On HIV in the elderly and vitamin B metabolism in HIV infection

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"Vi måste skydda de nya från att komma in i det här gänget,
och så måste vi ta hand om de gamla."

Torbjörn
Ur *Leva Livet – Att åldras med hiv*

ABSTRACT

The evolution of the human deficiency virus (HIV) field is unparalleled in the history of infectious diseases. From the first cases in the beginning of the 1980s, when an HIV diagnosis was a death sentence, through the discovery of the first effective medicines, up till today when people living with HIV (PLHIV) with access to antiretroviral therapy (ART) can lead a near normal life. The aim of this thesis was to investigate further into two areas where knowledge is still lacking, and important questions remain. We investigated HIV in the elderly and the role of vitamin B metabolism in HIV-associated central nervous system (CNS) disease.

In paper I and II we studied HIV infection in the elderly (≥ 65 years of age) compared to a control group (≤ 49 years of age). In a study of cross-sectional design 100 elderly PLHIV and 99 controls, on ART regimens containing atazanavir, darunavir, or efavirenz were included. In paper I we showed that elderly had a higher number of concomitant medications, comorbidities, and potential drug-drug interactions, than the younger controls. In the darunavir arm, the elderly had higher steady-state concentrations. This was also found in the atazanavir arm, although not statistically different, but suggesting a possible class effect of protease inhibitors. Paper II investigated the role of ART regimen on markers of inflammation and immune activation in elderly PLHIV. The regimens had different inflammatory profiles with lower interleukin-6 levels in the atazanavir arm, and lower ICAM-1 in the efavirenz arm. The darunavir arm had higher CXCL10 levels compared to the efavirenz arm.

Paper III and IV studied the role of homocysteine and vitamin B metabolism in CNS injury in HIV infection. Paper III describes an association between plasma homocysteine, a marker of vitamin B_{12} and folate deficiency, and cerebrospinal fluid neurofilament light protein (NfL), a sensitive marker of neuroaxonal damage in HIV infection. In paper IV this association was further studied in a randomised controlled clinical trial. Sixty-one virally suppressed PLHIV were randomised either to the active treatment arm (treatment with vitamin B_{12}, B_6, and folate) or control arm. After 12 months the levels of homocysteine had decreased, and the plasma B_{12} and folate levels had increased in individuals in the treatment arm. However, no difference in plasma levels of NfL was found compared to the control arm at 12 months. Furthermore, in the treatment arm, no difference in NfL was found after 24 months, compared to baseline plasma NfL levels.

In conclusion, we found that elderly PLHIV are at risk of adverse drug events through a high prevalence of concomitant medications, potential drug-drug interactions, and higher drug concentrations of protease inhibitors. In addition, we found different inflammatory profiles of efavirenz, atazanavir, and darunavir, a finding that needs to be confirmed in future studies. Furthermore, a novel finding of an association between homocysteine and NfL was made. However, supplementation with B vitamins did not decrease NfL, suggesting a non-vitamin B dependent cause of the association.

Keywords: HIV-1, elderly, drug levels, potential drug-drug interactions, inflammation, immune activation, antiretroviral therapy, homocysteine, neurofilament light protein.

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SAMMANFATTNING PÅ SVENSKA

I juni 1981 rapporterades det om de första fallen av vad som skulle bli känt som förvärvat immunbristsyndrom, AIDS. De första fallen diagnosticerades i USA men snart förstod man att det rörde sig om en global pandemi. 1983 lyckades man för första gången med att isolera viruset som orsakar AIDS, humant immunbristvirus (HIV). I slutet av 1980-talet utvecklades de första medicinerna och sedan mitten av 90-talet har det funnits effektiva mediciner. Idag kan en person med HIV, som står på behandling, leva ett normalt liv. Detta har lett till nya frågeställningar kring långtidseffekter av behandling och åldersrelaterade sjukdomar. Denna avhandling fokuserar på två områden där det saknas viktig kunskap, HIV hos de äldre och B-vitamin metabolismens relation till nervskada hos de som lever med HIV.

I takt med att fler och fler fått tillgång till effektiva mediciner mot HIV så har de som lever med HIV blivit äldre. I Sverige är runt hälften över 50 år gamla och allt fler blir äldre än 65 år. Med åldern ökar risken för att utveckla åldersrelaterade sjukdomar och sannolikheten för att ta flera olika läkemedel. Kroppen förändras vilket gör att man kan vara känsligare för läkemedel. Det saknas viktig kunskap om vad detta innebär för de som lever med HIV. För att undersöka detta genomförde vi den tvärsnittsstudie som är grunden för arbete I och II. I arbete I jämförde vi en grupp personer som lever med HIV som var 65 år eller äldre med en grupp som var 49 år eller yngre. Som förväntat hade de äldre fler sjukdomar och tog fler läkemedel än de yngre. Vi fann att koncentrationerna i blodet av en grupp HIV-läkemedel, proteashämmare, var högre hos de äldre. Hos personer som tog ett av dessa läkemedel, darunavir, var biverkningar vanligare. Vidare upptäckte vi att de äldre oftare hade en kombination av läkemedel som kan påverka varandra på ett ofördelaktigt sätt och att det var vanligt med polyfarmaci, dvs. att man använder fler än fem läkemedel varje dag.

Jämfört med sina jämnåriga har personer som lever med HIV en ökad risk att utveckla åldersrelaterade sjukdomar. Forskning talar för att en anledning till detta kan vara att de som lever med HIV har en högre nivå av inflammation i kroppen trots välfungerande behandling än de som inte har HIV. För att undersöka om valet av läkemedel påverkar denna inflammation undersökte vi i delarbete II 10 olika inflammationsmarkörer hos de äldre baserat på läkemedelsregim. Vi fann skillnader för tre olika markörer. De som behandlades med atazanavir hade lägre IL-6, de som behandlades med efavirenz hade lägre ICAM-1 och de som behandlades med darunavir hade högre CXCL10.

Utan antiretroviral behandling skulle många som lever med HIV utveckla en särskild form av demens på grund av infektionen. Även innan symptom utvecklas kan man se tecken på nervskada i hjärnan som ökar i takt med att sjukdomen förvärras. Antiretroviral behandling hindrar utvecklingen av demens och nervskadan minskar. Vissa personer har trots det kvarvarande tecken på en låggradig pågående nervskada, som kan mätas med biomarkören neurofilament light protein (NfL). Orsaken till denna kvarvarande skada är inte helt klarlagd men man kan också se tecken på immunaktivering hos personer med välbehandlad HIV. För att utreda denna kvarvarande skada mätte vi i delarbete III nivåer av NfL i ryggmärgsvätska och nivåer av homocystein i blod. Homocysteinnivåer har tidigare kopplats till demens och kognitiv påverkan hos HIV-negativa personer. Vi fann ett samband där högre NfL-nivåer var kopplat till högre nivåer av homocystein. Detta utredde vi vidare i delarbete IV. Homocysteinnivåerna i blodet är beroende av B-vitaminerna B_{12}, B_6 och folsyra och homocysteinnivåer sjunker vid behandling med B-vitaminer. I delarbete IV genomförde vi en randomiserad kontrollerad studie av personer som lever med HIV där vi gav B-vitaminer till den ena gruppen medan den andra gruppen inte fick någon behandling med B-vitaminer. Vi mätte NfL-nivåer och homocysteinnivåer innan och under behandlingen och såg att homocysteinnivåerna sjönk hos de som fick behandling men inte NfL-nivåerna. Detta talar emot att låga B-vitaminnivåer är en bidragande orsak till kvarvarande nervskada hos personer som lever med HIV med välfungerande behandling.

Sammanfattningsvis har denna avhandling ökat kunskapen kring HIV och läkemedelsbehandling hos äldre personer med HIV. Vi har visat att denna grupp är i risk för ogynnsamma effekter av läkemedel, genom ökade läkemedelskoncentrationer av vissa HIV-läkemedel, risk för interaktioner mellan läkemedel och polyfarmaci. Vi har också visat att val av antiretroviralbehandling kan ha betydelse för inflammationsnivåer. Utöver det har vi funnit ett samband mellan tecken på nervskada och homocysteinnivåer hos personer med HIV med behandling, men kunde in se någon effekt av behandling med B-vitaminer vilket talar för att sambandet beror på en annan orsak.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Tyrberg E, Edén A, Eriksen J, Nilsson S, Treutiger CJ, Thalme A, Mellgren Å, Gisslén M, Andersson LM.

