively). Table 1 shows additional baseline characteristics of the study population. During the 3 years before diagnosis, 72.4% of cases and 46.3% of controls had at least one prescription with any of the antimicrobial drugs included in the analysis ($p<0.001$) (Table 1). Beta-lactams and macrolides were the two most frequently prescribed antimicrobial drug classes for both cases and controls in the study period. For all antimicrobial drug classes, we found a substantially higher prescription rate in cases than controls. Despite the overall higher prescription for women compared with men, this trend was independent of sex (Figure 1).

Analysis by antimicrobial drug prescription levels

For all analysed antimicrobial drugs, we found a significantly higher fraction of cases than controls with a high drug prescription based on the predefined DDD levels

per PY. While drug prescription in HIV cases increased substantially the closer the date was to the HIV diagnosis, it remained stable among controls throughout the 3 years (Figure 2 and Table 2). This difference was statistically significant for beta-lactams, macrolides, quinolones, acyclovir, azoles and nystatin ($p<0.01$, Cochran-Armitage trend test).

We found a statistically significant association between the level of prescription of any of the analysed antimicrobial drugs and the risk of subsequent HIV diagnosis, which increased with a higher level of prescription and a shorter time to HIV diagnosis (Table 2).

In order to use estimates that could better translate into clinical practice, we analysed the exposure (i.e. consumption level) during the entire 3-year period as a binary exposure. The different DDD/PY cut-offs were based on the average estimated number of DDD for one or more treatments for the most commonly treated infections; the following results therefore illustrate the risk of a subsequent HIV diagnosis that was associated with the redemption of at least one treatment as compared with no treatment: beta-lactams (OR = 2.13; 95% CI: 1.97–2.30), macrolides (OR = 3.12; 95% CI: 2.87–3.40), acyclovir (OR = 7.20; 95% CI: 6.29–8.25), azoles (OR = 3.25; 95% CI: 2.84–3.72), quinolones (OR = 4.14; 95% CI: 3.52–4.81), nystatin (OR = 10.55; 95% CI: 8.27–13.46), doxycycline (OR = 4.61; 95% CI: 3.77–5.65) and UTI drugs (OR = 1.80; 95% CI: 1.54–2.09) (Figure 3). We found an overall strong and linear association between the magnitude of drug prescription and the risk of subsequent HIV diagnosis for all antimicrobial drugs with the exception of antibiotics used for UTI. For UTI drugs, even a higher cut-off (>10 vs≤10 DDD) had a low OR (2.02; 95% CI: 1.61–2.53). The analysis of the effectiveness factor showed a statistically significant association between targeted screening and subsequent HIV diagnosis, confirming how effective the different interventions would be compared with testing at random the whole population. The same analysis including only the second and third year before the HIV diagnosis is shown in Supplementary Table S2.

Sensitivity analyses

We performed several sensitivity analyses, i.e. stratifying results according to sex, age, mode of infection and HIV subgroups, for the data with statistically significant interactions and/or clinical interest.

Firstly, we observed a similar association for men and women, although for most of the drugs, the association was significantly higher for men. However, it needs to be noted that the number of women in this study was small (Figure 4 and Supplementary Table S3).
Secondly, a similar association was found for beta-lactams, macrolides, nystatin, doxycycline and UTI drugs irrespective of age and sex, when stratifying for both these variables. In contrast, in association with prescription of acyclovir, a much stronger association was found for middle-aged men (40–59 years;>2 DDD/3 PY: OR: 13.22; 95% CI: 10.20–17.13). Regarding the prescription of azoles, the association increased with increasing age for both men and women. For the prescription of quinolones, the association was strongest in younger men (Supplementary Tables S4 and S5).

Thirdly, a stronger association between the prescription of antimicrobials and subsequent HIV diagnosis was observed for men who have sex with men (MSM) than for heterosexuals. This association was most pronounced for macrolides, quinolones and doxycycline (Supplementary Tables S6 and S7).

Finally, a strong association was observed for all HIV subgroups. Nevertheless, for some antimicrobial drugs (macrolides, acyclovir and azoles) a more pronounced association was observed for cases presenting with a late diagnosis (LHIV and VLHIV) (Supplementary Table S8).

Discussion

In this nationwide, population-based nested case–control study, including 2,784 cases and 36,192 controls, we identified significantly higher levels of antimicrobial drug prescription for cases than controls in the 3 years preceding a new HIV diagnosis.

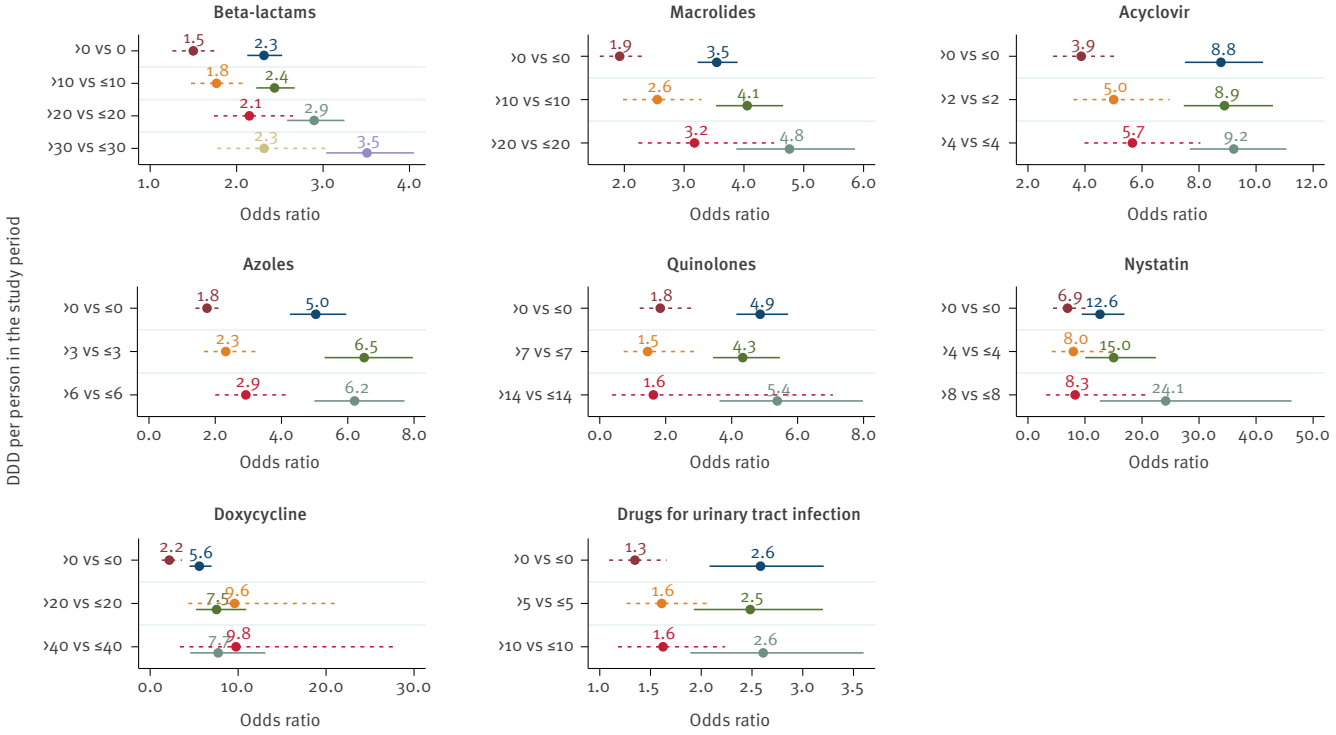
For all antimicrobial drug classes analysed, we found a statistically significant association between drug prescription and the risk of a subsequent HIV diagnosis. This association paralleled the level of prescription (i.e. increase in DDD/PY) and the proximity to the HIV diagnosis. The association was independent of age or Danish origin (data not shown), but more pronounced among men and those presenting with late-stage HIV infection. Overall, the prescription of antimicrobial drugs during the 3-year period was high for both cases and controls (72.4% of cases vs 46.3% of controls), and beta-lactams and macrolides were the most frequently used antimicrobial classes.

In a recent study [25], we established that HIV-infected individuals had frequent contacts with PHC in the years preceding the HIV diagnosis. Identifying increased prescription of one of these antimicrobial drugs should raise awareness among primary care physicians of a potential occult HIV infection. Older age and heterosexual HIV-transmission were associated with late HIV diagnosis, probably because the clinical suspicion of occult HIV infection in these situations is lower. Proactive HIV screening based on a surrogate marker such as antibiotic prescription could therefore be useful.

Although, we had no information on the disease indication for the prescriptions in this study, we presume that the use of antimicrobial drugs may in many cases represent a proxy for missed HIV indicator conditions such as community-acquired pneumonia (CAP), STI, herpes simplex infections or candidiasis. Among MSM, we observed a stronger association between macrolides,

Figure 4

Association between antimicrobial prescription in the 3 years before HIV diagnosis and the risk of HIV diagnosis, stratified by sex, Denmark, 1998–2016 (n = 38,976)



CI: confidence interval; DDD: defined daily dose; UTI: urinary tract infection.

Dashed lines: women, solid lines: men.

quinolones and doxycycline use and subsequent HIV diagnosis compared with other antimicrobials, and in the case of quinolones, particularly among younger MSM. Although the controls were not matched for sexual preference, this could indicate that the consumption in these cases was driven by STI, which in itself should prompt an HIV test. In addition, we assume that acyclovir was used for herpes simplex and zoster virus infections and azoles mainly for *Candida* infections and these antimicrobials could therefore serve as proxies for indicator conditions. Different clinical indications are plausible for beta-lactam and macrolides use; however, we suspect that a large proportion may have been given for CAP.

Our study provides additional evidence concerning missed opportunities for earlier HIV diagnosis, regarding both the identification of indicator conditions and the identification of behavioural aspects and risk factors for HIV infection. These findings support that a new targeted strategy is needed to find people with undiagnosed HIV infection in the general population in order to improve timely diagnosis and avoid new onward transmissions.

A recent analysis in Denmark has shown that a large percentage of people newly diagnosed with HIV has visited PHC or even hospitals 2 years before the diagnosis without being tested for HIV, although they presented with some clear indicator conditions [25,30]. Therefore, the strategy suggested in our analysis should be complementary to the mandatory HIV testing in people with indicator conditions.

The Centers for Disease Control and Prevention (CDC) in the US recommend universal HIV screening at least once during adulthood when in contact with any healthcare setting. However, this practice has so far not been widely implemented [31-33]. Furthermore, the individual HIV risk may vary during the lifetime if an individual develops new risk practices and one random HIV test may not capture the patient when at risk. Our data indicate that prescription of some antimicrobial drugs, and in particular repeated use over a short time interval, could be considered a marker of increased risk of occult HIV infection and act as a reminder in both primary and secondary healthcare to consider HIV testing; this would make the risk assessment a more dynamic process throughout the lifespan of sexually active adults.

In most European countries with a low HIV prevalence, targeted HIV testing is recommended based on identifying indicator conditions and risk groups. Nevertheless, many missed opportunities for HIV testing occur in these situations despite the existing recommendation, as highlighted in previous studies [21,22,34]. Based on our results, it seems reasonable to perform an HIV test after prescription of acyclovir, azoles, nystatin, doxycline, quinolones and macrolides. Even for women, whose risk was lower, the results were still statistically significant, although it has to be noted that the number of women in this study was small. Furthermore, recurrent beta-lactam use, where we suggest a threshold above two prescriptions in a 1–2-year period, may also be used as an indicator to perform an HIV test. The antimicrobial consumption in these situations was associated with a high risk of HIV with an OR›2, both in the analysis of the cumulative data for all the 3 years before HIV diagnosis and in the analysis including only the second and third year before diagnosis. Analysis of the effectiveness factor of these targeted prescriptions confirmed how effective the different interventions would be compared with testing at random. Given an HIV prevalence of 0.1% in the general Danish population, HIV prevalence in these subgroups could be estimated at above 0.2% (above 0.4% in the case of quinolone, doxycycline, acyclovir and nystatin consumption), which is regarded as a cost-effective strategy [13-15]. The prescription of these antimicrobials is an easily recognisable parameter, especially when using electronic health records. This might help the physician identify individuals at risk, and automatic reminders could easily be introduced into the system. However, in countries without electronic health records, these data may not be so easily available. Further studies are needed to confirm if this approach is cost-effective.

The main strengths of our study include its design with nesting in a well-established nationwide population-based HIV cohort and access to a well-matched control group from the population. We had full access to Danish registries of high quality, allowing us to look 3 years back in time from the established HIV diagnosis. With the DNPR, we had access to valid, nationwide, individual-level data on all dispensed antimicrobial prescriptions since 1995. Redemption data have proven to be a good proxy for consumption as primary non-adherence to antimicrobial drugs in Denmark is rare (6.5%) and antimicrobial drugs are available only on prescription from Danish physicians [35]. Furthermore, the DNPR includes information on redeemed rather than on issued prescriptions, which constitutes a more accurate surrogate measure for actual antimicrobial consumption, as around 10% of issued prescriptions in Denmark are not subsequently redeemed in the pharmacies [29]. Of note, our study includes vulnerable populations such as people who inject drugs and migrants. We show that even though these populations probably have risk factors that should have triggered an HIV test, they do attend primary healthcare and they could be captured by their antimicrobial consumption. To the best of our knowledge, this is the first study to analyse this.

Our study has some limitations. Some antimicrobial drugs (i.e. beta-lactams, macrolides, quinolones and doxycycline) may have several indications (e.g. CAP, skin infections, UTI, STI). Data on the disease indication for the drug prescriptions or presence of indicator conditions is not available in the DNPR. As a result, the DDD cut-offs for each antimicrobial class, were chosen based on the average estimated number of DDDs for one treatment for the most common conditions, may not be entirely correct. However, the same sources of data were used for both cases and controls, minimising differential misclassification. Unfortunately, we had no information regarding sexual orientation among controls and hence no knowledge of the percentage of MSM among controls. Another potential shortcoming is that we included only individuals living in Denmark 3 years or more before HIV diagnosis in the matched case. Excluding individuals who emigrated or immigrated in this period should, however, not affect our results, as we did not observe significant differences in antimicrobial consumption between non-Danish and Danish cases.

Conclusion

This case–control study nested in the nationwide DHCS identified a significantly higher consumption of many antimicrobial drugs along the 3 years preceding a new HIV diagnosis. The antimicrobial drug consumption was associated with a subsequent risk of HIV diagnosis. In many cases, the consumption may represent a proxy for an HIV indicator condition and thus would clearly identify a missed opportunity for timely diagnosis of HIV infection and ART initiation. We suggest that antimicrobial drug prescription, a parameter easily monitored in electronic medical records, could be considered as part of the individual risk assessment and trigger proactive HIV testing in PHC. Further studies are needed to confirm the relevance and cost-benefit of such an approach.