Higher plasma drug levels in elderly people living with HIV treated with darunavir

PLoS ONE 2021; 16(2): e0246171

II. Tyrberg E, Skovbjerg S, Samuelsson E, Nilsson S, Edén A, Treutiger CJ, Thalme A, Mellgren Å, Gisslén M, Andersson LM

Markers of inflammation and immune activation in elderly HIV-1 infected individuals on stable ART treatment with efavirenz, darunavir, or atazanavir

In manuscript

III. Ahlgren E, Hagberg L, Fuchs D, Andersson LM, Nilsson S, Zetterberg H, Gisslén M

Association between Plasma Homocysteine Levels and Neuronal Injury in HIV infection

PLoS ONE 2016; 11(7): e0158973

IV. Tyrberg E, Hagberg L, Andersson LM, Nilsson S, Yilmaz A, Mellgren Å, Blennow K, Zetterberg H, Gisslén M

The effect of vitamin B supplementation on neuronal injury in PLHIV – a randomised controlled trial

Submitted manuscript

CONTENT

ABBREVIATIONS

5-methyl-THF	5-methyl-tetrahydrofolate
AIDS	Acquired immunodeficiency syndrome
ANI	Asymptomatic neurocognitive impairment
ART	Antiretroviral therapy
ATV	Atazanavir
CCR5	CC chemokine receptor 5
CD	Cluster of differentiation
CDC	Centers for Disease Control and Prevention
CNS	Central nervous system
CSF	Cerebrospinal fluid
CXCL10	C-X-C motif chemokine ligand 10
CXCR4	CXC chemokine receptor 4
DRV	Darunavir
EI	Entry inhibitor
EFV	Efavirenz
HAD	HIV-associated dementia
HAND	HIV-associated neurocognitive disorder
HIV	Human immunodeficiency virus
ICAM-1	Intercellular adhesion molecule-1
IL-6	Interleukin-6

INSTI	Integrase strand transfer inhibitor
IF	Intrinsic factor
MMA	Methylmalonic acid
MND	Mild neurocognitive disorder
NfL	Neurofilament light protein
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NRTI	Nucleoside reverse transcriptase inhibitor
P	Plasma
PDDI	Potential drug-drug interaction
PI	Protease inhibitor
PLHIV	People living with HIV
s	Soluble
SAH	S-adenosylhomocysteine
SAM	S-adenosylmethionine
SIV	Simian immunodeficiency virus
SMART	Strategies for Management of AntiRetroviral Therapy
START	Strategic Timing of AntiRetroviral Treatment
STOPP	Screening tool of older people's prescriptions

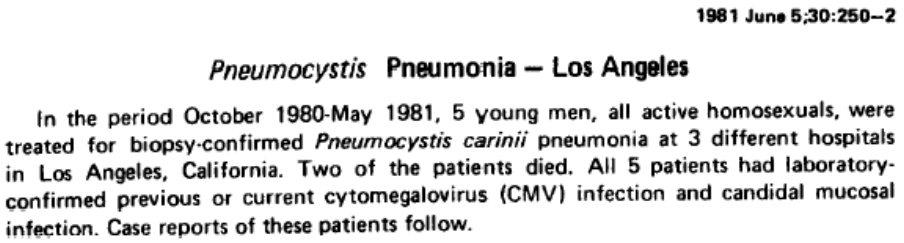
Introduction

1 INTRODUCTION

When looking back on the last 40 years the picture drawn is remarkable. The evolution of the human immunodeficiency virus (HIV) field is unparalleled in the history of medicine. From the first cases in the beginning of the 1980s, when an HIV diagnosis was a death sentence, through the discovery of the first effective medicines, up till today when people living with HIV (PLHIV) with access to antiretroviral therapy (ART) can lead a near normal life.

1.1 THE PANDEMIC

The first notion of the emerging epidemic was when the Morbidity and Mortality Weekly Report on Friday 5th of June 1981 published a report of five young men in Los Angeles presenting with pneumocystis pneumonia, a kind of pneumonia associated with immunodeficiency.[1]



1981 June 5;30:250–2

Pneumocystis Pneumonia — Los Angeles

In the period October 1980-May 1981, 5 young men, all active homosexuals, were treated for biopsy-confirmed *Pneumocystis carinii* pneumonia at 3 different hospitals in Los Angeles, California. Two of the patients died. All 5 patients had laboratory-confirmed previous or current cytomegalovirus (CMV) infection and candidal mucosal infection. Case reports of these patients follow.

Figure 1. Extract of the first published report on AIDS.[1]

A month later, in July, it was reported that 26 men living in New York and California were diagnosed with diseases related to immunosuppression, such as pneumocystis pneumonia and Kaposi sarcoma.[2] This was soon followed by several additional reports describing a similar clinical syndrome of low $CD4^+$ cell counts and diseases related to immunodeficiency. In September 1982 the Centers for Disease Control and Prevention (CDC) in the United States named the disease acquired immunodeficiency syndrome, AIDS.[3]

Initially, it was not known that AIDS was caused by a virus. The recent finding that retroviruses could cause infection in humans[4] led the way to the discovery of HIV-1 in 1983, by French scientists Francoise Barré-Sinoussi and Luc Montagnier, who were later awarded the Nobel prize in 2008.[5] During the next

year Robert Gallo and his group showed that HIV was the causative agent of AIDS which was confirmed by others.[6-8] The groups all named the virus differently, and it was not until 1986 that the newly discovered virus was officially named the human immunodeficiency virus-1 (HIV-1).[9]

The first American reports described cases of men who have sex with men, but soon cases of AIDS were also reported in individuals with haemophilia (receiving blood products), intravenous drug users, and heterosexual individuals.[3] Parallel to the unrevealing of the epidemic in the US, a report came from Belgium of immigrated men from Africa who presented with AIDS.[10] It was soon evident that AIDS affected several countries in Africa.[11-14] The reports from Africa portrayed a disease equally affecting women.[11, 12] In 1983 the first publications on vertical transmission, from mother to child, were published.[15]

The search for the origin of the pandemic led researchers to the African continent. A group of viruses, simian immunodeficiency viruses (SIVs), genetically related to HIV-1 was found among nonhuman primates living in sub-Saharan Africa.[16, 17] The virus with the closest resemblance to HIV-1 was found among chimpanzee.[17] It is believed that cross-species transmission from chimpanzee to humans have occurred at least four times, giving rise to the four known groups of HIV-1 (M, N, O, and P). M and N strains are known to originate from SIV infecting chimpanzee of the subspecies *Pan troglodytes troglodytes*.[18] The specific origin of O and P strains is not established.[19] How HIV-1 was first transmitted to humans is not known, but it is proposed to be through consumption and handling of bushmeat.[19, 20] Based on studies of the evolution of HIV researchers have localised the cradle of the pandemic to Kinshasa (Leopoldville at the time),[19] and the oldest diagnosed case derives from retrospective analysis of a plasma sample from 1959.[21] It is believed that the initial transmission occurred at the beginning of the 20th century.[19, 22]

Some years after the discovery of HIV-1, in 1986, another virus capable of causing AIDS in humans was found. The virus was named HIV-2,[23] and it originated from a SIV strain infecting sooty mangabey.[20, 24] Compared to HIV-1, HIV-2 is less pathogenic and less transmissible. It constitutes a smaller epidemic primarily localised to West Africa.[19, 23] This thesis will only cover HIV-1 (hereafter called HIV).

1.2 THE HUMAN IMMUNODEFICIENCY VIRUS

HIV belongs to the family of retroviridae, and the genus lentivirus.[25] Retroviruses are unique in that they contain the enzyme reverse transcriptase that translates RNA to DNA,[26] in contrast to the human transcription enzymes that translate

DNA to RNA. The lentiviruses are characterised by the slow disease progression that they give rise to.[25, 26]

The HIV virion is approximately 100 nm in diameter.[25, 27] It is made up of a lipid membrane envelope carrying the glycoproteins gp120 and gp41. Inside the envelope is a cone-shaped capsid containing the viral genome, consisting of two identical single stranded RNA molecules, the important viral enzymes (reverse transcriptase, integrase, protease), and accessory proteins.[25, 27] The genome includes the three major genes *gag, pol*, and *env* that in turn are responsible for encoding the structural proteins, viral enzymes, and envelope glycoproteins. In addition, the genome includes genes encoding the different regulatory and accessory proteins (Tat, Nef, Rev, Vif, Vpu and Vpr) important for e.g. viral replication and intracellular transport.[25, 27]

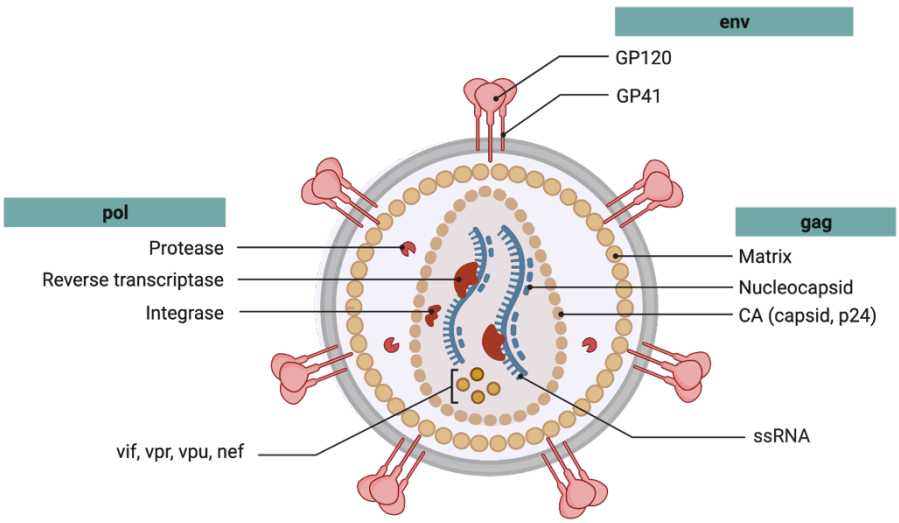


Figure 2. The human immunodeficiency virus.[28]

HIV is divided into four groups, based on genetic differences and origin, M (major), O (outlier), N (non-m/non-O), and P.[29] Group M constitutes the virus responsible for the pandemic, whereas N, O, and P are found primarily in western Africa. The M group is divided in turn into nine subgroups A–D, F–H, J, K.[19] In addition, recombinant forms of different subtypes of HIV exist.[30, 31] The different subtypes are unevenly spread globally where subgroup B predominate in Europe and North America, C in India, whereas a diverse panorama of types is present in Africa.[29]

1.2.1 LIFE CYCLE

HIV target cells that present the CD4 receptor ($CD4^+$ T cells) on their cell surface. These cells include T lymphocytes, monocytes, macrophages, microglia, and dendritic cells.[25, 32] The gp120 glycoprotein on the viral surface binds to the CD4 receptor resulting in a conformational change of the gp120 that enables binding to a co-receptor on the cell surface, CC chemokine receptor 5 (CCR5) or CXC chemokine receptor 4 (CXCR4).[25, 27, 30, 33] The virus envelope hereafter fuses with the cell membrane and the viral capsid is released into the cytoplasm.[25, 27, 30] In the next step viral RNA is translated into DNA by the viral enzyme reverse transcriptase and uncoating occurs.[25, 27] For a long time it has been believed that the uncoating occurs in the cytoplasm, either soon after the viral entry into the cell, stepwise, or at the nucleus.[34, 35] Interestingly, new data propose that the capsid disassembles in the nucleus and that the transcription process is completed within the capsid inside the nucleus.[34, 36, 37] In the nucleus the second viral enzyme, integrase, integrates the proviral DNA into the host genome.[25, 30] The viral DNA is hereafter transcribed by the cell RNA polymerase II to viral RNA and transported to the cytoplasm.[27] The ribosome translates viral RNA to three precursor polyproteins, Gag, Gag-pol, and Env, and the accessory and regulatory proteins.[27, 38] These assemble with viral RNA at the cell membrane and subsequently bud of as a new virion.[27, 38] Concomitantly, the third viral enzyme, the protease, splits the precursor proteins to the structural and enzymatic proteins resulting in a conformational change and the final maturation of the virus.[27, 30, 38]

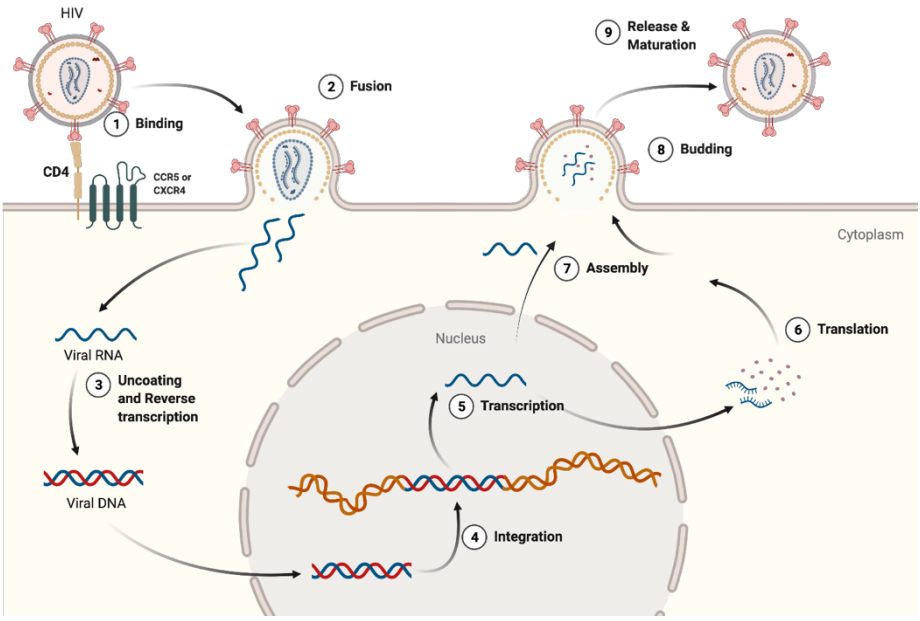


Figure 3. The life cycle of HIV.[39] *The virion binds to the CD4 receptor and a co-receptor (CCR5 or CXCR4) (1) and then enters the cell by fusion (2). In the next step uncoating and reverse transcription of viral RNA to DNA by the reverse transcriptase takes place (3). The viral DNA is then integrated into cell DNA by the viral integrase (4). After transcription of viral RNA (5), RNA is translated to viral proteins (6). The viral proteins and two strands of viral RNA assemble at the cell membrane (7) where it buds of as a new virion (8). In the last step the viral protease splits the precursor proteins and a new infective virion is produced (9).*

The HIV reverse transcriptase lacks proof reading, making it prone to errors. In combination with the high rate of replication (est. 10^{10} per day in untreated HIV infection), this gives rise to frequent mutations.[30] Furthermore, frequent recombination occurs.[40] The high variability is the basis for how the virus evades the immune response, develops resistance to ART, and one of the reasons why developing a vaccine is so challenging.[25]

1.2.2 TROPISM

The HIV strains are divided into two major groups, R5 and X4 viruses. The basis is their use of co-receptor, where R5 virus use the CCR5 receptor and X4 virus the CXCR4 receptor.[33, 41] The R5 virus is dominant during establishment of infection and the early stages of infection.[33, 42] Interestingly, individuals with a homozygous mutation in the gene coding the CCR5 receptor (CCR5Δ32) are protected from HIV infection with R5 virus.[33, 43] The emergence of X4 virus is

seen in many individuals during the course of infection, and is associated with disease progression and loss of immune function.[30, 33, 44] The CCR5 receptor is in addition to T cells found on macrophages, monocytes, dendritic cells, giving rise to the older term macrophage tropic (M-tropic) for R5 virus.[42] Similarly, X4 virus was previously termed T lymphocyte tropic (T-tropic), since the CXCR4 receptor is primarily found in T cell lines.[31, 33] In addition, dual tropic, able to bind both CXCR4 and CCR5 receptors, exist.[25, 33]

1.3 FROM HIV TO AIDS

HIV transmission can occur through three different pathways. The most common route is sexual contact. Other routes of transmission are vertical transmission from mother to child and exposure to blood or blood products (e.g. intravenous drug use or iatrogenically).[45]

How HIV crosses the mucosal barrier is not known in detail but infection can occur both by free virus and cell-associated virus.[32] HIV initially infects dendritic cells, macrophages, and T cells in the submucosa but rapidly spreads to local lymph nodes and is subsequently disseminated to the bloodstream and other organs such as the gastrointestinal tract, spleen and bone marrow where infection of a large number of cells occurs.[25, 30, 42] In this initial stage of infection, the lack of specific immune responses allow the virus to rise rapidly to peak levels, resulting in a simultaneous decline in $CD4^+$ cell count. This coincides with the acute retroviral syndrome seen 2–4 weeks after transmission in a majority of individuals.[45] Clinically, acute infection often presents with flu-like or mononucleosis-like symptoms, but can present with a range of other symptoms such as rash, meningitis, or diarrhoea.[25, 45, 46] Because of the unspecific picture, acute symptomatic infection is often not identified as HIV, and hence often passes undiagnosed. The symptoms in acute infection are self-limiting, and resolve in the course of one to two weeks.[25]

As the immune response awakens, the immune system takes partial control over the infection, viral levels drop, and the infection enters its asymptomatic chronic phase.[25, 31] During this phase the viral levels reach a steady-state, called the viral set point, probably related to the emergence of HIV-specific $CD8^+$ cytotoxic T cells.[30, 47] The set point level predicts the progress to advanced disease.[48] In some individuals the viral levels drop to very low levels. These individuals are called elite controllers.[49] Parallel to the decrease in viral load, the $CD4^+$ cell count recovers but usually not to pre-transmission levels.[25]

Although clinically asymptomatic, active viral replication continues throughout the chronic phase, eventually resulting in progressive CD4+ T cell loss, both in peripheral blood and mucosal tissues, and destruction of the lymphoid tissue.[25, 30] The number of infected CD4+ T cells are too few to solely explain the quantity of cell loss. It is considered that the chronic activation of the immune system plays a crucial role in the depletion of T cells and pathogenesis of HIV. This is supported by the impact not only on CD4+ T cells but also on CD8+ T cells, B cells and NK cells.[50] Over time, the immune system weakens and the immune function deteriorates to a point where the individual is at risk of contracting opportunistic infections (infections that normally do not affect the immunocompetent host) and malignancies, such as Kaposi sarcoma and lymphomas. HIV has developed to AIDS.[25, 30] There are a range of AIDS-defining diagnoses stipulated by the CDC (table 1), which usually occur when CD4+ cell levels drop below 200 cells/mm^3. The time lapse from acute infection to onset of AIDS has a large interindividual variation, but is approximately 10 years.[25] (Fig. 4)

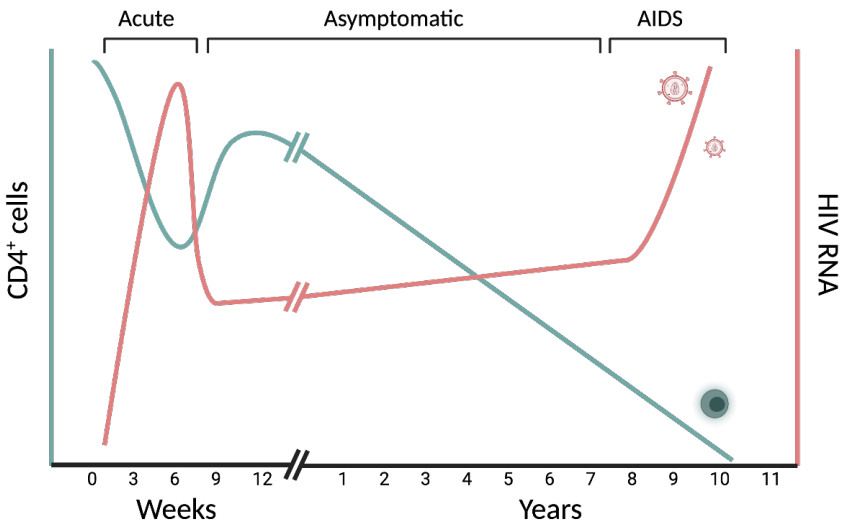


Figure 4. The natural course of HIV infection.[51]

Table 1. AIDS-defining diagnoses.