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We hereby declare that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as originally planned have been explained.

Conflict of interest

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: no support from any organisation for the submitted work, no financial relationships with any organisations that might have an interest in the submitted work in the previous three years and no other relationships or activities that could appear to have influenced the submitted work.

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Authors' contributions

RMI conducted the research and analysed the data. RMI wrote the first draft of the manuscript and Court Pedersen, Josep M Llibre, Jens Søndergaard, Frederik Veitland Ilkjær, Janne Jensen, Niels Obel, Isik Somuncu Johansen and Line Dahlerup Rasmussen contributed to the interpretation of the results and revision of the manuscript, and gave their final approval to the manuscript.

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Determinants of long-term survival in late HIV presenters: The prospective PISCIS cohort study

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Summary

Background Late HIV diagnosis (i.e CD4≤350 cells/μL) is associated with poorer outcomes. However, determinants of long-term mortality and factors influencing immune recovery within the first years after antiretroviral treatment (ART) initiation are poorly defined.

Methods From PISCIS cohort, we included all HIV-positive adults, two-year survivors after initiating ART between 2005–2019. The primary outcome was all-cause mortality according to the two-year CD4 count. We used Poisson regression. The secondary outcome was incomplete immune recovery (i.e., two-year CD4<500 cells/μL). We used logistic regression and propensity score matching.

Findings We included 2,719 participants (16593·1 person-years): 1441 (53%) late presenters (LP) and 1278 non-LP (1145 non-LP with two-year CD4 count >500 cells/μL, reference population). Overall, 113 patients (4·2%) died. Mortality was higher among LP with two-year CD4 count 200–500 cells/μL (aMRR 1·95[95%CI:1·06-3·61]) or <200 cells/μL (aMRR 4·59[2·25-9·37]).

Conversely, no differences were observed in participants with two-year CD4 counts >500 cells/μL, regardless of being initially LP or non-LP (aMRR 1·05[0·50-2·21]). Mortality rates within each two-year CD4 strata were not affected by the initial CD4 count at ART initiation (test-interaction, p = 0·48). The stronger factor influencing immune recovery was the CD4 count at ART initiation. First-line integrase-inhibitor-(INSTI)-based regimens were associated with reduced mortality compared to other regimens (aMRR 0·54[0·31-0·93]) and reduced risk of incomplete immune recovery in LP (aOR 0·70[0·52-0·95]).

Interpretation Two-year immune recovery is a good early predictor of long-term mortality in LP after surviving the first high-risk 2 years. Nearly half experienced a favorable immune recovery with a life expectancy similar to non-LP. INSTI-based regimens were associated with higher rates of successful immune recovery and better survival compared to non-INSTI regimens.

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Keywords: HIV; Late presenters; Delayed HIV diagnosis; Immune recovery; Immune response; Integrase inhibitors; Mortality

Research in context

Evidence before this study

Three cohort studies and two randomized controlled trials (RCT) have indicated that integrase strand transfer inhibitors (INSTI)-based regimens might offer a benefit in immune recovery compared to other ART regimens. However, these cohort studies included both late and non-late presenters, and the last is under-represented in RCT. Knowledge on determinants of long-term mortality and immune recovery in late presenters is scant.

Added value of this study

Our results provide new evidence that ART initiation with an INSTI-based regimen is associated with a more favorable immune response in late presenters and with improved long-term survival especially compared to protease inhibitors.

Besides, PLWH surviving the first two years after ART initiation and reaching a CD4 cell count threshold above 500 cells/μL achieved similar survival rates to non-late presenters, irrespective of their baseline CD4 count at ART initiation. Thus, ART-associated immune recovery at two years was a good early predictor of long-term mortality.

Implications of all the available evidence

The long-term survival prognosis for late presenters can be assessed upon immune recovery seen two years after ART initiation. Those achieving an early favorable response improve their life expectancy to the same level as non-late presenters, thus narrowing the gap between late and non-late presenters. INSTI-based ART is associated with a more favorable immune response and significantly improved survival, supporting the growing body of evidence in clinical trials. Based on all knowledge available, health care programs should consider using two-year CD4 cell counts after ART initiation as an early prognostic factor for long-term survival in late presenters. Also, INSTI-based regimens should be considered the preferred treatment choice, particularly in late HIV presenters.

Introduction

HIV-associated morbidity and mortality have decreased dramatically since the introduction of antiretroviral therapy (ART) in 1996. Today, people living with HIV (PLWH) timely diagnosed and starting treatment with CD4 counts ≥350 cells/μL have life expectancies close to the general population.[1–8] Still, despite the enormous advances achieved in the field, approximately half of PLWH in Western countries are diagnosed at a late disease stage, defined as CD4 cell count below 350 cells/μL or presentation with an AIDS-defining event, regardless of the CD4 cell count.[9]

This late HIV diagnosis significantly reduces life expectancy while increasing the frequency of HIV-related comorbidities,[10–14] it is associated with higher health care costs,[15] and onward transmission in the population.[16,17] Furthermore, 10–29% of PLWH presenting late to care fail to achieve a satisfactory immune recovery despite virologically effective ART. These patients, categorized as "immunological non-responders", have higher mortality and a higher risk for AIDS- and non-AIDS-related morbidity.[12,18,19]

Various observational studies of late presenters (LP) have reported high mortality rates within the first year after ART initiation, mainly due to advanced AIDS-defining conditions present at that time, which tends to decrease after this period.[20,21] Thus, CD4 counts at ART initiation, might not precisely predict long-term survival in these patients as the estimation might be distorted by this initial high-mortality period.

This study explores the performance of an early predictor for long-term survival after ART initiation, skipping this initial high-risk time. Furthermore, the influence of the first-line ART on the clinical trajectories of LP is poorly understood. Various studies have described that first-line ART with integrase strand-transfer inhibitor (INSTI)-based regimens could be associated with an improved immune response with greater CD4 gain.[22–25] However, how these first-line regimens influence adequate immune recovery and long-term survival in LP has not been assessed.

In this prospective multicentre, ongoing cohort of PLWH in our area, we aimed to investigate whether CD4 cell counts 2 years after ART initiation was a good early predictor of long-term survival in LP compared with a non-LP group with an optimal two-year immune response and explored determinants of survival in these patients, including the potential impact of INSTI as first-line regimen. Furthermore, we assessed the predictors of adequate immune recovery within this period.

Methods

Study design and data sources

This is a multicentre, prospective, population-based longitudinal analysis conducted in Catalonia, an autonomous region located in north-easter Spain. On January 1, 2021, Catalonia had a population of 7·7 million citizens and an estimated HIV prevalence among the adult population of 0·4%.[26] The Catalan healthcare system provides universal, tax-funded healthcare to all citizens. ART is provided free-of-charge.

Data were retrieved from the PISCIS Cohort project (Catalonian and Balearic Islands HIV cohort) and PADRIS (Public Data Analysis for Health Research and Innovation Program). For this study, only data from the Catalan PISCIS cohort were used. Briefly, PISCIS is an ongoing, prospective, multicentre, population-based cohort ongoing from 1998 that includes all PLWH aged ≥16 years followed in one of the 16 collaborating hospitals from Catalonia, representing 84% of all diagnosed PLWH.[27] Data are updated yearly and include demographics, date of HIV diagnosis, AIDS-defining events, ART, and measurement of CD4 cell count and plasma HIV-RNA over time. PADRIS is a central research-oriented data repository which gathers, and cross matches real-world health data generated by the public health system of Catalonia (SISCAT), provided by the Catalan Agency for Health Quality and Evaluation (AQuAS). The database includes comorbidity data from hospital discharge diagnoses and primary health care from 2005 according to the International Classification of Disease 10th revision (ICD-10).[28] Mortality data was obtained cross matching the information with PISCIS and PADRIS databases and the national mortality registry.

Ethics approval

The PISCIS cohort has been approved by the ethics committee of the coordinating centre, and patient data extraction is allowed by the 203/2015 Decree from the Catalan Health Department. PISCIS data is owned by each individual patient, and data is eliminated if requested by the hospital or by a patient. All data is pseudo-anonymized before arriving at the coordinating center, and confidentiality is guaranteed in accordance with the provisions of the Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016 on the protection of natural persons regarding the processing of personal data and on the free movement of such data and the new national Organic Law 3/2018 of December 5 on Protection of Personal Data and Digital Rights.

Study population

We screened the *PISCIS* cohort for treatment naïve PLWH ≥18 years, who initiated ART between January 1, 2005, and June 30, 2019. Of these, we included all 2-year survivors with active follow-up and available CD4 cell count at both timepoints, ART initiation and two years after treatment start.

Patients were classified according to the CD4 cell count at ART initiation into late HIV presenters (i.e., CD4 count ≤350 cells/μL) and non-LP (i.e., CD4 count >350 cells/μL). LP were further stratified according to their CD4 count at two different time points: at ART initiation (<100 cells/μL, 100−199 cells/μL, and 200−350 cells/μL) and at 2 years after ART initiation (<200 cells/μL, 200−500 cells/μL, and >500 cells/μL). Non-LP were also stratified according to their immune recovery two years after ART initiation (≤500 cells/μL and >500 cells/μL).

Outcomes

The primary outcome was time to all-cause mortality, setting the baseline time at two years after ART initiation. We chose a two-year time-point expecting to get rid of the bias introduced in the analysis by initial mortality related to baseline conditions in this cohort of PLWH with advanced HIV infection. The outcome was assessed for the different groups of LP based on the CD4 count immune recovery two years after ART initiation; non-LP with CD4 >500 cells/μL two years after ART initiation was used as the reference population.

The secondary outcome was incomplete immune recovery two years after ART initiation, defined as CD4 cell count ≤500 cells/μL. This was assessed in a nested case-control study design.

Statistical analysis

Continuous variables were described as the median and the interquartile range (IQR), whereas categorical variables were presented as the frequency and percentage over available data. Missing data were not imputed because the essential variables for the analysis (i.e., CD4 count available at ART initiation and 2 years after) were among the selection criteria for the study population. We used the chi-squared test, the t-test, and the Mann−Whitney U test to compare variables between late and non-LP. For polycothomous categorical variables with a statistically significant chi-square test of homogeneity, we further provided a significance test of the difference between proportions across categories of the variable between LP and non-LP.

The survival analysis considered all-cause death, loss-to-follow-up, or end of the observation period, whichever occurred first, between the 2-year time point after the date of ART initiation to June 30, 2021. The cumulative survival of PLWH in the different groups of two-year CD4 cell count was compared using the Log-rank test. We used a Poisson regression analysis to control for the following potential confounders and to explore for prognostic factors for survival: age, gender,

HIV risk group (men who have sex with men (MSM), heterosexual men, women, history of injection drug use (IDU), and other/unknown), region of birth (Spain, Europe excluding Spain, Africa, America, Asia), two-year CD4 cell count recovery (<200, 200–500, and >500 cells/μL), two-year HIV viral load (>200 vs ≤ 200 copies/mL), modified Charlson comorbidity index (CCI) at two years after ART initiation, prior AIDS-defining disease at two years after ART initiation, time-updated calendar period (2005–2009, 2010–2014, 2015–2019, according to pre-INSTI-available period, INSTI-available period, and change in guidelines to immediate ART initiation,[29] respectively), INSTI-based regimen as ART regimen initiated in the first two years, and socioeconomic level based on AQuAS composite socioeconomic indicator used to classify the primary health areas (no economic deprivation, mild economic deprivation, and moderate/severe economic deprivation).[30] We provide mortality rate (MR) and MR ratios (MRR) and a 95% confidence interval (CI).

CD4 cell counts at ART initiation and two years after were defined as the closest value to the index date, with a time window of 12 months before and 3 months after ART initiation and 3 months before and 12 months after for the time point at two years after ART initiation. The CCI was modified according to the information available on comorbidities from the PADRIS and excluding AIDS defining events, which was assessed separately (Table S1, Supplementary data).

We conducted a sensitivity analysis using propensity score matching (1:1 nearest-neighbor matching with caliper 0·2 on each sample) for gender, age, CD4 cell count, modified CCI, and AIDS-defining event, all at ART initiation, to assess the impact of initiation of INSTI as first- line regimen on long-term mortality. The application of these probabilities to the study population created a subpopulation in which these potential confounders were more equally distributed across both groups. The same analysis was performed for the whole database population of individuals who initiated ART, regardless of survival in the first two years or availability of CD4 counts at 2 years after ART.

To identify risk factors in the group of LP associated with incomplete immune recovery 2 years after ART initiation (defined as CD4 cell count ≤500 cells/μL), we performed a logistic regression analysis with propensity score matching on age (continuous), gender, CD4 cell count at ART initiation (<100 cells/μL, 100–199 cells/μL, and 200–350 cells/μL)) and modified CCI. We assessed the following covariates at ART initiation: age, gender, CD4 cell count, INSTI as first ART regimen, modified CCI, AIDS-defining event within 90 days after ART initiation. We provide the odds ratio (OR) and a 95% CI.

All analyses were conducted using the STATA software (v.16; Stata Corp, College Station, TX, USA).

Role of funding

This work was supported by scholarships from the University of Southern Denmark, the Danish AIDS foundation, and Public Regional Funds from the Region of Southern Denmark. The study was investigator-driven and thus independent of any pharmaceutical company. The funding sources were not involved in study design, data collection, analyses, report writing, or decision to submit the paper.

Results

Study participants

From the PISCIS cohort, we identified 4036 PLWH starting ART between 2005 and 2019. Of them, 2719 (67·4%) were eligible for the analysis, giving rise to 16593·1 person-years of follow-up (median follow-up 5·8 years [IQR 3·2-8.9]) (Figure 1). Table S2 summarizes and compares the characteristics of included and excluded participants. Of the analysis population, 1441 (53%) were LP and 1,278 were non-LP.

Table 1 summarizes the demographic and clinical characteristics of individuals included in the analysis population, according to the defined CD4 count groups. Compared with non-LP, LP were more frequently older, heterosexual men, women, individuals with a history of IDU, of African origin, with lower educational level and higher economic deprivation. The percentage of late HIV presentation decreased in time from 77% in 2005–2009 to 41% in 2015–2020 (p<0·001). Among LP, 27% initiated ART with a CD4 cell count <100 cells/μL, 21·8% with 100–199 cells/μL and 51·6% with 200–350 cells/μL, and 13·2% had an AIDS-defining event at baseline. Six hundred thirty-seven (44·2%) of late presenters achieved CD4 cell counts >500 cells/μL two years after ART start. The comparator population of non-LP with two-year CD4 cell count >500 cells/μL (n =1145) had a median two-year CD4 count of 837 (IQR: 697-1030).