AIDS-defining diagnoses for adults
Candidiasis of bronchi, trachea, lungs, or esophagus
Cervical cancer, invasive
Coccidioidomycosis, disseminated or extrapulmonary
Cryptococcosis, extrapulmonary
Cryptosporidiosis, chronic intestinal (>1 month)
CMV disease (other than liver, spleen, or nodes)
CMV retinitis
Encephalopathy, HIV-related
Herpes simplex: chronic ulcers (>1 month); bronchitis, pneumonitis, esophagitis
Histoplasmosis, disseminated or extrapulmonary
Isosporiasis, chronic intestinal (>1 month)
Kaposi sarcoma
Lymphoma (Burkitt, immunoblastic, or primary, of brain)
Mycobacterium avium complex or *M. kansasii*, disseminated or extrapulmonary
Mycobacterium tuberculosis, of any site
Mycobacterium, other species or unidentified species, disseminated or extrapulmonary
Pneumocystis jirovecii pneumonia
Pneumonia, recurrent
Progressive multifocal leukoencephalopathy
Salmonella septicemia, recurrent
Toxoplasmosis of brain
Wasting syndrome due to HIV

1.4 ANTIRETROVIRAL THERAPY – THE PARADIGM SHIFT

Before the introduction of ART, HIV infection was a fatal disease in almost every case. This led to intense research efforts to find effective drugs. The first drug was approved in 1987, the nucleoside reverse transcriptase inhibitor (NRTI) zidovudine. The first randomised trial with a duration of 24 weeks showed benefit of treatment in a group of individuals with late stage disease.[52] But subsequent trials of treatment in earlier stages of disease reported disheartening results,

without benefit on disease progression.[53] During the early 90s zidovudine was followed by additional NRTIs, also these without lasting effect.

The big breakthrough came with the introduction of the first protease inhibitors (PIs) in 1995 and the use of combined ART. During the following years, studies showed the durable viral suppression and clinical benefit of combining a PI with two NRTIs compared to earlier NRTI regimens.[54-56] The dramatic effect of combination therapy was well illustrated by the decline in mortality from 29.4 deaths per 100 person-years in 1995 to 8.8 in 1997, showed by Palella et al.[55] Based on the rate of viral decay during treatment, it was estimated that HIV could be cured after 2–3 years of ART but this initial hope was turned into disappointment with the discovery of latent viral reservoirs that were not susceptible to available treatment regimens, as discussed later.[57, 58]

Although effective, ART was also associated with side effects and adverse events. The early drugs induced metabolic changes such as lipodystrophy and there was a fear of increased cardiovascular risk.[59, 60] This gave rise to the idea of treatment-sparing strategies,[61] but initial studies showed discordant results.[62] The SMART (Strategies for Management of AntiRetroviral Therapy) study compared continuous ART with deferred treatment. In the deferred treatment arm, treatment was guided by $CD4^+$ cell counts, whereby an individual who dropped below 250 cells/mm^3 in $CD4^+$ cell count initiated ART and subsequently stopped when the $CD4^+$ cell count raised above 350. In January 2006 the SMART trial was prematurely ended when it became clear that the continuous treatment arm not only experienced less AIDS-related morbidity and mortality, but also less non-AIDS-related morbidity and mortality.[62]

Almost a decade later the results of the START (Strategic Timing of AntiRetroviral Treatment) study were published adding to the knowledge of the beneficial effects of ART. At the time, initiation of ART was recommended to start at a $CD4^+$ cell count of 350 cells/mm^3 in asymptomatic individuals. The START study randomised participants to either immediate initiation of ART, regardless of $CD4^+$ cell levels, or to commence ART when the $CD4^+$ cell count was ≤ 350, with the aim of studying the risks and benefits of early ART. In May 2015 the study was stopped early because of the benefits of early ART seen regarding serious AIDS-related events and serious non-AIDS-related events.[63] This knowledge led the way to the revision of treatment guidelines globally to recommend treatment to all, independent of $CD4^+$ cell count.

Today the arsenal of ART in Sweden consists of five different drug groups with different mechanisms of action. In addition to the above mentioned NRTIs and PIs they include: non-nucleoside reverse transcriptase inhibitors (NNRTIs), integrase inhibitors (INSTIs) and entry inhibitors (EIs). Their mechanisms of action are described in figure 5.

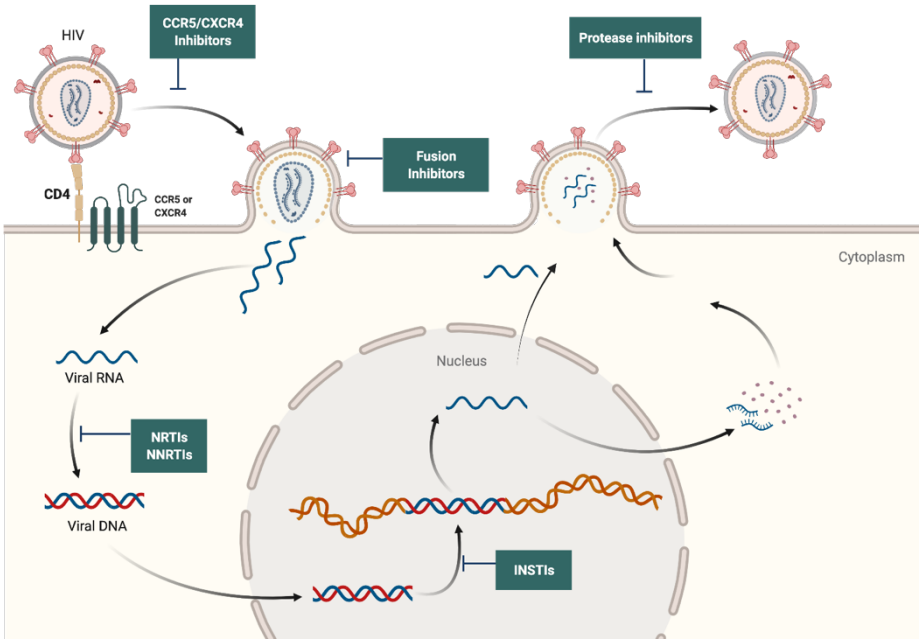


Figure 5. Entry inhibitors (CCR5/CXCR4 and fusion inhibitors) block the receptor-binding, fusion and entry of the virus into the cell. NRTIs/NNRTIs inhibit reverse transcription, and thus the translation of viral RNA to DNA. INSTIs interfere with the viral enzyme integrase, and thus inhibit the incorporation of viral genome in the DNA of the cell. PIs block the enzymatic cleavage of precursor proteins, inhibiting maturation of functional virions.[64]

The first Swedish national recommendations for the treatment of HIV were written in the late 1990s and have since been updated on a regular basis. The standard regimen still today consists of a backbone of two NRTIs and one third agent, either an INSTI, a PI, or NNRTI. When starting ART in a treatment naïve individual, the first line recommendation today in Sweden is a combination of the two NRTIs tenofovir and emtricitabine and the INSTI dolutegravir or the NNRTI doravirine, although treatment can be individualised as needed. In the course of the last years a two-drug regimen consisting of lamivudine and dolutegravir has been shown to be effective and is now available as a switch therapy after an initial three drug regimen in patients with a high level of compliance and no pre-existing drug resistance. The most recent additions to the

arsenal are long-acting intramuscular injection formulas. The recommended antiretroviral drugs in Sweden today are presented in table 2.

Group	Generic name	Abbreviation	Trade name
NRTI			
	abacavir	ABC	Ziagen / Abacavir
	emtricitabine	FTC	Emtriva
	lamivudine	3TC	Epivir / Lamivudine
	tenofovir disoproxil	TDF	Viread / Tenofovir disoproxil
	tenofovir alafenamide	TAF	Vemlidy
NNRTI			
	efavirenz	EFV	Stocrin / Efavirenz
	nevirapine	NVP	Viramune / Nevirapine
	etravirine	ETR	Intelence
	rilpivirine	RPV	Edurant / Rekambys (injection)
	doravirine	DOR	Pifeltro
PI			
	atazanavir	ATV	Reyataz / Atazanavir
	darunavir	DRV	Prezista / Darunavir
	ritonavir	RTV	Norvir / Ritonavir
INSTI			
	raltegravir	RAL	Isentress
	dolutegravir	DTG	Tivicay
	cabotegravir	CAB	Vocabria
	bictegravir	BIC	(Not available as single drug)
	elvitegravir	EVG	(Not available as single drug)
EI			
	maraviroc	MVC	Celsentri
	enfuvirtide	T-20	Fuzeon
	fostemsavir	FTR	Rukobia
	ibalizumab	IBA	Trogarzo

Table 2. Recommended antiretroviral drugs in Sweden 2021.

1.4.1 TREATMENT AS PREVENTION

With neither a cure nor a potent vaccine in the near horizon, effective ART remains our best way of preventing HIV transmission and halting the epidemic. Transmission risk is related to HIV RNA viral load.[65] The first notion of ART as a prevention strategy came with a publication in 1994 by Connor et al., who showed that zidovudine treatment to the mother peripartum and to the child postpartum reduced the risk of vertical transmission of HIV.[66]

In the last decade several studies have investigated the risk of transmission of HIV during ART by studying heterosexual and homosexual serodiscordant couples.[67-69] These studies have presented mounting evidence that HIV is not sexually transmittable when HIV RNA viral load is undetectable in standard assays.[68-72]

This has subsequently led to the revision of the Swedish Communicable Diseases Act, and the rules of conduct, such as the requirement of condom use.

Furthermore, prophylactic ART, preexposure prophylaxis (PrEP), has been proven to effectively prevent HIV transmission in high-risk groups.[73-75]

1.5 LATENCY AND RESERVOIRS

In 1995 the first study showing evidence of latent infection was published.[76] Latency is defined as resting cells with integrated proviral DNA that can give rise to viral replication upon stimulation and subsequent activation of the resting cell.[77] This latent infection allows viral persistence despite ART,[78-80] and also evades elimination by the immune system. Latently infected cells are primarily resting memory T cells, but latency can also occur in other cell types such as monocytes, macrophages, and follicular dendritic cells.[81] These latently infected cells constitute the cellular reservoir.