Mortality risk

Overall, 113 (4·2%) died during the study period (crude all-cause MR 6·8/1000 person-years; 95%CI: 5·7-8·2). Compared with non-LP with two-year CD4 count >500 cells/μL (reference population), LP with two-year CD4 count ≤500 cells/μL had higher mortality (adjusted MRR 1·95 [1·06-3·61] and 4·59 [2·25-9·37] in the groups of CD4 200–500 cells/μL and <200 cells/μL, respectively). Conversely, no significant differences were observed between the adjusted MR of non-LP and LP with two-year CD4 count >500 cells/μL (1·05 [0·50-2·21]) (Table 2 and Table S3). Within the individual strata of immune-recovery (two-year CD4 cell count >500, >350–500, 200–350, <200 cells/μL), MR were not significantly different when stratifying by initial CD4 cell count at ART initiation (>350, 200–350, 100–199,

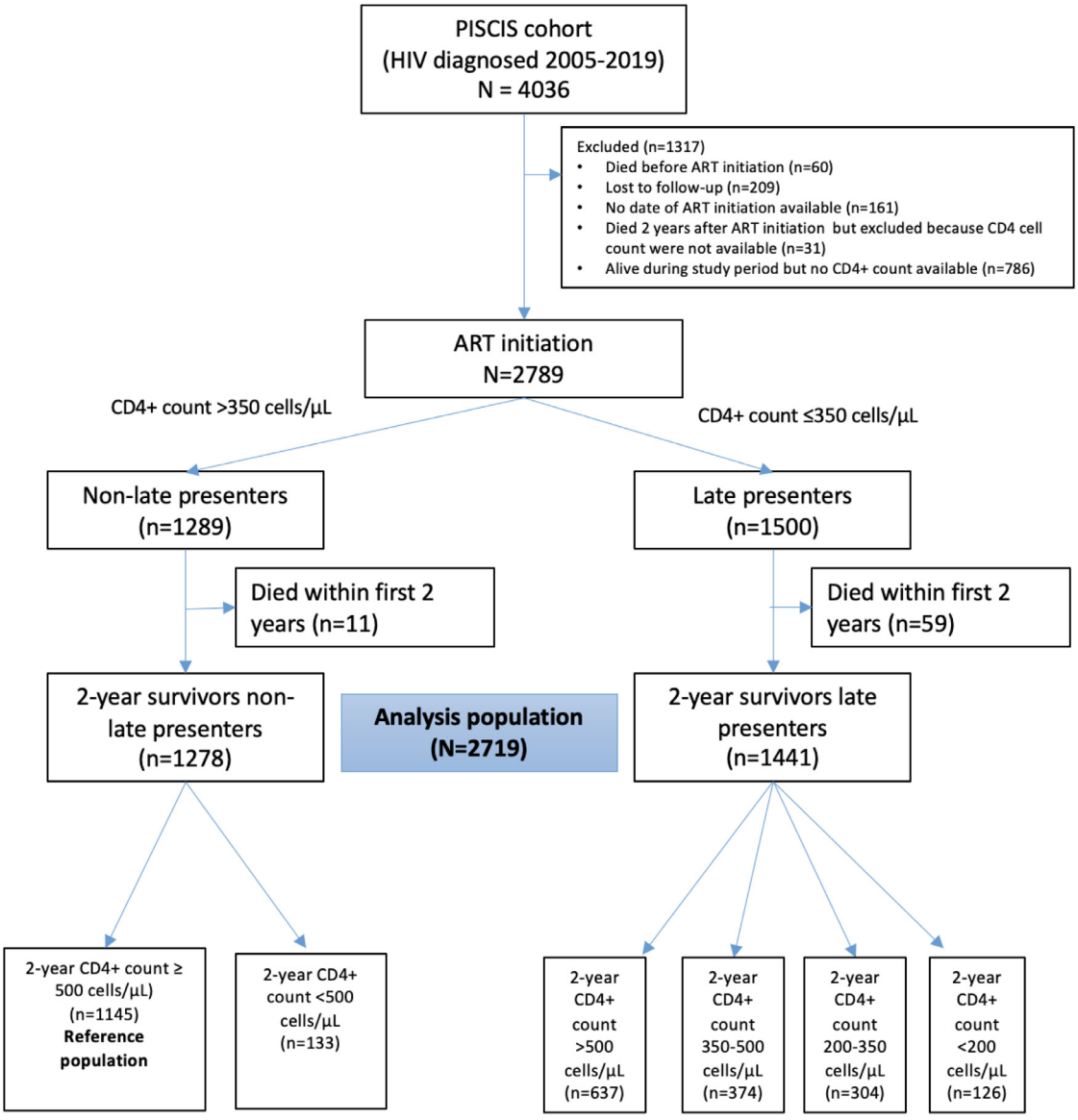


Figure 1. Flowchart of cohort construction.

<100 cells/µL) (test for interaction $p = 0{\cdot}48$) (Figure 2, Table S4). HIV viral load >200 copies/mL two years after ART initiation was also associated with increased mortality risk (1·78 [0·99-3·19]). Other factors associated with increased mortality according to the adjusted analysis included a modified CCI score at ART start ≥4 *versus* 0 and HIV risk group (i.e., heterosexual men and IDU *versus* MSM).

INSTI-based regimens as first-line ART were associated with lower mortality than non-INSTI regimens (0·54 [0·31-0·93]). There was an improved survival trend when considering LP and non-LP separately, but it was not statistically significant (0·61 [0·33-1·13] and 0·25 [0·06-1·12], respectively). However, the sensitivity analysis with propensity score matching, conducted to address the potential channeling bias, did show a significant benefit of INSTI *versus* non-INSTI regimens (0·48 [0·25-0·90]) (Table S5). We did a second sensitivity analysis including all individuals starting ART regardless of survival the first two years or availability of CD4 cell count two years after ART. In line with the previous analysis, the sensitivity analysis showed that INSTI-based first-line regimens were associated with a survival benefit when compared to protease inhibitor (PI) (0·41 [0·23-0·72]) but not versus non-nucleoside reverse transcriptase inhibitors (NNRTI (0·88 [0·48-1·61]), Table S5).

	Study population according to CD4 cell count at ART initiation			Study population according to immune recovery 2 years after ART initiation				
	Non-late presenters *n* =1278 (47·0%)	Late presenters *n* =1441 (53·0%)	*p*-value	Late presenters *n* =1441				Non-late presenters
				CD4 cell count (cells/μl)				CD4 cell count (cells/μl)
				>500 *n* =637 (44·2%)	>350-500 *n* =374 (26·0%)	≥200-350 *n* =304 (21·1%)	<200 *n* =126 (8·7%)	>500 cells/μL *n* =1145
Male, n (%)	1122 (87·8)	1202 (83·4)	0·001	549 (86·2)	314 (84·0)	238 (78·3)	101 (80·2)	1022 (89·3))
Age at ART initiation (years), median (IQR)	35 (29-42)	38 (32-45)	<0·001	36 (30-43)	39 (32-46)	41 (35-48)	40 (34-48)	35 (29-42)
Birth area, n (%)			0·015					
Spain	598 (46·8)	743 (51·6)	0·013	330 (51·8)	180 (48·1)	163 (53·6)	70 (55·6)	531 (46·4)
Europe	124 (9·7)	97 (6·3)	0·005	47 (7·4)	31 (8·3)	15 (4·9)	4 (3·2)	111 (9·7)
Africa	43 (3·4)	72 (5·0)	0·035	18 (2·8)	20 (5·4)	22 (7·2)	12 (9·5)	36 (3·1)
America	249 (19·5)	289 (20·1)	0·71	141 (22·1)	81 (21·7)	49 (16·1)	18 (14·3)	221 (19·3)
Asia	14 (1·1)	15 (1·0)	0·89	10 (1·6)	3 (0·8)	0	2 (1·6)	14 (1·2)
Unknown	250 (19·6)	225 (15·6)	0·007	91 (14·3)	59 (15·8)	55 (18·1)	20 (15·9)	232 (20·3)
HIV risk group, n (%)			<0·001					
MSM	910 (71·2)	742 (51·5)	<0·001	415 (65·2)	198 (52·9)	94 (30·9)	35 (27·8)	842 (73·5)
Heterosexual men	127 (9·9)	266 (18·5)	<0·001	85 (13·3)	65 (17·4)	85 (28·0)	31 (24·6)	109 (9·5)
Women	128 (10·0)	195 (13·5)	0·005	74 (11·6)	51 (13·6)	54 (17·8)	16 (12·7)	103 (9·0)
IDU	56 (4·4)	131 (9·1)	<0·001	26 (4·1)	38 (10·2)	36 (11·8)	31 (24·6)	44 (3·8)
Unknown	57 (4·5)	107 (7·4)	0·001	37 (5·8)	22 (5·9)	35 (11·5)	13 (10·3)	47 (4·1))
ART- regimen in the first 2 years, n (%)								
NNRTI-based regimen	570 (44·6)	723 (50·2)	0·004	338 (53·1)	197 (52·7)	131 (43·1)	57 (45·2)	508 (44·4)
PI-based regimen	350 (27·4)	629 (43·7)	<0·001	240 (37·7)	164 (43·9)	147 (48·4)	78 (61·9)	300 (26·2)
INSTI-based regimen	544 (42·6)	435 (30·2)	<0·001	198 (31·1)	109 (29·1)	83 (27·3)	45 (35·7)	508 (44·4)
Viral load at HIV diagnosis (log_{10} copies/mL), median (IQR)	4·8 (3·9-4·9)	4·9 (4·7-5·7)	<0·001	4·9 (4·7-5·7)	4·9 (4·0-5·7)	4·9 (4·7-5·7)	4·9 (4·7-5·8)	4·8 (3·9-4·9)
CD4 cell count at ART initiation (cells/ μL), n (%)								
<100 cells/μL	-	383 (26·6)		67 (17·5)	92 (24·0)	133 (34·7)	91 (23·8)	-
100-199 cells/μL	-	314 (21·8)		109 (34·7)	97 (30·9)	88 (28·0)	20 (6·4)	-
200-350 cells/μL	-	744 (51·6)		461 (62·0)	185 (24·9)	83 (11·2)	15 (2·0)	-
>350-500 cells/μL	613 (48·0)	-		-	-	-	-	509 (44·5)
>500 cells/μL	665 (52·0)	-			-	-	-	636 (55·6)
Viral load at ART initiation (log^{10} copies/mL), median (IQR)	4·4 (3·6-4·9)	4·9 (4·0-5·5)	<0·001	4·9 (4·3-5·3)	4·8 (3·9-5·4)	4·9 (3·9-5·7)	4·9 (4·1-5·7)	4·4 (3·7-4·9)
CD4 cell count 2 years after ART initiation (cells/μL), median (IQR)	802 (632-998)	469 (320-627)	<0·001	-	-	-	-	-
Detectable viral load 2 years after ART initiation (>200 copies/mL), n (%)	61 (4·8)	94 (6·5)	0·049	25 (3·9)	15 (4·0)	24 (7·9)	30 (23·8)	35 (3·1)
Unavailable viral load 2 years after ART initiation	113 (8·8)	81 (5·6)	0·001	34 (5·3)	21 (5·6)	18 (5·9)	8 (6·4)	109 (9·5)
Individuals with AIDS defining events at ART initiation[a], n (%)	20 (1·6)	190 (13·2)	<0·001	49 (7·7)	47 (12·6)	56 (18·4)	38 (30·2)	14 (1·2)
Individuals with AIDS defining events in the first 2 years beyond ART initiation, n (%)	1 (0·1)	14 (1·0)	0·002	5 (0·8)	3 (0·8)	3 (1·0)	3 (2·4)	1 (0·1)
Modified Charlson comorbidity score at ART initiation, n (%)			0·080					
0	1158 (90·6)	1262 (87·6)		583 (91·5)	328 (87·7)	256 (84·2)	95 (75·4)	1041 (90·9)
1	76 (6·0)	108 (7·5)		32 (5·0)	25 (6·7)	31 (10·2)	20 (15·9)	67 (5·9)
2-3	36 (2·8)	60 (4·2)		20 (3·1)	18 (4·8)	14 (4·6)	8 (6·4)	30 (2·6)
≥4	8 (0·6)	11 (0·8)		2 (0·3)	3 (0·8)	3 (1·0)	3 (2·4)	7 (0·6)

Table 1 *(Continued)*

	Study population according to CD4 cell count at ART initiation			Study population according to immune recovery 2 years after ART initiation				
	Non-late presenters *n* =1278 (47·0%)	Late presenters *n* =1441 (53·0%)	*p*-value	Late presenters *n* =1441				Non-late presenters
				CD4 cell count (cells/μl)				CD4 cell count (cells/μl)
				>500 *n* =637 (44·2%)	>350-500 *n* =374 (26·0%)	≥200-350 *n* =304 (21·1%)	<200 *n* =126 (8·7%)	>500 cells/μL *n* =1145
Modified Charlson comorbidity score at 2 years after ART initiation, n (%)			<0·001					
0	1094 (85·6)	1146 (79·5)	0·012	551 (86·5)	301 (80·5)	221 (72·7)	73 (57·9)	988 (86·3)
1	107 (8·4)	158 (11·0)	0·11	52 (8·2)	39 (10·4)	42 (13·8)	25 (19·8)	90 (7·9)
2-3	62 (4·9)	106 (7·4)	0·058	29 (4·6)	26 (7·0)	32 (10·5)	19 (15·1)	53 (4·6)
≥4	15 (1·2)	31 (2·2)	0·67	5 (0·8)	8 (2·1)	9 (3·0)	9 (7·1)	14 (1·2)
Calendar time of ART initiation, n (%)			<0·001					
2005-2009	180 (23·4)	588 (76·6)	<0·001	232 (36·4)	148 (39·6)	147 (48·4)	61 (48·1)	141 (12·3)
2010-2014	651 (54·5)	543 (45·5)	0·001	264 (41·4)	145 (38·8)	94 (30·9)	40 (31·8))	586(51·2)
2015-2019	447 (59·1)	306 (41·0)	<0·001	141 (22·1)	81 (21·7)	63 (20·7)	25 (19·8)	418 (36·5)
Educational level, n (%)			<0·001					
None or primary education only	226 (39·2)	350 (60·8)	<0·001	134 (28·3)	90 (33·6)	92 (45·1)	34 (46·0)	190 (21·7)
Secondary education	396 (51·0)	380 (49·0)	0·008	187 (39·5)	106 (39·6)	64 (31·4)	23 (31·1)	361 (41·2)
University	350 (55·7)	278 (44·3)	<0·001	153 (32·3)	67 (25·0)	42 (20·6)	16 (21·6)	319 (36·4)
Unknown	8 (40·0)	12 (60·0)	0·53	0	5 (1·9)	6 (2·9)	1 (1·4)	7 (0.8)
Income, n (%)			0·010					
No economic deprivation	678 (53·1)	691 (48·0)	0·008	337 (52·9)	176 (47·1)	126 (41·5)	52 (41·3)	613 (53·5)
Mild economic deprivation	215 (16·8)	309 (21·4)	0·002	129 (20·3)	71 (19·0)	77 (25·3)	32 (25·4)	184 (16·1)
Moderate/severe economic deprivation	353 (27·6)	399 (27·7)	0·97	152 (23·9)	118 (31·6)	93 (30·6)	36 (28·6)	319 (27·9)
Unknown	32 (2·5)	42 (2·9)	0·51	19 (3·0)	9 (2·4)	8 (2·6)	6 (4·8)	29 (2·5)
Death 2 year after ART initiation, n (%)	23 (1·8)	90 (6·3)	<0·001	14 (2·2)	25 (6·7)	22 (7·2)	29 (23·0)	17 (1·5)

***Table 1*: Baseline characteristics.**

ART, antiretroviral therapy; INSTI, integrase strand transfer inhibitor; IQR, interquartile range (percentiles 25th and 75th); MSM, men who have sex with men; IDU, injection drug use; NNRTI, non-nucleoside reverse transcriptase inhibitors; PI, protease inhibitor.