Latent infection is established very early after transmission, probably within days. ART initiation during the acute phase does not hinder the establishment of the reservoir but diminish the size.[81] Only one out of a million resting T cells are latently infected[82] but due to their long half-life ART would have to continue for over 70 years to eradicate the reservoir.[58, 83] Consequently, the reservoir makes cure of HIV through ART unfeasible.

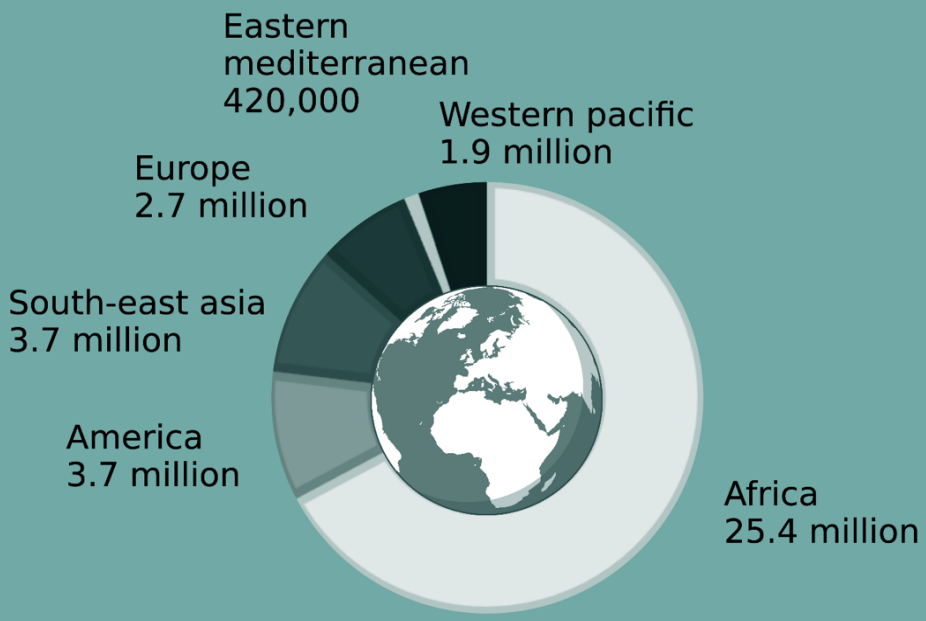
In addition, the reservoir concept is also used to describe anatomical sites where persistence of replication-competent virus may occur.[77] Lymph nodes, gut-associated lymphoid tissue, central nervous system (CNS) and the genital tract are proposed to be such anatomical reservoirs.[81]

1.6 HIV TODAY

Up till today the HIV pandemic has claimed more than 36 million lives. Today 37.7 million people live with HIV globally, whereof approximately 8,000 in Sweden (InfCare HIV, December 2021). Even though there is much work left to do, the testing, treatment and prevention strategies are successful. Compared to 2010, 30% fewer people were newly diagnosed and 47% fewer died of HIV-related causes in 2020.[84] In large, this is attributable to the increasing access to ART. The current global situation can be viewed on the next page.[51, 84]

HIV Globally 2020

37.7 million people living with HIV



73% had access to ART

1.5 million people were infected with HIV

680,000 people died of HIV-related causes

1.7 HIV AND AGING

As a result of the success of ART, many PLHIV now reach an older age. In the US today over 50% of PLHIV are 50 years of age or older,[85] and it has been estimated that by 2030 73% of PLHIV in the Netherlands will be over 50 years old.[86] Notably, the prevalence in the age group over 65 years increased with 48% from 2015–2019.[85] In Sweden today 49.5% of those living with HIV are aged 50 years or older and 12.4% are aged 65 years or older (InfCare HIV December 2021). In countries with good access to modern ART and care, the life expectancy of PLHIV is approaching the life expectancy of the general population.[87-89] However, globally, the effect on life expectancy and mortality differs between countries.[90, 91]

There is a general lack of data on elderly PLHIV due to underrepresentation and exclusion from trials. With the increasing age of PLHIV, understanding the particular aspects of aging with HIV has emerged as a research field of great importance.

1.7.1 COMORBIDITIES

Aging is associated with an increasing prevalence of comorbidities independent of HIV status.[92-94] However, PLHIV have a higher prevalence of comorbidities compared to HIV negative individuals in the same age group.[94-98] The association between HIV and other comorbidities have been most extensively studied in cardiovascular diseases,[95, 99-101] but associations have also been found with other diseases such as hypertension,[94] chronic renal disease,[94, 95] dyslipidemia,[94] osteoporosis,[102] and cancer.[103] The mechanism underlying this elevated risk is not completely understood but several contributing factors have been proposed, including both HIV-associated and non-HIV-associated.[104] A) A higher frequency of traditional risk factors, such as smoking, in PLHIV.[105, 106] B) A higher frequency of other lifestyle related risk factors, such as alcohol consumption/abuse and substance use.[97, 98] C) A higher frequency of infection with other viruses, such as hepatitis B, hepatitis C and cytomegalovirus.[94, 97] D) ART related toxicity.[107, 108] E) HIV-related causes, such as direct effect of the virus, $CD4^+$ nadir, and time with immunodeficiency.[95, 96] F) Ongoing low-grade inflammation and immune activation.[109-111]

1.7.2 ART AND NON-ART DRUGS

The increased prevalence of comorbidities associated with aging goes hand in hand with an increased number of concomitant drugs. Concomitant medications in turn increase the risk for potential drug-drug interactions (PDDIs).[112] This is of particular importance in PLHIV as they have a higher frequency of

polypharmacy (not including ART) compared to HIV negative individuals in the same age group.[113] During the last years several international guidelines have included recommendations on HIV in the older person. They all highlight the need to take comorbidities and concomitant medications into consideration when caring for the older individual living with HIV.[114-116] Possible ART drug toxicities need to be carefully considered since older individuals are especially vulnerable to drug toxicities.[117]

Aging is related to numerous physiological changes affecting organ function. These changes include alterations that may affect the pharmacokinetics of drugs. With age kidney function decreases with a decline in glomerular filtration rate affecting the clearance of drugs. Furthermore, the mass of, and the blood flow to the liver is reduced, affecting the metabolism and elimination of drugs. Currently, data do not support relevant age-related differences in enzymatic function.[118, 119] Additionally, alterations in body composition with reduced total body water and lean body mass lead to a relative increase in body fat and altered volume of distribution for water-soluble and lipid-soluble drugs.[118] Albumin levels may not be decreasing with age per se but are decreased in acute illness and malnutrition, and may affect protein binding of drugs. Pharmacodynamic age-related effects often result in increased sensitivity to drugs. Altogether these alterations may lead to toxic drug effects, particularly for drugs with a narrow therapeutic window.[118] In addition, the interindividual variability increases with age making effects of drugs less predictable and comorbidities may in turn affect organ function.[118, 120] Although the pharmacokinetic effects of aging in PLHIV have not been studied it is reasonable to believe that studies on HIV-negative individuals can be applied on PLHIV, with the addition of possible organ damage related to HIV infection.[120]

1.7.3 INFLAMMATION AND IMMUNE ACTIVATION

The grade of inflammation and immune activation caused by HIV decline during ART. But despite viral suppression a low grade of chronic inflammation persists which is elevated compared to HIV negative individuals.[121-123] As stated above this inflammation is suggested to be a contributor to the increased risk of non-communicable diseases seen in PLHIV.[109-111] Different mechanisms have been proposed to give rise to the persistent inflammation such as low level residual viremia, co-infections (e.g. cytomegalovirus), lifestyle factors, and microbial translocation.[124, 125]

The choice of markers of inflammation and immune activation analysed in paper II were based on findings of earlier studies.[126] They are briefly described below.

Soluble (s) intercellular adhesion molecule-1 (ICAM-1) is present on endothelial cells and can be used as a marker of endothelial activation. It is upregulated after inflammatory stimuli and helps leukocytes migrate to the tissues.[127] High-sensitive C reactive protein (hsCRP) is an acute phase protein produced in the liver and is a marker of systemic inflammation.[128] Interleukin-6 (IL-6) is a proinflammatory cytokine that is involved in several physiologic processes, as the acute phase response.[129] It is secreted by a variety of cells, particularly monocytes/macrophages on stimulation.[104] C-X-C motif chemokine ligand 10 (CXCL10) is a chemokine induced by interferon gamma and secreted by various cells but predominantly by monocytes.[130] An important function is to recruit leukocytes to sites of infection.[131] s-cluster of differentiation 163 (sCD163) and sCD14 are markers of monocyte/macrophage activation.[125, 132] sCD14 is particularly associated with microbial translocation.[125] Both s-tumour necrosis factor receptor-II (sTNFRII) and sCD27 are soluble cytokine receptors belonging to the TNF superfamily and markers of T cell activation.[126, 133, 134] Matrix metalloproteinase-3 (MMP-3) is upregulated by cytokines and involved in tissue remodeling.[135] s-glycoprotein130 (sgp130) is involved in IL-6 signalling.[136]

1.8 HIV IN THE CENTRAL NERVOUS SYSTEM

Neurological symptoms were described in AIDS patients prior to the identification of HIV as the causative agent.[137] In the years after the discovery of HIV, the symptoms and neuropathology of a form of dementia affecting HIV-infected were described.[138, 139] If left untreated at least 20–30% of HIV-infected would develop HIV-associated dementia (HAD), initially called AIDS dementia complex.[140, 141] HAD is caused by HIV replication within the CNS, leading to HIV encephalitis. This is a disease unrelated to the opportunistic infections or malignancies such as cryptococcal meningitis, cerebral toxoplasmosis, progressive multifocal leukoencephalopathy caused by JC virus, or lymphoma that can affect the CNS in the late stages of HIV infection.[142]

Even though HIV infects the CNS shortly after disease transmission, HAD develops during the late immunocompromised stages.[139] HAD gives rise to a triad of cognitive, motor, and behavioural symptoms. Early symptoms include impaired memory and concentration, loss of balance, and social withdrawal which during weeks to months develop to severe dementia.[139]

Although ART prevents the development of HAD, PLHIV still experience milder forms of cognitive symptoms.[143, 144] To adjust to this new situation the neurocognitive complications of HIV are now brought together under the umbrella diagnosis termed HIV-associated neurocognitive disorder, HAND. During the pre-ART era no specific objective diagnostic markers for the diagnosis

of HAD were available. Indeed, the diagnosis relied on criteria based on symptoms, and the exclusion of other diagnoses. This issue still remains and in 2007 a set of criteria, the Frascati criteria, were defined to try to overcome some of the diagnostic difficulties and establish criteria useful for research purposes.

In addition to HAD, the Frascati criteria introduced two new subgroups of HAND, that included HIV-associated mild neurocognitive disorder (MND) and asymptomatic neurocognitive impairment (ANI). The basis of these criteria is neurocognitive testing of at least five domains. To fulfill the criteria of ANI an individual need to perform at least one standard deviation below adjusted normative scores in at least two cognitive domains. For the diagnosis of MND the criteria of ANI need to be fulfilled and in addition give rise to symptoms that interfere with daily functioning. For the most severe form, HAD, neurocognitive testing needs to show a result at least two standard deviations below adjusted normative scores in at least two domains, and the individual must experience a distinct interference with everyday functioning. For all diagnoses the presence of another condition that explains the symptoms is an exclusion criterion.[145]

Studies in the ART era (of note, not all included PLHIV were on ART) have found a high prevalence of HAND, primarily ANI.[146, 147] In addition, studies have suggested ANI to be a predictor of development of symptomatic HAND.[144, 148] Notably, the clinical relevance of ANI, and the results of these studies are controversial.[149, 150] Indeed, the use of neuropsychological testing as the basis of diagnosis is complicated since the result can be confounded by comorbidities and life style factors. Furthermore, the results cannot distinguish ongoing injury from an effect related to previous events. This has led to the search for biomarkers that can facilitate the diagnostics and to identify individuals at risk of progressive disease, which is discussed further below.

1.8.1 NEUROPATHOGENESIS – A TROJAN HORSE?

The neuropathogenesis of HIV is still not completely understood. The CNS is probably infected during the first weeks after transmission.[151, 152] It is believed that the virus infects circulating blood monocytes, which in turn migrate to the CNS trough the blood-brain barrier and carry the virus like a Trojan horse. Other plausible mechanisms for CNS infection are via infected T cells, or through the transcytosis of free virions through endothelial cells.[153-155]

Monocytes further differentiate into perivascular macrophages, which are the main cell type for HIV infection in the CNS. Virus from these macrophages can in turn infect microglia. Additionally, astrocytes might be infected but do not give rise to viral replication. Infected cells can induce fusion with non-infected cells

expressing CD4- and co-receptors and create multinucleated giant cells. These cells can continue to replicate virus, and is a pathological hallmark of HIV encephalitis.[154]

Neurons, however, are not directly infected by HIV, which raises the question of, how CNS HIV infection can give rise to neurological symptoms. Two different pathways have been proposed. First, a direct neurotoxic effect of viral proteins has been suggested. Vpu, Vpr, gp120 and Tat have all been described as possibly neurotoxic, where at least gp120 and Tat are neurotoxic in vitro. However, it is not certain that the concentrations needed are present in vivo. Second, and more probable, the infection of macrophages and microglia induces secretion of inflammatory mediators and products that can have neurotoxic effects (i.e., quinolinic acid, tumor necrosis factor and arachidonic acid metabolites), induce activation of other cells, and attract additional T cells and macrophages further enhancing inflammation. This mechanism is further supported by the fact that few cells are infected, in comparison to the severity of the disease. In addition, several studies have found signs of macrophage activation in cerebrospinal fluid (CSF) of untreated PLHIV, and this has in turn been related to neuronal damage.[153, 154]

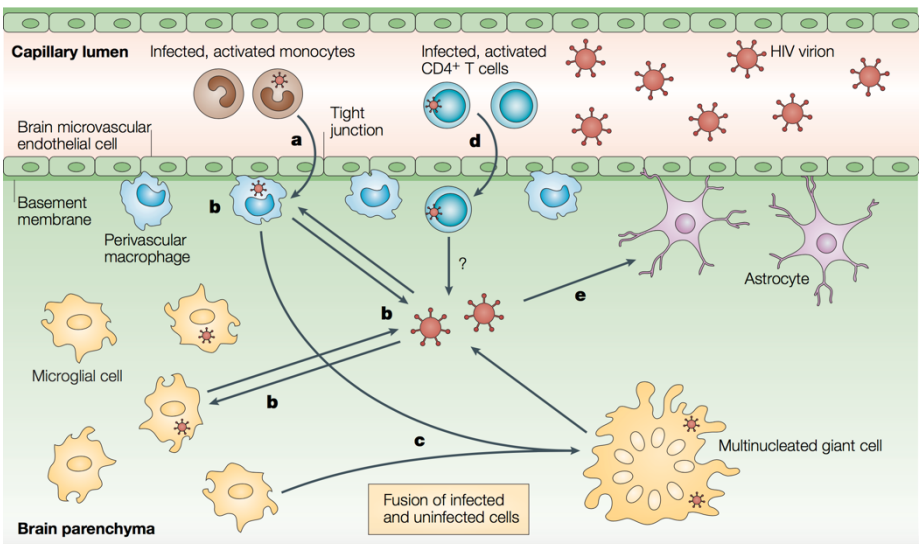


Figure 6. Neuropathogenesis of HIV. a) HIV-infected circulating blood monocytes migrate across the blood-brain barrier. b) Monocytes differentiate into perivascular macrophages. Virus derived from perivascular macrophages in turn infect microglia. c) Infected cells fuse with uninfected cells creating multinucleated giant cells. d) Another possible entryway of HIV is through infected CD4+ T cells. e) Astrocytes do not replicate virus, but their malfunction due to the inflammatory response caused by HIV, contribute to neuropathogenesis. Reprinted with permission from Springer Nature: Nature reviews Immunology. The Neuropathogenesis of AIDS, González-Scarano F et al., 2005.

1.8.2 BIOMARKERS OF CNS INFECTION

It is inherently hard to obtain samples from the brain parenchyma. CSF is, by lumbar puncture, accessible and is used as a proxy for pathological processes in the brain. A number of different biomarkers measurable in the CSF, and in some cases in plasma, have proven to be useful in the study of the HIV CNS infection. This section will focus on the biomarkers most relevant to this thesis.

1.8.3 NEUROFILAMENT LIGHT PROTEIN

The cytoskeleton in neurons consists of three different components; the microtubules, the microfilaments, and the neurofilaments. In turn the neurofilament is constructed from three neurofilament subunits (the heavy, medium, and light chain), alfa-internexin, and peripherin. They are found in the cytoplasm of all parts of the neuron, but most abundantly in the axon, and particularly of large myelinated neurons. The neurofilament determines the caliber of the axon and hence the conduction velocity.[156, 157] When damage to a neuron occurs, neurofilament is released to the extracellular space. The mechanism for transport to CSF or blood is not fully known.[157]

The neurofilament light protein (NfL) has proven to be a sensitive and specific marker of axonal damage. However, it is not disease specific and elevated levels can be found in the CSF in several neurological disorders such as; multiple sclerosis, amyotrophic lateral sclerosis, cerebral infarction, and traumatic brain injury.[158-160] Furthermore, CSF NfL is elevated in different types of dementia; Alzheimer's disease, vascular dementia, and frontotemporal dementia.[160] In 2016, an assay able to detect NfL in plasma was developed, with a strong correlation between CSF NfL and plasma (P-)NfL.[161] The results of elevated CSF NfL has then been reproduced in P-NfL.[160] In addition, P-NfL may reflect axonal damage of peripheral neurons.[160, 162, 163]

NfL is a sensitive biomarker of neuronal damage in HIV infection. Untreated HIV-infected individuals with HAD or opportunistic CNS infection exhibit very high CSF NfL levels.[164-166] Interestingly, NfL may be increased in neuroasymptomatic individuals, corresponding to the level of immunodeficiency, with higher levels in the late stages.[164-167] In addition, NfL levels may anticipate the development of HAD 1–2 years prior to diagnosis.[168] After initiation of ART, NfL levels decrease and in most, but not all, cases normalise.[165-167, 169, 170] Notably, one study found that PLHIV on ART had CSF NfL levels corresponding to HIV negative individuals 3.9 years older.[169]

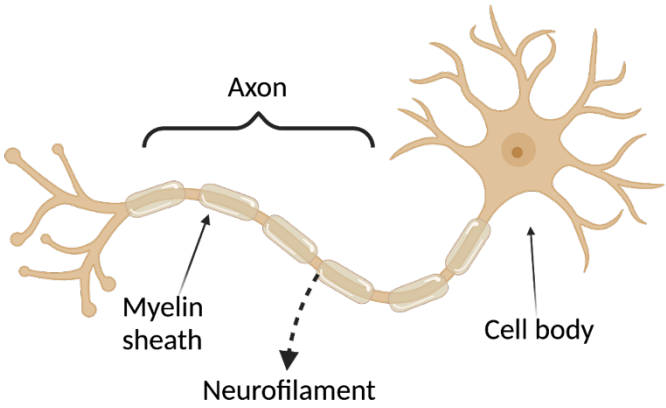


Figure 7. Schematic picture of a neuron.[51]

1.8.4 NEOPTERIN

Neopterin is a biomarker of macrophage/monocyte activation. It is released from macrophages on stimulation from interferons, primarily interferon-γ. Neopterin can be measured both in blood and CSF. In blood it has been found to predict HIV disease progression. In untreated HIV infection without neurological symptoms both blood and CSF neopterin rise with progression to immunodeficiency, with the highest levels in those with a $CD4^+$ cell count below 200 cells/mm^3. In individuals with HAD, CSF neopterin levels exceed the levels found in patients with neuroasymptomatic immunodeficiency, while a concomitant rise in blood neopterin is absent.[171] ART effectively reduce neopterin levels both in blood and in CSF but despite years of treatment elevated levels of neopterin may persist.[170]