[a] (before or within 90 days from ART initiation).

	Deaths (n)	MR (95%CI)	MRR (95% CI)[1]	aMRR (95% CI)[2]
All individuals, N=2719	113	6·8 (5·7-8·2)		
CD4 cell count 2 years after ART initiation (cells/µL)				
Non-LP, CD4 >500	17	2·9 (1·8-4·6)	Ref (1)	Ref (1)
Non-LP, CD4 ≤ 500	6	7·3 (3·3-16·2)	2·55 (1·00-6·46)	1·97 (0·75-5·21)
LP, CD4>500	14	3·3 (2·0-5·6)	1·16 (0·57-2·35)	1·05 (0·50-2·21)
LP, CD4 200-500	47	9·8 (7·4-13·1)	3·45 (1·98-6·00)	1·95 (1·06-3·61)
LP, CD4 <200	29	36·1 (25·1-52·0)	12·66 (6·96-23·03)	4·59 (2·25-9·37)
INSTI as first line ART-regimen vs non-INSTI				
No	92	7·2 (5·9-8·9)	Ref (1)	Ref (1)
Yes	21	5·5 (3·6-8·4)	0·76 (0·47-1·21)	0·54 (0·31-0·93)
HIV viral load >200 c/ml 2 years after ART				
No	89	6·0 (4·9-7·4)	Ref (1)	Ref (1)
Yes	15	13·6 (8·2-22·6)	2·28 (1·32-3·94)	1·78 (0·99-3·19)
AIDS defining event in the first 2 years after ART				
No	85	5·7 (4·6-7·0)	Ref (1)	Ref (1)
Yes	28	16·9 (11·6-24·4)	2·96 (1·93-4·54)	1·36 (0·86-2·15)
Modified CCI 2 years after ART				
0	63	4·5 (3·5-5·8)	Ref (1)	Ref (1)
1	20	13·2 (8·5-20·4)	2·91 (1·76-4·81)	1·40 (0·80-2·45)
2-3	17	17·6 (11·0-28·4)	3·90 (2·28-6·66)	1·57 (0·86-2·86)
≥ 4	13	67·3 (39·1-115·9)	14·86 (8·18-27·00)	3·96 (1·84-8·50)
Transmission mode				
MSM	30	3·1 (2·2-4·4)	Ref (1)	Ref (1)
Heterosexual men	37	14·6 (10·6-20·2)	4·74 (2·93-7·67)	1·94 (1·13-3·34)
Women	10	4·7 (2·6-8·8)	1·53 (0·75-3·13)	0·90 (0·42-1·93)
IDU	25	19·9 (13·4-29·4)	6·46 (3·80-10·98)	2·74 (1·49-5·03)
Unknown/other	11	11·5 (6·4-20·8)	3·74 (1·87-7·45)	1·51 (0·69-3·33)
Calendar time (time-updated)				
2015-2020	79	6·6 (5·3-8·2)	Ref (1)	Ref (1)
2010-2014	28	6·8 (4·7-9·8)	1·03 (0·67-1·59)	0·62 (0·38-1·02)
2005-2009	6	14·0 (6·3-31·2)	2·13 (0·93-4·89)	1·19 (0·51-2·80)

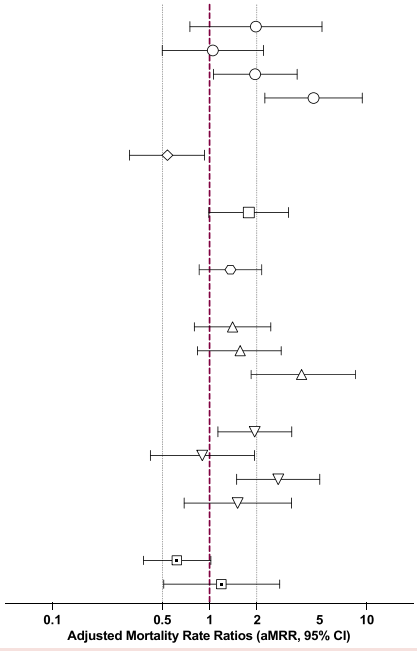


***Table 2*: Crude and adjusted mortality in treatment-naive individuals surviving the first 2 years after ART initiation.**
ART, antiretroviral therapy; INSTI, integrase strand transfer inhibitor; MSM, men who have sex with men; IDU, injection drug use.
[a] MRR, mortality rate ratio.
[b] aMRR, adjusted mortality rate ratio. The multivariate analysis was adjusted for age at baseline, transmission mode, CD4 cell count recovery at 2 years after ART initiation, HIV viral load > 200 c/ml vs ≤200 c/ml at 2 years, INSTI-based regimen as first-line regimen in the first 2 years, modified Charlson comorbidity score at baseline, history of AIDS-defining event at baseline, and calendar time (time-updated).

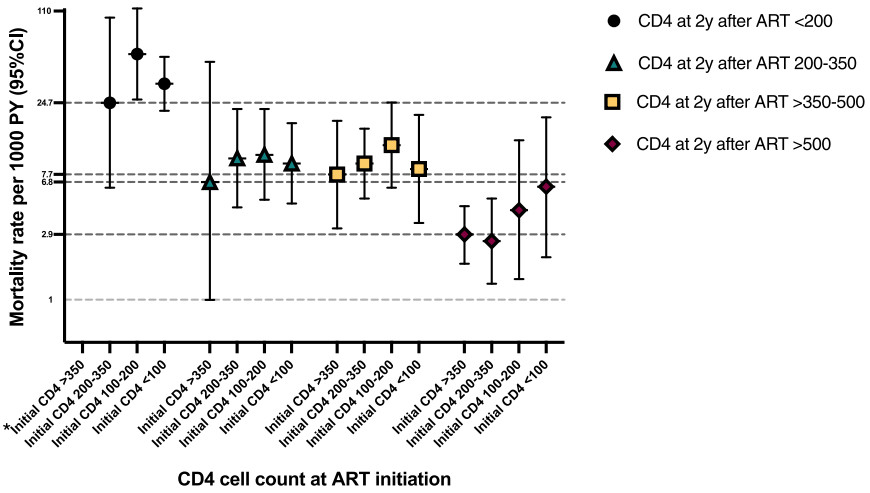
The log-rank test revealed significant differences between survival curves for all-cause mortality (Figure 3). However, while LP with CD4 ≤500 cells/µL at two years had remarkably lower survival, the curve of LP with CD4 >500 overlapped with that of non-LP. As a sensitivity analysis, we excluded patients with HIV viral load >200 copies/mL or unavailable (n =349), with similar results (Figure S1). The long-term survival analysis comparing non-LP achieving CD4 cell count >500–699 and those with ≥700 cells/µL did not reveal significant differences between groups (0·57 [0·21-1·51]) (Tables S6 and S7).

Risk factors for incomplete immune recovery

Table 3 summarizes the results of univariate and multivariate logistic regression analyses after propensity score matching assessing incomplete immune recovery (i.e., two-year CD4 counts ≤500 cells/µL) in LP. Lower initial CD4 counts at ART initiation strongly influenced the likelihood of incomplete immune recovery at two years, together with older age and modified CCI score 1. First-line INSTI-based therapy was associated with 30% lower odds of incomplete immune recovery in LP (adjusted OR 0·70 [0·52-0·95]). The distribution of baseline covariates in both groups of INSTI and non-INSTI before and after propensity score matching is presented in table S8. When including calendar time in the model, we found the same trend, but the results did not reach statistical significance (0·76 [0·52-1·13]). The same analysis including also non-LP with CD4 cell counts <500 cells/µL at ART initiation and using a threshold of two-year CD4 cell count ≥700 cells/µL for a satisfactory immune recovery, showed a similar benefit of first-line INSTI regimens also incorporating calendar time in the model (0·74 [0·57-0·97]) (Table S9).

Discussion

In this population-based prospective cohort study with 2719 PLWH initiating their ART between 2005 and 2019 and surviving the first two years, 53% of them were diagnosed at a late stage (i.e., CD4 count ≤350 cells/µL). Despite this delayed ART initiation in these patients presenting late to care, 44% achieved an optimal immune recovery with a CD4 cell count >500 cells/µL at two years. LP achieving two-year CD4 cell count >500 cells/µL had comparable long-term mortality rates to non-LP, irrespective of their initial CD4 cell count. CD4 cell count recovery two years after ART



Mortality rate per 1000 person-years (95% CI) in log2 scale.
Mortality rates upon immune recovery 2 years after initiation of antiretroviral therapy (ART) stratified by initial CD4 cell count at ART initiation (<100, 100-199, 200-350, >350 cells/μL).

No statistically significant differences in mortality rates in the different strata of two-year CD4 cell count, independently of initial CD4 cell count at ART initiation (test for interaction p=0·48).
*There were no participants with initial CD4 counts >350 cells/μL in the group of patients with two-year CD4 cell count <200 cells/μL.

Figure 2. Mortality rates upon immune recovery 2 years after initiation of antiretroviral therapy stratified by nadir CD4 cell count at ART initiation.

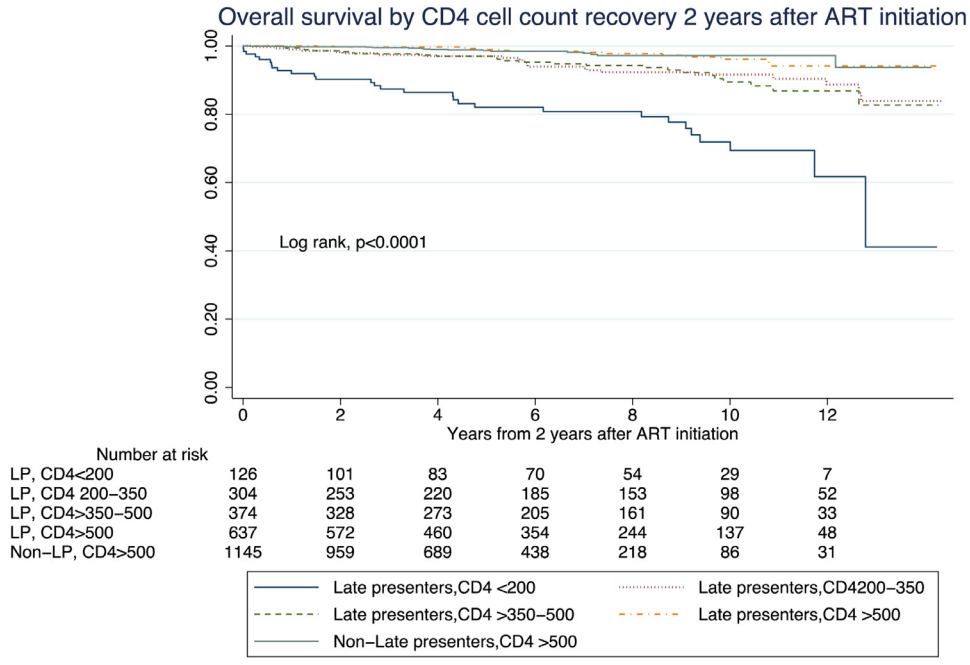


Figure 3. Kaplan-Meier curves for overall survival by immune recovery 2 years after antiretroviral therapy (ART) initiation (non-late presenter with CD4 counts >500 cells/μL versus late presenters with CD4 counts >500 cells/μL, late presenters with CD4 counts >350-500 cells/μL, late presenters with CD4 counts 200-350 cells/μL and late presenters with CD4 counts <200 cells/μL at 2 years after ART initiation.

initiation was a useful early predictor of long-term mortality. Exposure to an INSTI-based ART regimen in the first two years was associated with a significantly better two-year immune recovery and better long-term survival. A lower CD4 cell count, older age, and worse CCI score at ART initiation significantly increased the risk for incomplete two-year immune recovery.

Although LP have an overall increased mortality, earlier studies have shown that the risk of death and/or AIDS is highest in the first year after HIV diagnosis

and decreases hereafter.[20,21] There is less knowledge on the determinants of long-term survival after this initial high-risk period. Furthermore, many of the studies evaluating life expectancy in LP include patients initiating ART before the year 2012, thus exposed to older ART regimens.[6,31–35] The available evidence seems to indicate that the association between CD4 cell count at ART initiation and mortality fades over the years as patients survive. An ART-CC cohort study of PLWH initiating ART between 1996–2001, found that there were no differences in long-term survival after 5 years among those starting ART with CD4 cell count 200–350 cells/μL or >500 cells/μL.[31] Similarly, a Spanish cohort found that in five-year survivors without history of AIDS-defining events, there was no survival differences between those starting ART with CD4 count >350 cells/μL or with 200–350 cells/μL.[21] A cohort study from UK including patients from 2000–2010 showed that LP improved their life span if they achieved a good immune response and suppressed HIV-1 viral load. In fact, those achieving a good early immune response, with one-year CD4 cell count ≥350 cells/μL and virological suppression, experienced a life expectancy close to the background population.[36] However, in this study, half of the patients started ART before 2005 and no individual in the study was exposed to INSTI. Existing evidence supports that current CD4 count correlates strongly with mortality[15,33,34] and some studies have shown that PLWH with current CD4 count ≥500 cells/μL and without history of IDU have life expectancies close to the background population.[2] We investigated if CD4 cell count at 2-two years could be used as an early predictor of survival. Our results support that after this initial high-risk period of 2 years after HIV diagnosis in LP, likely associated with advanced AIDS-related medical conditions and complications present at diagnosis, two-year CD4 count recovery is a strong independent early predictor for long-term all-cause mortality, independently of the CD4 cell count at ART initiation. Actually, 44% of the two-year survivors successfully achieved a good immune recovery, with two-year CD4 cell counts >500 cells/μL. Importantly, the mortality rate in this subgroup of patients did not differ from that of non-LP with optimal immune response (i.e., CD4 cell counts > 500 cells/μL with a median 2-years CD4 837 [IQR: 697-1030]), thus narrowing the survival gap between late and non-LP. Only 8·7% of LP in our cohort remained with CD4 cell count <200 cells/μL at two years. These patients with immune discordance exhibited the highest mortality rates, approximately 5 times greater than non-LP. Of note, this group had a high percentage of detectable two-year viremia (24%), suggesting that increased mortality rates and immune discordance might be partially explained by ART non-adherence, aside from their low baseline CD4 count. Patients achieving CD4 cell counts between 200 and 500 cells/μL at two years still had an approximately double mortality risk compared to non-LP.

Our findings in immunological non-responders are in accordance with results from the COHERE cohort,[37] which also found increased mortality in patients with CD4 ≤200 cells/μL compared to those above this threshold after three years of successful suppressive ART (adjusted hazard ratio 2·60 [95%CI: 1·86–3·61]). Similarly, the Italian MASTER and the Dutch ATHENA cohorts reported higher mortality risks and non-AIDS-defining events in a subpopulation of immunological non-responders.[12,18] Participants in these studies initiated their ART in earlier calendar years (1996 to 2011 and 1998-2009, respectively), with older ART regimens. A recent published study found that in an overall cohort of PLWH initiating ART, five-year survivors with five-year CD4 <200 cells/μL remained with the worse prognosis.[32]

Several studies have described a better CD4 count recovery in patients exposed to INSTI-based regimens, mainly versus efavirenz-based regimens.[22–25,38–40] Initiation of ART with a INSTI-based regimen was associated with a higher CD4 count increase at three years compared to efavirenz in the SINGLE and STARTMRK phase 3 randomized studies.[23,38,39] However, no differences were observed between Dolutegravir and Darunavir in the FLAMINGO and SYMTRI randomized trials.[41,42] A recent collaborative cohort showed a more favourable immunologic response, defined as 25% increase in CD4 count or as reaching ≥750 cells/μL at one year, with INSTI- vs PI- or NNRTI-based regimens.[40] This study, however, included both treatment-naïve and treatment-experienced individuals, did not assess the effect of INSTI on long-term mortality nor the specific effect on immune responses in the subpopulation of LP, and residual confounding could not be fully excluded. In our analysis, incomplete immune recovery was defined as CD4 cell count <500 cells/μL two years after ART, given that this threshold was associated with increased mortality compared with non-LP in our analysis. Choosing a higher threshold of >700 cells/μL lead to similar results both in successful immune recovery and mortality. We found 30% lower odds of incomplete immune recovery in individuals starting INSTI-based regimens, both in LP and in individuals with an initial CD4 count ≤500 cells/μL, and approximately 46% decrease in long-term mortality in the whole study population.

To our knowledge, this is the first study to identify the exposure to INSTI-based regimen first-line ART with a significantly higher probability of reaching a successful immune recovery with a significant impact on long-term mortality in LP. Nonetheless, the strongest independent risk factor for incomplete immune recovery was lower CD4 cell count at ART initiation, reinforcing the importance of timely HIV diagnosis.

	Incomplete immune recovery (n, %)	OR (95% CI)	*aOR (95% CI)*[1]
Individuals with CD4 cell count at ART initiation ≤350 cells/μL (n=870)	524 (60·2)		
Age at ART initiation (years)			
<30	71 (41·0)	Ref (1)	Ref (1)
30-39	161 (55·0)	1·75 (1·20-2·56)	1·40 (0·92-2·12)
40-49	164 (60·7)	2·22 (1·51-3·28)	1·77 (1·15-2·71)
≥50	101 (75·4)	4·40 (2·68-7·22)	2·73 (1·57-4·74)
CD4 cell count nadir at ART initiation (cells/ μL)			
CD4 >200-350	168 (38·1)	Ref (1)	Ref (1)
CD4 100-199	119 (67·2)	3·33 (2·32-4·82)	3·22 (2·19-4·72)
CD4 <100	210 (83·3)	8·13 (5·54-11·92)	6·92 (4·60-10·41)
INSTI as first line ART regimen			
No	287 (66·0)	Ref (1)	Ref (1)
Yes	237 (54·5)	0·81 (0·62-1·05)	0·70 (0·52-0·95)
AIDS-defining event at ART initiation			
No	405 (53·8)	Ref (1)	Ref (1)
Yes	92 (78·6)	3·16 (1·99-5·03)	1·31 (0·78-2·20)
Gender			
Men	419 (55·8)	Ref (1)	Ref (1)
Women	78 (65·6)	1·51 (1·01-2·26)	0·84 (0·53-1·33)
Modified Charlson comorbidity score at ART initiation			
0	386 (53·7)	Ref (1)	Ref (1)
1	62 (75·6)	2·67 (1·58-4·52)	2·18 (1·22-3·89)
2-3	40 (69·0)	1·92 (1·08-3·41)	1·19 (0·63-2·25)
≥4	9 (81·8)	3·88 (0·83-18·09)	1·85 (0·36-9·54)

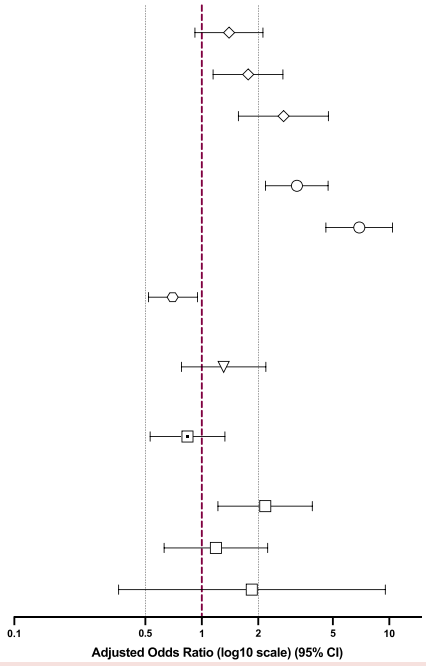


***Table 3*: Variables associated with incomplete immune restoration, defined as CD4 cell count ≤500 cells/μL, 2 years after ART initiation, in individuals with CD4 cell count ≤350 cells/μL at ART initiation.**

AIDS, Acquired Immune Deficiency Syndrome; ART, antiretroviral therapy; INSTI, integrase strand transfer inhibitor.

[a] Multivariate analysis adjusted for age (categorical), gender, CD4 cell count at ART initiation, INSTI-based regimen, Charlson comorbidity score at ART initiation, and AIDS-defining event at ART initiation.

Propensity score weighting on age, gender, CD4 cell count at ART initiation, and Charlson comorbidity index at ART initiation.

Our study is strengthened by the prospective, population-based, multicentre HIV-cohort design, including 84% of all PLWH followed in Catalonia, and the long-term observation time. We had access to high-quality, real-world health data generated by the public health system providing additional information on comorbidities and mortality. We compared a fully characterized group of LP regarding their CD4 count at ART initiation and two-years after with non-LP with optimal immune response (i.e. two-year CD4 >500 cells/μL, median CD4 count 837 (IQR: 697-1030)) also including more recent calendar years and treatments. A propensity score matching helped us to obtain more comparable treatment risk groups in the cohort regarding INSTI-exposure, reducing possible channeling bias.

On the other hand, some limitations must be considered. First, the definition of LP in our study included only CD4 cell count at ART initiation ≤ 350 cells/μL and not AIDS-defining events as we focused in CD4 responses. However, few non-LP experienced an AIDS-defining event (n =20), so including these in the group of LP would not have significantly change the results. Second, we excluded 817 individuals from the analysis because initial (n =223) or two-year CD4 counts (n =708) were not available. Of the last group, 41% (n =281) corresponded to individuals whose two-year blood samples had to be collected during the COVID-19 pandemic years 2020-2021, where hospital closure for non-COVID-19 patients prevented access to perform these blood analyses. Of those missing CD4 cell count at ART initiation, most (n =137, 61%) belonged to the initial calendar period 2005-2009. Thirty-one (3·8%) of the excluded individuals died after ART initiation with a high proportion having a history of IDU (58%). This population differs from our overall study population being probably more prone to non-adherence to care and ART. Overall, these differences in the excluded population represent small absolute numbers and it is unlikely that it would have affected our results. Third, the availability and prescribing practices of INSTI have varied over time. We added calendar time to our models to adjust for these differences over time. However, we cannot rule out bias, especially residual channelling bias. When assessing the risk of incomplete immune recovery (two-year CD4 cell count ≤ 500 cells/μL) in LP we found 30% lower odds when initiating ART with an

INSTI-based regimen. This trend was maintained when we also adjusted for calendar time, however, the results did not reach statistical significance, possibly due to lack of power. Other residual confounders such as viral resistance, lifestyle factors, or comorbidities other than the ones included were not examined, though these factors would be unlikely to have a significant impact on the results. Forth, the size of the study cohort is little with regard to a rare event like death, so risk of type II errors cannot be excluded. Finally, the lack of access to cause of death information is a limitation for the detailed interpretation of the mortality figures.

In conclusion, two-year CD4 count was inversely correlated with the mortality risk, and LP with a two-year CD4 cell count above 500 cells/μL, irrespective of their initial CD4 cell count, had the same long-term survival as non-LP. Immune recovery at this time point could be used as an early prediction of long-term survival. Despite being diagnosed at a late stage with severe immune damage, LP will regain the possibility of achieving a similar prognosis as non-LP if there is an early optimal immune recovery. In our cohort of LP, INSTI-based regimens were associated with a significantly improved two-year immune recovery and lower long-term mortality, thus being a modifiable factor to improve immune response and survival in these patients. Our data support positioning INSTI-based ART as preferred regimens, particularly in late HIV presenters, though further studies are needed to confirm these findings. Whether our results can be extrapolated to dolutegravir-based two-drug regimens will also need to be explored further.

Finally, our findings highlight the need for intensified public health efforts to improve timely HIV diagnosis and the need to continue with studies to identify modifiable determinants for immune recovery to improve survival in LP.

Contributors

J Aceitón, Y Díaz, S Moreno-Fornés had access to the raw data and performed the initial data management. R Martin-Iguacel conducted the research and analysed the data. R Martin-Iguacel wrote the first draft of the manuscript under the supervision of JM Llibre. All authors contributed to the interpretation of the results and revision of the manuscript and gave their final approval to the manuscript.

Data sharing statement

The data collected for this study are available from the Centre for Epidemiological Studies of Sexually Transmitted Diseases and HIV/AIDS in Catalonia (CEEIS-CAT), the coordinating centre of the PISCIS cohort study and from each of the collaborating hospitals upon request. Requests can be made via https://pisciscohort.org/contacte/.

The study protocol, the statistical codebook and dofiles for the analysis can be requested from RMI (raquel@bisaurin.org).

Declaration of interests

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: no support from any organization for the submitted work; no financial relationships with any organizations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

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We hereby declare that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as originally planned have been explained.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.eclinm.2022.101600.

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Original article

Tuberculosis incidence and mortality in people living with human immunodeficiency virus: a Danish nationwide cohort study

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ABSTRACT

Objectives: To explore changes over time in the epidemiology of tuberculosis (TB) in Denmark in people living with human immunodeficiency virus (HIV) (PLWH).
Methods: In this nationwide, population-based cohort study we included all adult PLWH from the Danish HIV Cohort Study (1995–2017) without previous TB. We estimated TB incidence rate (IR), all-cause mortality rate (MR), associated risk and prognostic factors using Poisson regression.
Results: Among 6982 PLWH (73 596 person-years (PY)), we observed 217 TB events (IR 2.9/1000 PY, 95% CI 2.6–3.4: IR 6.7, 95% CI 5.7–7.9 among migrants and IR 1.4, 95% CI 1.1–1.7 among Danish-born individuals; $p < 0.001$). The IR of concomitant HIV/TB remained high and unchanged over time. The IR of TB diagnosed >3 months after HIV diagnosis declined with calendar time, longer time from HIV diagnosis, and CD4 cell recovery. Independent TB risk factors were African/Asian/Greenland origin (adjusted incidence rate ratio (aIRR) 5.2, 95% CI 3.5–7.6, aIRR 6.5, 95% CI 4.2–10.0, aIRR 7.0, 95% CI 3.4–14.6, respectively), illicit drug use (aIRR 6.9, 95% CI 4.2–11.2), CD4 <200 cells/μL (aIRR 2.7, 95% CI 2.0–3.6) and not receiving antiretroviral therapy (aIRR 3.7, 95% CI 2.5–5.3). Fifty-five patients died (MR 27.9/1000 PY, 95% CI 21.4–36.3), with no improvement in mortality over time. Mortality prognostic factors were Danish-origin (adjusted mortality rate ratio (aMRR) 2.3, 95% CI 1.3–4.3), social burden (aMRR 3.9, 95% CI 2.2–7.0), CD4 <100 cells/μL at TB diagnosis (aMRR 2.6, 95% CI 1.3–4.9), TB diagnosed >3 months after HIV versus concomitant diagnosis (aMRR 4.3, 95% CI 2.2–8.7) and disseminated TB (aMRR 3.3, 95% CI 1.1–9.9).
Conclusion: Late HIV presentation with concomitant TB remains a challenge. Declining TB rates in PLWH were observed over time and with CD4 recovery, highlighting the importance of early and successful antiretroviral therapy. However, MR remained high. Our findings highlight the importance of HIV and TB screening strategies and treatment of latent TB in high-risk groups. **Raquel Martin-Iguacel, Clin Microbiol Infect 2022;28:570**

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Introduction

The use of antiretroviral therapy (ART) has sharply reduced the incidence of tuberculosis (TB) in people living with human immunodeficiency virus (HIV) (PLWH). TB is still the most common AIDS-defining condition, however, and is a leading cause of death among PLWH [1,2].

Identifying risk factors for TB development, treatment failure and death in PLWH is pivotal to achieve the goals of the End TB Strategy [3]. Previous studies have identified immigration from high-burden TB countries and HIV-related immunosuppression as risk factors for development of active TB in low-incidence countries [2,4]. However, improved ART coverage and changing migration dynamics over the last decade may have influenced TB epidemiology in countries with a low TB incidence.