1.9 B VITAMINS & HOMOCYSTEINE

The history of the B vitamins in medicine dates back to the 19th century. Then a fatal form of anemia with macrocytic red blood cells, related to atrophic gastritis, was described. It was named pernicious anemia. In addition to anemia, concomitant neurological symptoms were reported. In the mid 1920s it was discovered that raw liver could cure pernicious anemia, and in 1948 vitamin B_{12}, the compound that gave rise to the effect, was isolated and the chemical structure subsequently described.[172] Parallel to this discovery, the physician Lucy Wills investigated macrocytic anemia in pregnant women in Bombay and discovered

that yeast extract could cure the anemia. In the 1940s it was discovered that the active agent was folate, and it was subsequently industrially synthesised. After the isolation of both enzymes it was found that their deficiency gave rise to the same pathologic picture in the bone marrow, and later it was discovered that the vitamins are indeed metabolically closely related.[173]

Homocysteine, a non-essential amino acid, was first described in 1932. However, it was not until the 1960s that the implication in disease was made, when a number of genetic diseases caused by different enzyme defects affecting homocysteine metabolism were described. These diseases gave rise to homocysteinuria and high blood levels of homocysteine, and among other symptoms mental disturbance and arteriosclerotic changes.[172] This was the start of the field of research investigating the role of homocysteine in disease. Increased levels of homocysteine has since been related to over a hundred different conditions, predominantly cardiovascular disease and diseases of the CNS.[174]

Homocysteine is involved in two different metabolic pathways, the remethylation pathway in the methionine cycle and the transsulfuration pathway. In the remethylation pathway homocysteine is methylated to methionine, through a reaction that is both B_{12} and folate dependent. An adenosine group is then transferred to methionine, creating S-adenosylmethionine (SAM). SAM donates a methyl group, of importance to several reactions in cells, and is converted into S-adenosylhomocysteine (SAH). SAH is then converted through hydrolysis back to homocysteine. In the transsulfuration pathway homocysteine is metabolised to cysteine, in a reaction dependent of vitamin B_6. Cysteine is in turn used in the synthesis of the antioxidant glutathione.[175, 176] Since folate is a cosubstrate in the homocysteine metabolism, the metabolism of homocysteine and folate are closely related, which will be discussed below. These different metabolic pathways are described in figure 8.

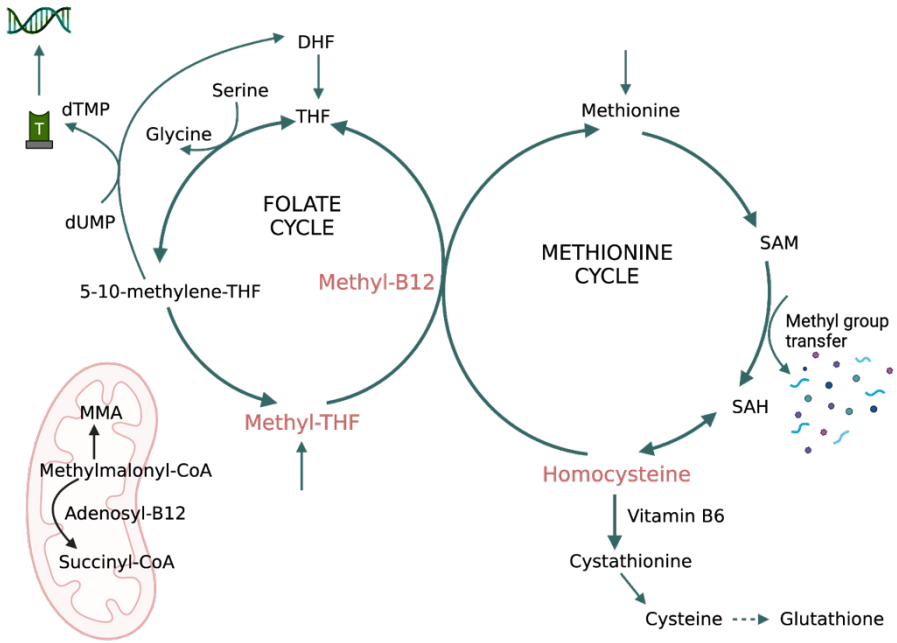


Figure 8. Homocysteine, folate, and vitamin B_{12} metabolism.[51]

As mentioned above, one of the causes of high levels of homocysteine, hyperhomocysteinemia, is genetic defects of enzymes involved in the metabolism of homocysteine. Furthermore, deficiency of B_{12}, folate, or B_6 hinders homocysteine metabolism and as a consequence increase the homocysteine levels. Levels of homocysteine also increase with age and are inversely related to renal function. In addition, several drugs such as trimethoprim, methotrexate, and anticonvulsants may increase homocysteine.[176, 177]

Homocysteine can be used as a marker of B_{12} and folate deficiency, as it increases when B_{12} and folate levels decrease. In addition, methylmalonic acid (MMA), described in the next section, can be used as a marker of B_{12} deficiency. With the introduction of assays capable of determining homocysteine and MMA in blood, the concept of subclinical deficiency was introduced.[172, 178-180] It was now possible to detect metabolic signs of intracellular deficiency through raised levels of homocysteine and MMA in a subset of individuals without clinical symptoms, and vitamin levels not traditionally regarded as deficient.[178, 179] Studies have followed that show that homocysteine and MMA concentrations start to increase at vitamin levels within the normal reference interval.[181] In the context of cognitive decline,

this has led to the discussion of which cut off levels are appropriate and whether subclinical deficiency may be of clinical relevance.[175, 179, 180]

There is still no consensus or gold standard for the diagnosis of B12 or folate deficiency.[177, 178, 180, 181] However, there is support for using of a combination of metabolic markers (i.e., homocysteine or MMA) and vitamin levels.[177, 178, 180, 182]

Closer descriptions of the two B vitamins of greatest importance for this thesis follow in the next sections.

1.9.1 VITAMIN B_{12}

Vitamin B_{12}, also called cobalamin, is an essential water-soluble vitamin found in animal source foods such as meat, egg, and dairy products. On the contrary, vitamin B_{12} is not found in vegetables.[178, 182, 183]

Upon ingestion vitamin B_{12} is released from animal proteins in the acid environment of the stomach, where it binds to haptocorrin to prevent its degradation. In the duodenum, vitamin B_{12} is released from haptocorrin, and forms a complex with intrinsic factor (IF) which is produced by parietal cells in the stomach. The B_{12}-IF complex is absorbed by receptor-mediated endocytosis in the distal ileum. In addition, a small fraction of vitamin B_{12} is absorbed through passive diffusion throughout the intestine. Once absorbed in the enterocyte the B_{12}-IF complex enters a lysosome where IF is degraded and vitamin B_{12} is released. From the basolateral membrane of the enterocyte vitamin B_{12} is released into the blood where it binds to one of two possible transport proteins. Approximately 20% of vitamin B_{12} in the blood is bound to transcobalamin and constitutes the active form that delivers vitamin B_{12} to the tissues. The remaining 80% is bound to haptocorrin and forms a complex that can only be taken up by hepatocytes where it is stored. A portion of the stored vitamin B_{12} is secreted in the bile every day and may again be absorbed in the ileum.[178, 182-184]

Inside the cell, vitamin B_{12} is converted into its two different biologically active forms. In the cytoplasm, vitamin B_{12} is converted into methylcobalamin and is involved in the methionine cycle described in figure 8. In the mitochondria vitamin B_{12} is converted into adenosylcobalamin, which is a cofactor for the enzyme methylmalonyl-CoA mutase in the conversion of methylmalonyl-CoA to succinyl-CoA.[178, 183, 184] In the absence of vitamin B_{12}, MMA accumulates and can be measured as a marker of B_{12} deficiency.

Causes for vitamin B_{12} deficiency can be divided into low intake or malabsorption (see table 3). As described above B_{12} deficiency can manifest as macrocytic anemia, through pathways related to folate metabolism, described in more detail

in the next section. It can, in addition to anemia, or as the sole manifestation, give rise to various neurologic symptoms such as polyneuropathy, paresthesia, cognitive impairment, and depression. The exact pathogenic mechanism is not clear, though it is known that deficiency can cause demyelination of central and peripheral neurons.[178, 182, 185, 186]

1.9.2 FOLATE

Folates is the common name of a family of closely related compounds of water-soluble vitamins. In contrast to vitamin B_{12}, folate is found in many plants such as asparagus, broccoli, green leafy vegetables, and whole grain, but also in animal products such as liver. Folate is absorbed in the proximal part of the jejunum through specific folate transporters. Inside the intestinal cells absorbed forms of folate are converted to 5-methyl-tetrahydrofolate (5-methyl-THF). 5-methyl-THF is the form that circulates in blood where it is either unbound or protein-bound, mainly to albumin.[187, 188]

The folate cycle is described in figure 8. 5-methyl-THF is a cosubstrate for methionine synthase, the enzyme converting homocysteine to methionine, and is thus part of the methionine cycle. This reaction converts 5-methyl-THF to THF. In the next step THF accepts a carbon unit from serine forming 5,10-methylene-THF. 5,10-methylene-THF is required for the synthesis of thymidylate and purines, and subsequently important for the synthesis of DNA and RNA. The interruption of this step by the deficiency of folate gives rise to errors in DNA synthesis, the cause of the macrocytic anemia seen in folate deficiency. Even if sufficient levels of folate is available the conversion of 5-methyl-THF to THF is dependent of vitamin B_{12} and hence vitamin B_{12} deficiency hampers the folate cycle, a phenomenon called the folate trap, and through this pathway leads to macrocytic anemia. 5,10-methylene-THF converts to dihydrofolate which in turn is converted back to THF. Concomitantly 5,10-methylene-THF is converted to 5-methyl-THF in an irreversible reaction.[178, 188, 189]

Similar to B_{12}, the classical manifestation of folate deficiency is macrocytic anemia. It may also give rise to neurological symptoms, such as cognitive impairment and depression.[190]

Table 3. Causes of vitamin B_{12} and folate deficiency.