We conducted a Danish nationwide cohort study from 1995 to 2017 among PLWH to estimate the incidence rate (IR) of TB, the associated mortality rates (MR) and the associated predictive and prognostic factors.

Materials and methods

Study design

In this Danish nationwide population-based cohort of HIV-1-infected individuals, we estimated the incidence and prognosis of TB. The study design had two parts. First, we investigated the IR and risk factors for Danish adult PLWH to develop the first TB episode through Danish nationwide prospective cohorts. Second, we evaluated MR and associated prognostic factors in the cohort of patients with HIV/TB co-infection identified as well in the Danish HIV Cohort Study (DHCS) cross-checked with the Danish National Hospital Registry (DNHR) and the Danish Civil Registration System (DCRS).

Infectious diseases physicians reviewed all hospital records from patients coded with a mycobacterial infection in either DHCS (both hospitalized and outpatient diagnosis) or DNHR (according to International Classification of Disease 10th revision codes at discharge, see Supplementary material, Appendix S1). They validated the TB diagnosis and gathered additional information (see Supplementary material, Fig. S1). Both databases were used to improve case detection.

Setting

In May 2017, Denmark had a population of 5.8 million with an estimated HIV prevalence among the adult population of 0.1% [5] and a TB IR of 0.05/1000 person-years (PY) [6]. The Danish healthcare system is universal and tax-payer funded. All PLWH are followed in one of the eight hospital-based specialized departments of infectious diseases. ART and TB therapy are provided according to national guidelines and are free of charge.

Study period

The study ran from 1 January 1995 to 1 May 2017.

Data sources

We used the Danish personal identification number, which is assigned to all individuals at birth or immigration, to link individuals in the following national registries.

The DHCS is an ongoing nationwide, prospective, population-based cohort of all PLWH treated at Danish hospitals since 1 January 1995. Data are updated yearly and include demographics, date of HIV diagnosis, AIDS-defining events, cause/date of death, ART, CD4 cell count and plasma HIV-RNA measurements [7].

The DCRS, established in 1968, contains information on vital status, residency and migration for all residents in Denmark [8]. We extracted data on emigration, immigration and date of death.

The DNHR, established in 1977, contains information about hospitalization in non-psychiatric Danish hospitals, including discharge diagnosis codes according to the International Classification of Disease 10th revision from 1994 onwards [9].

Study population

For TB incidence, from the DHCS, we included all PLWH ≥18 years of age, alive and living in Denmark at study inclusion, followed for HIV care during 1995–2017, and not diagnosed with TB before to study inclusion.

For TB mortality, we included all PLWH diagnosed with TB during the study period.

Outcomes

For TB incidence, the primary outcome measure was time to TB diagnosis.

We classified TB as either diagnosed concomitantly (within 1 month before and up to 3 months after HIV diagnosis) or diagnosed >3 months thereafter. We included both definitive and presumptive TB diagnoses [10]. The diagnosis was classified as definitive when *Mycobacterium tuberculosis* was identified by culture of *M. tuberculosis* complex and/or detection by nucleic acid amplification or in case of detection of acid-fast bacilli by microscopy and compatible TB clinical symptoms. A presumptive diagnosis was based on histopathological, clinical and/or radiographic criteria compatible with TB.

For TB mortality, the primary outcome measures were time to all-cause mortality and to TB-related mortality.

Statistical analysis

Chi-squared test, *t* test, and Mann–Whitney *U* test were used to compare differences between groups.

For TB incidence, the date of study inclusion was defined as the most recent of the following: 1 January 1995 (initiation of DHCS), date of HIV diagnosis, or date of immigration for individuals with a known HIV infection. We calculated the time from study inclusion to TB diagnosis, death, emigration, loss to follow up, or 1 May 2017, whichever occurred first.

We calculated the crude IR per 1000 PY and 95% CI. The TB IR was further assessed by (a) calendar time:1995–2002, 2003–2009, 2010–2014, ≥2015 (time-updated), (b) time after HIV diagnosis (>3 months to 1 year, >1–5 years, >5–10 years, and >10 years, and (c) recovery of the CD4 cell count.

We used Poisson exponential regression analysis to control for the following potential confounders and to explore risk factors for TB: age, gender, continent of birth, race, route of HIV transmission, CD4 cell count and HIV viral load at study inclusion, AIDS-defining events before study inclusion, and not being on ART (time-updated).

For TB mortality, we assessed the time from TB diagnosis to death, emigration, loss to follow up or 1 May 2017, whichever occurred first, and calculated crude MR and 95% CI. Kaplan–Meier analysis was used to estimate survival according to social burden (defined as alcohol consumption >7/14 drinks per week, illicit drug use (IDU), homelessness, stay in shelter or prison facilities, and/or prostitution), TB localization (pulmonary, extrapulmonary and disseminated), and HIV viraemia copy-years before TB diagnosis

(≥5 versus <5 median log_{10} copy-year/mL). Viraemia copy-years is a time-updated measure of cumulative HIV viral load exposure [11]. The definition of social burden included in this study is not a validated definition but summarizes risk factors for TB exposure that reflect the social vulnerability of these patients.

We used Poisson exponential regression analysis to control for the same confounders described above plus: Charlson co-morbidity score, alcohol/tobacco consumption, social burden, TB localization (pulmonary, extrapulmonary and disseminated), calendar time, TB diagnosed >3 months after HIV, and first 6 months after TB diagnosis.

Causes of death were classified as (a) TB-related, (b) HIV-related, (c) neither HIV- nor TB-related, (d) unknown.

The analyses were stratified by known HIV status at TB diagnosis. STATA software (v.16; StataCorp, College Station, TX, USA) was used for data analyses.

The study was approved by the Danish Data protection Agency (2012-41-0005). Ethical approval and individual consent are not required by Danish legislation for this type of research.

Results

From the DHCS, we identified 6982 PLWH followed for HIV care during 1995–2017, giving rise to 73 596 PY (median follow-up time 9.6 years; interquartile range (IQR) 3.8–16.9 years). We observed 217 (3.1%) incident TB cases in the study period, with an IR of 2.9/1000 PY (95% CI 2.6–3.4): 6.7/1000 PY (95% CI 5.7–7.9) for migrants and 1.4/1000 PY (95% CI 1.1–1.7) for Danish-born individuals (excess IR for migrants 5.3/1000 PY, p < 0.001). In the group of patients co-infected with TB and HIV, there were fewer males compared with mono-infected individuals (58.5% versus 75.9%), and a higher percentage of heterosexuals (58.5% versus 34.9%) and non-European individuals (57.6% versus 20.4%, mainly from Africa (39.2%) and Asia (16.1%)). The median time from arrival in Denmark to HIV diagnosis was 5.9 years (IQR 2.8–9.1 years) for the migrants. Additional characteristics are provided in Table 1.

Ninety-nine patients (45.6%) were diagnosed concomitantly with HIV/TB (median time from HIV to TB diagnosis 7.3 days, IQR 0–21.9 days). Of these patients, three-quarters were migrants, and the median time from arrival in Denmark to HIV/TB diagnosis was 2.7 years (IQR 0.5–6.9 years). In contrast, 118 patients (54.4%) with HIV infection were diagnosed with TB > 3 months after the HIV diagnosis (median time from HIV to TB diagnosis 5.1 years, IQR 2.0–9.6 years). These patients were significantly older, more likely to be of Danish origin, with higher social burden, tobacco consumption, CD4 cell count and Charlson co-morbidity scores (mainly driven by liver and other non-TB AIDS illnesses, see Supplementary material, Table S1), and had higher rates of pulmonary TB (Table 1). Only 41.5% of these PLWH with a subsequent diagnosis of TB had HIV viral load <200 copies/mL at the time of TB diagnosis. More of these individuals interrupted the TB treatment compared with those diagnosed concomitantly (5.1% versus 0%, p 0.02) whereas the rate of TB relapse was similar (6.8% versus 8.1%, p 0.72) in the two groups (Table 1). Only 44.9% ($n = 53$) of these patients had either a Mantoux test ($n = 21$) and/or an interferon-γ release assay ($n = 43$) performed before TB diagnosis, mainly migrants (62.3%, $n = 33$), finding a positive result in 53.8% of cases ($n = 35$). However, none of these patients received treatment for latent tuberculosis infection (LTBI) (Table 1).

Fig. 1 shows that concomitant TB/HIV IR remained unchanged throughout the study period (IR 58.1/1000 PY; 95% CI 47.7–70.8). However, a decline was observed for TB diagnosed >3 months after HIV between 1995–2002 and 2015–2017 (p < 0.01). Investigation of time trends in TB IR in PLWH showed a peak during the first 3 months after HIV diagnosis followed by a substantial decline thereafter (p < 0.001). Hence, the risk of TB decreased significantly beyond 3 months from HIV diagnosis (adjusted incidence rate ratio (aIRR) 0.05; 95% CI 0.04–0.08). Following the initiation of ART, TB incidence declined substantially and correlated inversely with CD4 cell recovery (p < 0.001) (Figs. 1a–f; see Supplementary material, Tables S2 and S3).

In multivariate analyses, African/Asian/Greenland origin (aIRR 5.16, 95% CI 3.52–7.57; aIRR 6.49, 95% CI 4.20–10.01; and aIRR 7.03, 95% CI 3.38–14.59, respectively), IDU (aIRR 6.87, 95% CI 4.21–11.24), heterosexual route of transmission (aIRR 2.99, 95% CI 1.88–4.74), CD4 count ≥100–200 cells/μL (aIRR 2.47, 95% CI 1.64–3.72) or <100 cells/μL (aIRR 2.86, 95% CI 2.05–4.05), and not being on ART (aIRR 3.65, 95% CI 2.53–5.26) remained associated with an increased risk of TB (Table 2).

Fifty-five patients (25.4%) died during the study period (27 866 PY, crude all-cause MR 27.9/1000 PY, 95% CI 21.4–36.3). From 2007 to 2017, the mortality rate was 56% higher in PLWH developing TB compared with HIV mono-infected (MR 22.7/1000 PY versus 14.6/1000 PY; IRR 1.56, 95% CI 1.11–2.19, p 0.01). No changes in MR were observed over calendar time in TB/HIV co-infected patients (Table 3), whereas a clear decline was observed for HIV mono-infected patients (from 34.2/1000 PY before 2007 to 14.6/1000 PY from 2007 onwards; IRR 0.43, 95% CI 0.39–0.47, p < 0.01). TB-related deaths accounted for 29% of all deaths (Table 1).

Mortality rates were higher in patients diagnosed with TB > 3 months after HIV diagnosis compared with those diagnosed concomitantly (adjusted mortality rate ratio (aMRR) 4.34, 95% CI 2.17–8.66) despite the higher rates of late HIV diagnosis in the latter (13% versus 36%, p < 0.001) (Table 3). Thirty-three per cent of the deaths in the first group (versus 15% in the latter) occurred within the first 6 months and were mainly TB-related (Table 1).

In multivariate analyses, the following factors remained associated with an increased risk of death: Danish-origin (aMRR 2.34, 95% CI 1.27–4.33), social burden (aMRR 3.87, 95% CI 2.15–6.97), CD4 count <100 cells/μL at TB diagnosis (aMRR 2,57, 95% CI 1.34–4.94), TB diagnosed >3 months after HIV (aMRR 4.34, 95% CI 2.17–8.66), higher HIV viraemia/copy years (aMRR 3.58, 95% CI 1.56–8.22) and disseminated TB (aMRR 3.32, 95% CI 1.11–9.94) (Table 3, Fig. 2). Fig. 2 shows Kaplan–Meier survival curves for all-cause mortality stratified by social burden, HIV viraemia copy-years and TB.

Similar results were found when stratifying analyses by HIV status at TB diagnosis (see Supplementary material, Tables S3 and S4).

Discussion

In this Danish, nationwide population-based cohort study, the risk of concomitant HIV/TB diagnosis remained high and unchanged over calendar time, affecting mainly migrants at an advanced stage of HIV infection at diagnosis. For individuals with known HIV, we observed a steady decline in TB rates over time. Overall, the risk of TB in PLWH was associated with migration, vulnerable subgroups (IDU, social burden, not receiving ART) and with HIV-induced immunosuppression. All-cause mortality in PLWH co-infected with TB remained high over time. HIV-associated immunosuppression, disseminated TB and social burden were found to be significant predictors of death.

Our data suggest that individuals with concomitant HIV/TB are a different population from those with previously known HIV infection who develop TB > 3 months after their HIV diagnosis. Whereas those diagnosed concomitantly were mainly migrants and HIV late-presenters, many of those with a later TB diagnosis had social burden, higher co-morbidity and sub-optimal linkage to care and ART. Patients in the latter group were also more prone to poor

Table 1
Characteristics of the study population

	All HIV-infected individuals without TB ($n = 6765$)	All TB cases 1995–2017 ($n = 217$)	p value	Concomitant TB/HIV $n = 99$	TB diagnosed >3 months after HIV ($n = 118$)	p value
Observation time (years), median (IQR)	9.9 (4.2–17.3)	0.4 (0.03–4.1)		0.02 (0.001–0.06)	4.0 (1.3–8.2)	
Male, *n* (%)	5169 (76.4)	127 (58.5)	<0.0001	60 (60.6)	67 (56.8)	0.57
Age at baseline (years), median (IQR)	37 (30–45)	35 (30–42)	0.08	36 (31–41)	35 (29–42)	0.34
Caucasian, *n* (%)	5224 (77.2)	81 (37.3)	<0.0001	28 (29.5)	53 (45.3)	0.001
Route of HIV transmission, *n* (%)						
MSM	3156 (46.7)	30 (13.8)	<0.0001	14 (14.1)	16 (13.6)	0.90
Heterosexual	2363 (34.9)	127 (58.5)	<0.0001	64 (64.7)	63 (53.4)	0.09
IDU	593 (8.8)	35 (16.1))	<0.0001	8 (8.1)	29 (24.6)	0.001
Unknown	653 (9.7)	23 (10.6)	0.643	13 (13.1)	10 (8.5)	0.27
HCV, *n* (%)	1359 (20.1)	54 (24.9)	0.08	17 (17.2)	37 (31.2)	0.02
HBV, *n* (%)	385 (5.7)	19 (8.8)	0.06	7 (7.1)	12 (10.2)	0.42
HIV diagnosis before 1995, *n* (%)	2010 (29.7)	38 (17.5)	<0.0001	1 (1.0)	37 (31.4)	<0.0001
Non-TB AIDS before inclusion, *n* (%)	463 (6.8)	12 (5.5)	0.449	8 (8.1)	4 (3.4)	0.13
Non-TB AIDS before TB diagnosis, *n* (%)		33 (15.2)		7 (7.1)	26 (22.0)	0.002
$CD4^+$ cell count at study inclusion (cells/μL), median (IQR)	290 (106–499)	190 (65–373)	<0.0001	80 (40–175)	291 (205–522)	<0.0001
HIV VL at study inclusion (log_{10} copies/mL), median (IQR)	4.8 (4.1–5.4)	5.0 (4.5–5.7)	0.002	5.2 (4.9–5.7)	4.6 (4.1–5.2)	0.004
Emigration during the study period, *n* (%)	395 (5.8)	13 (6.0)	0.925	10 (10.1)	3 (2.5)	0.019
Loss to follow-up, *n* (%)	32 (0,5)	2 (0.9)	0.35	2 (2.0)	0	0.12
Region of origin						
Denmark	4745 (70.1)	72 (33.2)	<0.0001	23 (23.2)	49 (41.5)	0.01
Greenland	64 (1.0)	9 (4.1)	<0.0001	3 (3.0)	6 (5.2)	0.6
Europe	510 (7.5)	11 (5.1)	0.17	6 (6.1)1	5 (4.3)2	0.2
Africa	888 (13.1)	85 (39.2)	<0.0001	42 (42.4)	43 (36.4)	0.37
Asia	336 (5.0)	35 (16.1)	<0.0001	23 (23.2)	12 (10.2)	0.009
America	153 (2.3)	5 (2.3)	0.967	2 (2.0)	3 (2.6)	0.51
Unknown	69 (1.0)	0	—			
Age at TB diagnosis (years), median (IQR)				35 (31–41)	40 (33–48)	0.005
Time from arrival in Denmark to TB diagnosis (years), median (IQR)		4.1 (1.2–8.6)		2.7 (0.5–6.9)	5.9 (2.8–9.1)	0.004
Alcohol, *n* (%)		65 (30.0)		31 (31.3)	34 (28.8)	0.69
<7/14 per week		25 (11.8)		16 (16.8)	9 (7.8)	0.05
>7/14 per week		33 (15.6)		14 (14.7)	19 (16.4)	0.69
Unknown		159 (73.3))		69/70.0)	90/76.3)	0.28
Smoking, *n* (%)		98 (45.2)		37 (37.4)	61 (51.7)	0.04
<10 cigarettes/day		8 (3.8)		5 (5.3)	3 (2.6)	0.33
10–20 cigarettes/day		34 (16.2)		10 (10.6)	24 (20.7)	0.04
>20 cigarettes/day		24 (11.4)		7 (7.5)	17 (14.7)	0.09
Unknown		151 (69.6)		77 (77.8)	74 (62.7)	0.02
Social burden[a]		71 (32.7)		26 (26.3)	45 (38.1)	0.06
$CD4^+$ cell count, at TB diagnosis (cells/μL), median (IQR)		170 (70–371)		80 (40–175)	260 (140–460)	<0.0001
Viral load at TB diagnosis (log_{10} copies/mL), median (IQR)		4.9 (1.8–5.3)		5.2 (4.9–5.7)	3.0 (1.3–5.0)	<0.0001
Virological suppression at TB diagnosis (<200 copies/mL), *n* (%)		51 (23.5)		2 (2.0)	49 (41.5)	<0.0001
Viraemia copy-years from HIV diagnosis to TB diagnosis (log_{10} copy-years/mL), median (IQR)		4.9 (4.2–6.0)		4.0 (3.9–4.2)	5.0 (4.2–6.0)	0.06
On ART at TB diagnosis, *n* (%)		61 (28.1)		0	61 (51.7)	<0.0001
Charlson co-morbidity score at TB diagnosis						
0		135 (62.2)		75 (75.8)	60 (50.9)	<0.0001
1		31 (14.3)		14 (14.1)	17 (14.4)	0.96
2–5		16 (7.4)		3 (3.0)	13 (11.0)	0.03
≥6		35 (16.1)		7 (7.1)	28 (23.7)	0.001
TB diagnosis, *n* (%), based on						
Microbiology (PCR or culture)		180 (83.7)				
Histopathology		33 (15.4)				
Clinical suspicion		2 (0.9)				
Autopsy		2 (0.9)				

(*continued on next page*)

Table 1 (*continued*)

	All HIV-infected individuals without TB ($n = 6765$)	All TB cases 1995–2017 ($n = 217$)	p value	Concomitant TB/HIV $n = 99$	TB diagnosed >3 months after HIV ($n = 118$)	p value
Performance of interferon-γ release assays, *n* (%)	na	67 (30.9)		24 (24.2)	43 (36.4)	0.05
Positive	na	40 (59.7)		15 (62.5)	25 (58.1)	0.25
Negative	na	25 (37.3)		9 (37.5)	16 (37.2)	0.30
Performance of Mantoux test, *n* (%)	na	32 (14.7)		11 (11.1)	21 (17.8)	0.17
Positive	na	21 (65.6)		7 (63.6)	14 (66.7)	0.23
Negative	na	11 (34.4)		4 (36.4)	7 (33.3)	0.53
TB localization, *n* (%)						
Pulmonary		102 (47.0)		34 (34.3)	68 (57.6)	0.001
Extrapulmonary		47 (21.7)		21 (21.2)	28 (22.0)	0.88
Disseminated		68 (31.3)		44 (44.4)	24 (20.3)	<0.0001
Treatment outcome[b], *n* (%)						
Cure		5 (2.3)		1 (1.0)	4 (3.4)	0.25
Treatment completed		187 (86.2)		94 (95.0)	93 (78.8)	0.001
Died in the study period	1772 (25.4)	55 (25.4)	0.991	13 (13.1)	42 (35.6)	<0.0001
Treatment failure		0		0	0	—
Treatment interrupted		6 (2.8)		0	6 (5.1)	0.02
Relapse		16 (7.4%)		8 (8.1)	8 (6.8)	0.72
Time from TB to death (years), median (IQR)		2.1 (0.3–8.4)		5.8 (1.4–8.7)	1.8 (0.1–7.3)	0.16
Causes of death, *n* (%)		total	first ½ year	beyond ½ year	first ½ year	beyond ½ year
TB-related		16 (29.0)	2[c]	0	12[d]	2[e]
HIV-related		3 (5.5)	0	1	0	2
Not TB- or HIV-related[f]		27 (49.1)	0	9	2	16
Unknown		9 (16.4)	0	1	0	8

Abbreviations: ART, antiretroviral therapy; HBV, hepatitis B virus infection; HCV, hepatitis C virus infection; HIV, human immunodeficiency virus; IDU, illicit drug use; IQR, interquartile range; MSM, men having sex with men; NA, data not available; TB, tuberculosis; VL, viral load.

[a] Social burden defined as alcohol consumption >7/14 drinks per week, injection drug use (IDU), homelessness, stay in shelter or prison facilities, and/or prostitution.

[b] Outcome for TB treatment according to WHO guidelines [10].

[c] Due to disseminated TB.

[d] Six cases of pulmonary TB, one extrapulmonary TB and five disseminated TB.

[e] One pulmonary and one disseminated.

[f] Five due to malignancies (two lung, one oropharynx, one breast, one anal cancer), five other infections (one aspergillus, three pneumonia sepsis, one staphylococcal sepsis), one terminal liver disease, one terminal chronic obstructive pulmonary disease, three ischaemic heart disease, one bleeding from liver biopsy, one fire accident and ten drug abuse.

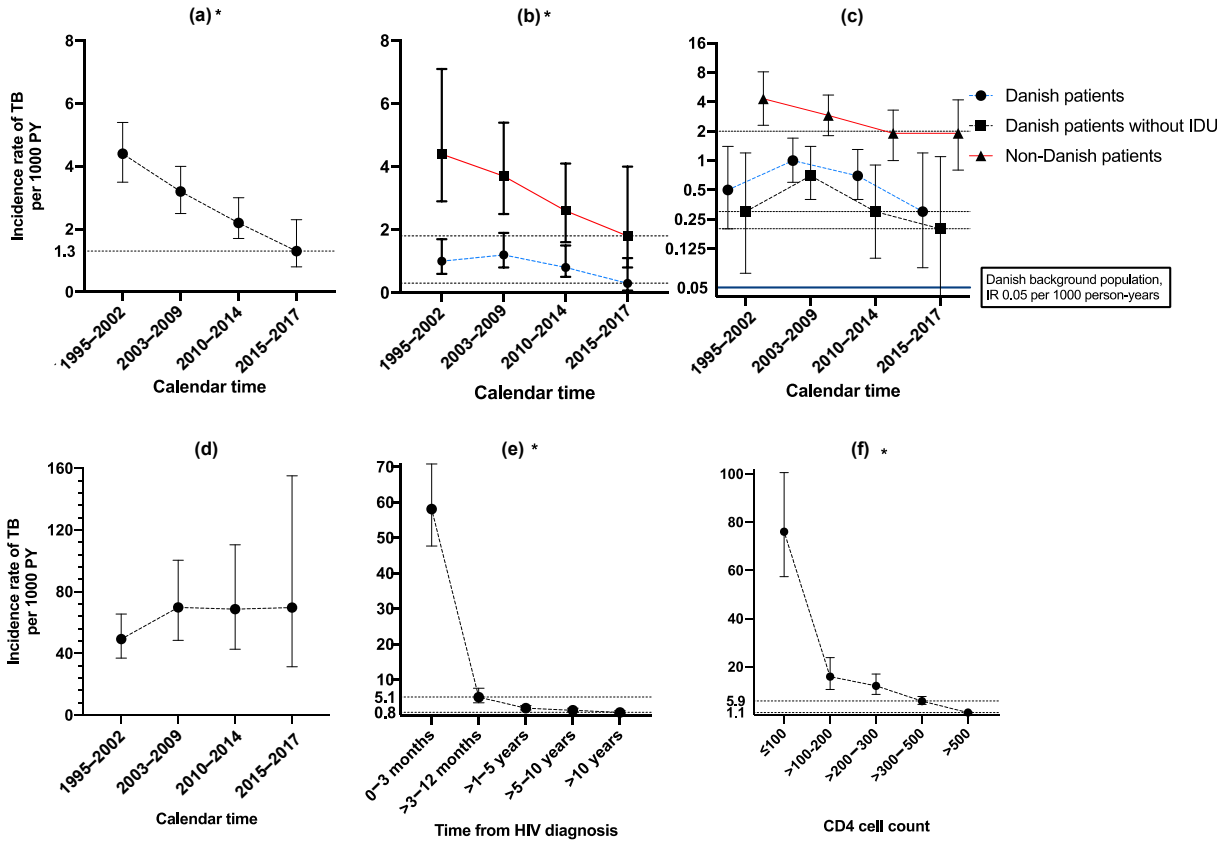

Fig. 1. Incidence rates of tuberculosis (TB) stratified by calendar time, time after human immunodeficiency virus (HIV) diagnosis, and CD4 cell count recovery. Incidence rates of TB are defined as incident TB cases per 1000 person-years (PY) of follow up. IDU, illicit drug use. (a) Incidence rates of TB over calendar time. (b) Incidence rates of TB in patients with known HIV infection over calendar time stratified by Danish origin. (c) Incidence rates of TB in patients with known HIV infection on antiretroviral therapy over calendar time stratified by Danish origin (incidence rate in log scale base 2). The blue line represents TB incidence in the Danish background population (source www.ssi.dk). (d) Incidence rates of TB diagnosed concomitantly with HIV infection over calendar time. (e) Incidence rates of TB by time after HIV diagnosis. (f) Incidence rates of TB by CD4 cell count recovery (time updated). The dotted grey lines in every figure represent the point incidence rate in the recent calendar period. * Statistically significant test for trend (<0.001).

TB treatment outcomes and death, suggesting that individualized care programmes are still needed for this subset.

The IR for concomitant HIV/TB diagnosis remained high and showed no significant changes over time. This trend is in line with high and persistent rates of late HIV presentation worldwide. This has a negative impact on morbidity and survival for the individual and fuels ongoing transmission of both TB and HIV at the population level [12–15]. These findings highlight the importance of timely HIV diagnosis by improving awareness of HIV risk and effectiveness of TB screening strategies. Three-quarters were migrants, mostly from countries with high HIV and TB incidence, which in itself should have triggered a proactive HIV and TB screening upon immigration.

We observed a statistically significant decline in TB IR over time among individuals with known HIV. The improved ART coverage and earlier ART initiation [16] might have contributed to this decline. The risk of TB also declined progressively with time from HIV diagnosis and paralleled CD4 recovery in those successfully treated for HIV.

The interaction between social burden and lack of adherence to ART/TB-treatment and health-care follow up may explain the increased TB IR and MR seen in a subset of Danish-born patients with previously known HIV infection. This vulnerable and difficult-to-treat population should probably be the focus of targeted public health strategies to encourage relinkage to care and treatment.

Current guidelines recommend systematic testing for and treatment of LTBI in PLWH once active TB is excluded, as PLWH have a lifelong risk of developing active TB [17–19]. Treatment of LTBI is estimated to reduce the overall risk of developing active TB by around 33%, up to 64% in those with positive screening tests [19]. Although the level of evidence is high for middle and high TB burden countries [20], the evidence for low-incidence countries with good access to ART is less robust [21–26]. With the high efficacy and immediate ART initiation, the benefit of treating LTBI is perceived as less important. For these reasons, the implementation of these guidelines is poor in Denmark and in many European countries with low TB incidence [27]. This is demonstrated by the low rates of LTBI screening and the absence of LTBI treatment despite positive screening tests in our population. The low awareness among physicians of the *a priori* risk of TB development in PLWH was more pronounced for patients of Danish origin, as shown by the lower rates of LTBI screening among Danish-born individuals.

Although the rate of active TB disease in PLWH declined over the study period, it remained 16-fold higher than in the background Danish population. Although the absolute country incidence remains low, the incremental relative risk is high. Our results support the ongoing need for intensified routine targeted screening and treatment of LTBI in PLWH, the last especially in those with the highest risk, i.e. migrants and individuals with IDU, low CD4 counts, social risk factors, irregular follow up and poor ART adherence.

Main strengths of our study include the design, a population-based, nationwide HIV cohort with high coverage, long observation time and almost complete follow up with a linked review of

Table 2
Predictive factors for developing tuberculosis in Danish HIV-infected patients

	TB events	PRY	IR per 1000 pyr (95% ci)	IRR (95% CI)	aIRR[a] (95% CI)
Total	217	73 595.8	2.9 (2.6–3.4)		
Demographic factors					
Gender					
Female	90	18 954.7	4.7 (3.9–5.8)	ref (1)	ref (1)
Male	127	54 641.1	2.3 (2.3–2.8)	0.49 (0.37–0.64)	1.44 (1.05–1.97)
Age (time-updated)[b]					
≥40 years	93	47 428.5	2.0 (1.6–2.4)	ref (1)	ref (1)
<40 years	124	26 167.3	4.7 (4.0–5.7)	2.42 (1.85–3.16)	1.06 (0.78–1.43)
Region of birth					
Denmark	74	52 237.9	1.4 (1.1–1.8)	ref (1)	ref (1)
Europe	11	4772.0	2.3 (1.3–4.2)	1.63 (0.86–3.07)	1.69 (0.89–3.18)
Africa	82	9944.5	8.2 (6.6–10.2)	5.82 (4.25–7.97)	5.16 (3.52–7.57)
Asia	36	3644.5	9.9 (7.1–13.7)	6.97 (4.68–10.38)	6.49 (4.20–10.01)
Greenland	8	743.4	10.8 (5.4–21.5)	7.60 (3.66–15.75)	7.03 (3.38–14.59)
Other	6	2253.5	2.7 (1.2–5.9)	1.88 (0.82–4.32)	2.15 (0.93–4.96)
Route of HIV transmission					
MSM	30	34 911.0	0.9 (0.6–1.2)	ref (1)	ref (1)
IDU	37	6128.6	6.0 (4.4–8.3)	7.03 (4.34–11.37)	6.87 (4.21–11.24)
Heterosexual	127	27 489.3	4.6 (3.9–5.5)	5.38 (3.61–8.00)	2.99 (1.88–4.74)
Other	23	5067.0	4.5 (3.0–6.8)	5.28 (3.07–9.09)	2.62 (1.48–4.64)
HIV factors					
CD4 cell count at baseline					
CD4 cell count ≥200 cells/μL	138	58 040.3	2.4 (2.0–2.8)	ref (1)	ref (1)
CD4 cell count <200, ≥100 cells/μL	30	6539.3	4.6 (3.2–6.6)	1.93 (1.30–2.86)	2.47 (1.64–3.72)
CD4 cell count <100 cells/μL	48	9016.2	5.4 (4.1–7.2)	2.29 (1.65–3.17)	2.86 (2.05–4.05)
VL ≥ 100 000 copies/mL at study inclusion					
No	60	19 726.9	3.0 (2.4–3.9)	ref (1)	
Yes	67	12 411.5	5.4 (4.2–6.9)	1.78 (1.25–2.51)	
Baseline VL, $\log_{10}$ copies/mL				1.23 (1.06–1.43)	
Non-TB AIDS before inclusion					
No	205	70 514.3	2.9 (2.5–3.3)	ref (1)	
Yes	12	3081.5	3.9 (2.2–6.9)	1.34 (0.75–2.40)	
ART initiation (time-updated)					
Time before ART initiation	133	15 963.0	8.3 (7.0–9.9)	5.72 (4.35–7.51)	3.65 (2.53–5.26)
Time after ART initiation	84	57 632.8	1.5 (1.2–1.8)	ref (1)	ref (1)
Time-related factors					
Calendar time (time-updated)					
1995–2002	82	18 786.4	4.4 (3.5–5.4))	3.25 (1.84–5.72)	0.91 (0.49–1.68)
2003–2009	75	23 613.0	3.2 (2.5–4.0)	2.36 (1.34–4.18)	1.34 (0.74–2.42)
2010–2014	46	20 781.7	2.2 (1.7–3.0)	1.65 (0.91–3.00)	1.34 (0.73–2.44)
2015–2017	14	10 414.7	1.3 (0.8–2.3)	ref (1)	ref (1)
HIV status at TB diagnosis					
Concomitant diagnosis[c]	99	1703.6	58.1 (47.7–70.8)	ref (1)	ref (1)
Known HIV infection[d]	118	71 892.2	1.6 (1.4–2.0)	0.03 (0.02–0.04)	0.05 (0.04–0.07)

Abbreviations: aIRR, adjusted incidence rate ratio; ART, antiretroviral therapy; HIV, human immunodeficiency virus; IDU, illicit drug use; IR, incidence rate; IRR, incidence rate ratio; MSM, men having sex with men; PYR, person-years at risk; TB, Tuberculosis, VL, viral load.
[a] Adjusted for gender, age, race (Caucasian versus non-Caucasian), route of HIV transmission, CD4 cell count at study inclusion, ART initiation during the study period (time-updated), calendar time (time-updated), and HIV status at TB diagnosis.
[b] Age ≥40 years versus <40 years at time of study inclusion (time updated).
[c] TB was diagnosed up to 1 month before or within 3 months after HIV diagnosis.
[d] TB was diagnosed >3 months after HIV diagnosis.

validated national databases. We had full access to Danish registries providing high-quality, individual-level data on vital status, residency and migration. This allowed the estimation of longitudinal trends in TB incidence and survival. Moreover, all medical records coded with any mycobacterial infection were reviewed by specialists to confirm TB diagnosis and cause of death. An additional strength is the high number of definitive TB cases included in the study.

Fifty-nine medical records of patients coded with mycobacterial infection had been sent to shredding from the archives, 78% of these before the year 2000. Forty-six of them were coded as atypical mycobacterial infection, but misclassification cannot be ruled out. This could have led to underestimation of the TB incidence in the first period (1995–2002), and thus the real decline over time in TB incidence could have been steeper.

In conclusion, we observed declining rates of TB in PLWH over time, which paralleled CD4 cell recovery. This highlights the importance of early and successful ART. Late HIV presentation with concomitant TB diagnosis remained a challenge with persistently high rates, underlining the need for intensified strategies to ensure earlier diagnosis of HIV and TB. Migration, IDU and HIV-induced immunosuppression were identified as important risk factors for TB, supporting the need for routine screening and treatment of LTBI in these high-risk groups in countries with low TB incidence. Finally, mortality in TB/HIV co-infected individuals remained unchanged over time, and immunosuppression and social burden had an important impact on prognosis.

Transparency declaration

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: no support from any organization for the submitted work, no financial relationships with any organizations that might have an interest in the submitted

Table 3
Prognostic factors for mortality in HIV-infected patients developing TB

	Deaths	PYR	MR per 1000 PYR (95% CI)	MRR (95% CI)	aMRR[a] (95% CI)
Total	55	27 866	27.9 (21.4–36.3)		
Gender					
Female	15	851.7	17.6 (10.6–29.2)	ref (1)	ref (1)
Male	40	1122.1	35.6 (26.1–48.6)	2.02 (1.12–3.66)	1.26 (0.66–2.39)
Age (time-updated)[b]					
≥40 years	38	1248.4	30.4 (22.1–41.8)	ref (1)	ref (1)
<40 years	17	725.3	23.4 (14.6–37.7)	1.30 (0.73–2.30)	1.08 (0.58–2.02)
Migrants					
No	38	607.6	62.5 (45.5–86.0)	5.03 (2.84–8.90)	2.34 (1.27–4.33)
Yes	17	1366.2	12.4 (7.7–20.0)	ref (1)	ref (1)
IDU					
No	31	1803.8	17.2 (12.1–24.4)	ref (1)	
Yes	24	170.0	141.2 (94.6–210.7)	8.22 (4.82–14.00)	
High alcohol consumption[c]					
No	40	1751.9	22.8 (16.7–31.1)	ref (1)	
Yes	15	221.8	67.6 (40.8–112.2)	2.96 (1.64–5.36)	
High tobacco consumption[d]					
No	32	1496.8	21.4 (15.1–30.2)	ref (1)	
Yes	23	477.0	48.2 (32.0–72.6)	2.26 (1.32–3.85)	
Social burden[e]					
No	21	1541.7	13.6 (8.9–20.9)	ref (1)	ref (1)
Tes	34	432.1	78.7 (56.2–110.1)	5.78 (3.35–9.95)	3.87 (2.15–6.97)
CD4 cell count at TB diagnosis					
≥200 cells/μL	25	836.9	29.9 (20.2–44.2)	ref (1)	ref (1)
<200, ≥100 cells/μL	10	462.9	21.6 (11.6–40.1)	0.72 (0.35–1.51)	1.17 (0.55–2.48)
<100 cells/μL	20	673.9	29.7 (19.1–46.0)	0.99 (0.55–1.79)	2.57 (1.34–4.94)
Viraemia copy-years from HIV to TB, median log_{10} copies/mL[f]					
≥log_{10} 5 copy-years/mL	19	307.0	61.9 (39.5–97.0)	2.27 (1.03–5.02)	
<log_{10} 5 copy-years/mL	9	353.2	25.5 (13.3–49.0)	ref (1)	
Charlson co-morbidity score					
0	29	1371.4	21.1 (14.7–30.4)	ref (1)	
1	9	221.3	40.7 (21.2–78.2)	1.92 (0.91–4.06)	
2–5	5	97.2	51.4 (21.4–123.6)	2.43 (0.94–6.28)	
≥6	12	283.8	42.3 (24.0–74.4)	2.0 (1.02–3.92)	
Non-TB AIDS before TB diagnosis					
No	45	1691.0	26.6 (19.9–35.6)	ref (1)	
Yes	10	282.8	35.4 (19.0–65.7)	1.33 (0.67–2.64)	
ART initiation (time-updated)					
Time before ART initiation	11	126.8	86.7 (48.1–156.7)	3.64 (1.88–7.05)	
Time after ART initiation	44	1847.0	23.8 (17.7–32.0)	ref (1)	
Calendar time (time–updated)					
1995–2002	9	269.3	33.4 (17.4–64.2)	ref (1)	
2003–2009	23	701.4	32.8 (21.8–49.3)	0.98 (0.45–2.12)	
2010–2014	15	661.5	22.7 (13.7–37.6)	0.68 (0.30–1.55)	
2015–2017	8	341.5	23.4 (11.7–46.8)	0.70 (0.27–1.82)	
HIV status at TB diagnosis					
Concomitant diagnosis[g]	13	1044.8	12.4 (7.2–21.4)	ref (1)	ref (1)
Known HIV infection[h]	42	928.9	45.2 (33.4–61.2)	3.63 (1.96–6.77)	4.34 (2.17–8.66)
Time from TB diagnosis (time-updated)					
First 6 months	16	101.7	157.3 (96.3–256.7)	7.54 (4.22–13.51)	5.37 (2.92–9.84)
Beyond 6 months	39	1872.0	20.8 (15.2–28.5)	ref (1)	ref (1)
TB localization					
Extrapulmonary	4	511.5	7.82 (2.9–20.8)	ref (1)	ref (1)
Pulmonary	31	819.6	37.8 (26.6–53.8)	4.84 (1.71–13.70)	2.74 (0.94–8.05)
Disseminated	20	642.7	31.1 (20.1–48.2)	3.98 (1.36–11.64)	3.32 (1.11–9.94)
Recurrence of TB during the study period					
No	47	1829.1	25.7 (19.3–34.2)	ref (1)	
Yes	8	144.7	55.3 (27.7–110.6)	2.15 (1.02–4.55)	

Abbreviations: aMRR, adjusted mortality rate ratio; ART, antiretroviral therapy; HIV, human immunodeficiency virus; IDU, illicit drug use; MMR, mortality rate ratio; MR, mortality rate; PYR, person-years at risk; TB, tuberculosis.

[a] Adjusted for age, gender, migrant, social burden, CD4 cell count, HIV status at TB diagnosis, first half year from TB diagnosis (time-updated), and TB localization. Same results were found when age and gender were not included in the final model.

[b] Age, ≥40 years versus <40 years at time of TB diagnosis (time updated).

[c] Alcohol consumption defined as >7/14 drinks per week for women and men respectively.

[d] Tobacco consumption defined as ≥10 cigarettes daily.

[e] Social burden defined as alcohol consumption >7/14 drinks per week, illicit drug use, homelessness, stay in prison or shelter and/or prostitution.

[f] Viraemia copy-years from HIV to TB diagnosis, median Log_{10} copies/mL in those patients with known HIV infection, ≥ log_{10} 5 copy-years/mL versus < log_{10} 5 copy-years/mL.

[g] TB was diagnosed at up to 1 month before or within 3 months after HIV diagnosis.

[h] TB was diagnosed >3 months after HIV diagnosis.

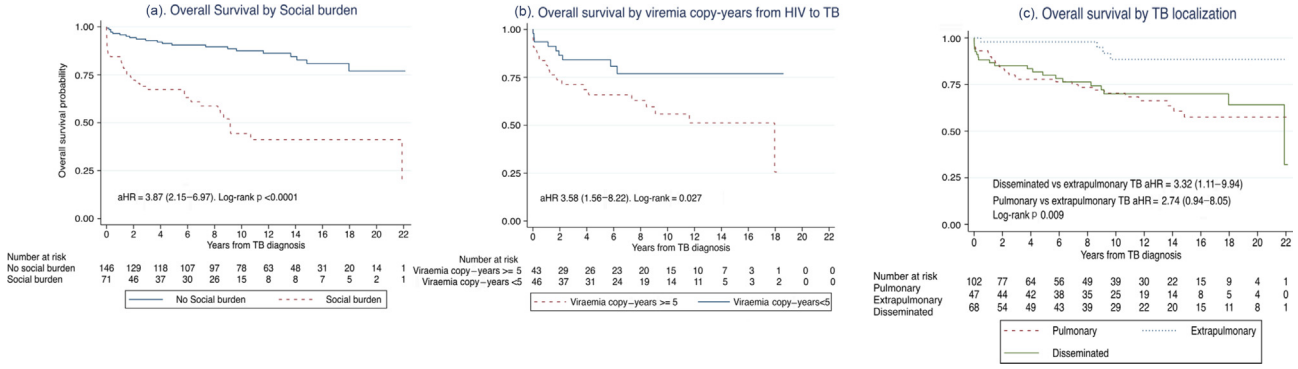

Fig. 2. Kaplan–Meier curves for overall survival after tuberculosis diagnosis in human immunodeficiency virus (HIV) -infected individuals stratified by social burden, viraemia copy-years and tuberculosis (TB) localization. (a) Overall survival by social burden. Social burden was defined as alcohol consumption >7/14 units per week, illicit drug use, homelessness, stay in shelter or prison, and/or prostitution. (b) Overall survival by HIV viraemia copy-years to TB diagnosis in patients with previously diagnosed HIV. Viraemia copy-years ≥ 5 $\log_{10}$ copy-years/mL versus <5 $\log_{10}$ copy-years/mL. Adjusted hazard ratio (aHR) 3.58 (model adjusted for age (time-updated), gender, social burden, viraemia copy-years, HIV status at TB diagnosis and TB localization). (c) Overall survival by TB localization.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2021.07.036.

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