	B_{12}	Folate
Insufficient intake	Dietary factors (e.g. malnutrition, vegan diet, alcoholism)	Dietary factors (e.g. malnutrition, alcoholism)
Impaired absorption or metabolism	Pernicious anemia (autoimmune gastritis)	Gastrointestinal disorders (e.g. Crohn's and coeliac disease)
	Chronic atrophic gastritis	High alcohol intake
	Gastrointestinal surgery	Gastrointestinal surgery
	Pancreatic disease	Tobacco smoking
	Inherited disorders	Inherited disorders
	Medications (e.g. proton pump inhibitors, metformin)	Antifolate medications (e.g. methotrexate, trimethoprim, anticonvulsants)
	Bacterial or parasitic infection	
	Inflammatory bowel disease	
Increased demand		Pregnancy

1.9.3 HOMOCYSTEINE AND NEUROCOGNITIVE DISEASE

The development of cognitive impairment and dementia is multifactorial, where homocysteine may have a contributing role. In the early 1990s two articles were published presenting the hypothesis that homocysteine metabolism might play a role in the etiology of Alzheimer's disease.[191] This was followed by a large number of cross-sectional studies that reported a relationship between homocysteine, and cognitive impairment and dementia.[191] These results have been confirmed by both prospective observational studies and meta-analyses, reporting an increased risk of cognitive impairment or dementia in individuals with elevated homocysteine levels.[192-195] In several studies the association remains after adjusting for B vitamin status, arguing against vitamin deficiency as the sole mechanism.[192, 196, 197] It is possible that the relationship is influenced by another unidentified risk factor or that the risk is due to the combination of several pathways.[198, 199]

Several clinical trials have been performed to examine the effect of vitamin B supplementation on the levels of homocysteine and cognitive decline. These studies have unquestionably showed the homocysteine lowering effect of B

vitamins, but they have had contradictory results on the effect on cognition.[200-205] It has been argued that the lack of effect in many trials is due to inadequate trial design.[174, 206]

Aims

2 AIMS

The general aim of this thesis was to describe HIV infection in the elderly, focusing on drug levels, side effects, drug-drug interactions, comorbidities and inflammation, and to investigate the role of homocysteine (as a marker of vitamin B deficiency) in neuronal injury in PLHIV.

The specific aims were:

- To examine drug levels, side effects, adherence, concomitant medications, drug-drug interactions, and comorbidities in PLHIV aged 65 years or older compared to a group of PLHIV aged 49 years or younger.

- To analyse levels of inflammatory markers in PLHIV aged 65 years or older in individuals with three different ART regimens.

- To explore the relationship of homocysteine levels and signs of neuronal injury in PLHIV.

- To investigate the effect of vitamin B supplementation on signs of neuronal injury in PLHIV with raised levels of homocysteine.

Study population and design

3 STUDY POPULATION AND DESIGN

The four papers in this thesis are based on three studies. All PLHIV included in the studies attended the Department of Infectious Diseases at Sahlgrenska University Hospital, Gothenburg; South Älvsborg Hospital, Borås; Karolinska University Hospital, Huddinge; or Stockholm South General Hospital, Stockholm. The distribution of study participants is shown in figure 9.

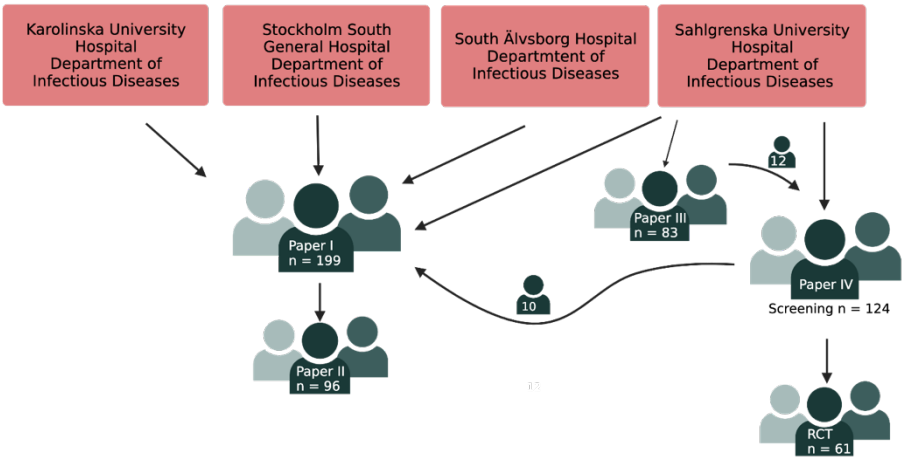


Figure 9. The distribution of study participants in the thesis papers.

3.1 PAPER I & II

The basis of paper I and II is a multi-center cross-sectional study performed at four sites in Sweden: the Department of Infectious Diseases at Sahlgrenska University Hospital in Gothenburg; the Department of Infectious Diseases at South Älvsborg Hospital in Borås; the Department of Infectious Diseases at Karolinska University Hospital Huddinge in Stockholm; and the Department of Infectious Diseases at Stockholm South General Hospital in Stockholm. The aim was to study aspects of HIV infection in elderly individuals, defined as aged 65 years or older, compared to PLHIV under the age of 50. The inclusion criteria were age (≥ 65 years for the study group and 18–49 years in the control group), HIV infection, and stable ART for at least 6 months, with a drug regimen containing atazanavir (ATV), darunavir (DRV), or efavirenz (EFV).

Inclusion began in November 2013. At that time 3568 PLHIV were receiving HIV care at the study sites. Two hundred fifty-six (7.2%) were 65 years or older, whereof 38 (14.8%) were treated with ATV, 56 (21.9%) with DRV, and 78 (30.4%) with EFV. Patients were offered to participate during ordinary routine clinical follow-up. If enrolled, a blood sample for analysis of drug concentrations (paper I), markers of inflammation (paper II), and data on concomitant medications, side effects, and adherence were collected. Further information on comorbidities and routine blood test results were recorded through systematic review of medical charts. PDDIs were analysed using drug-drug interaction databases.

From November 2013 to August 2015, 100 individuals were included in the study group and 99 in the control group. In paper I, 27 individuals were excluded from the drug concentration analysis, due to sample management (3) or dosing (24) issues. All enrolled individuals were included in the other analyses in paper I. Only individuals over the age of 65 years were included in paper II, whereof 4 were excluded due to ongoing bacterial or fungal infections (3) or treatment with two of the study drugs (1).

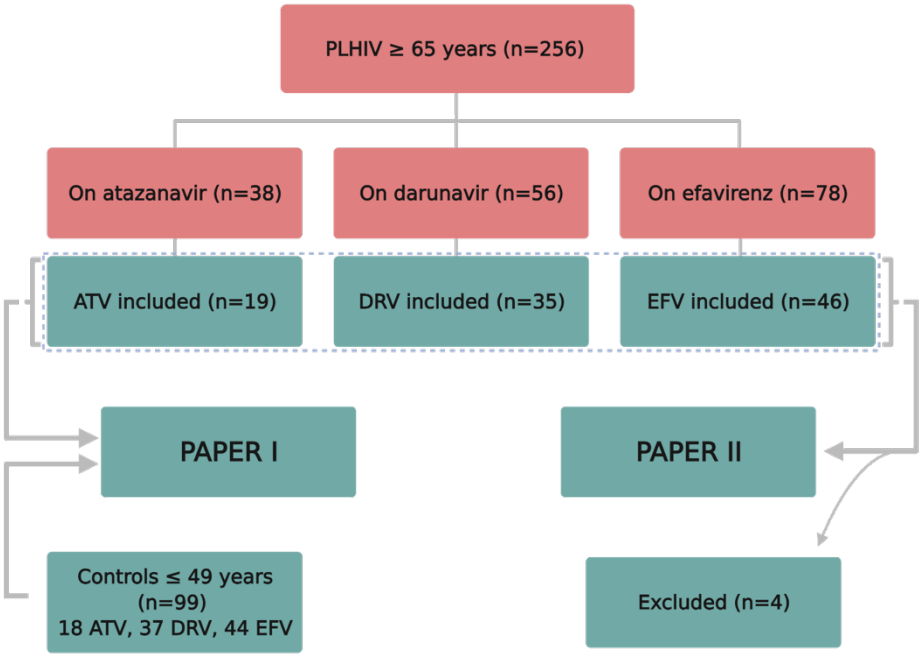


Figure 10. Study design of the multi-center cross-sectional study.[51]

3.2 PAPER III

Since 1985, a longitudinal prospective study of the effects of HIV on the CNS has been ongoing at the department of Infectious diseases at Sahlgrenska University Hospital in Gothenburg. The study includes blood and CSF sampling with lumbar punctures performed before and after treatment initiation, after change of ART regimen, and annually during follow-up. Currently, 648 PLHIV have been included in the longitudinal study. In 2014, at the time for the study described in paper III, 494 had been included.

The aim of the study was to investigate the relationship between P-homocysteine and signs of neural injury in PLHIV. Based on the inclusion criteria (untreated HIV infection, age 18 ≥ years, no opportunistic CNS infection) 83 HIV-infected individuals from the prospective study were randomly included in the retrospective study described in paper III. A paired CSF and serum sample was analysed, and in 22 individuals a second paired sample was analysed after ART initiation.

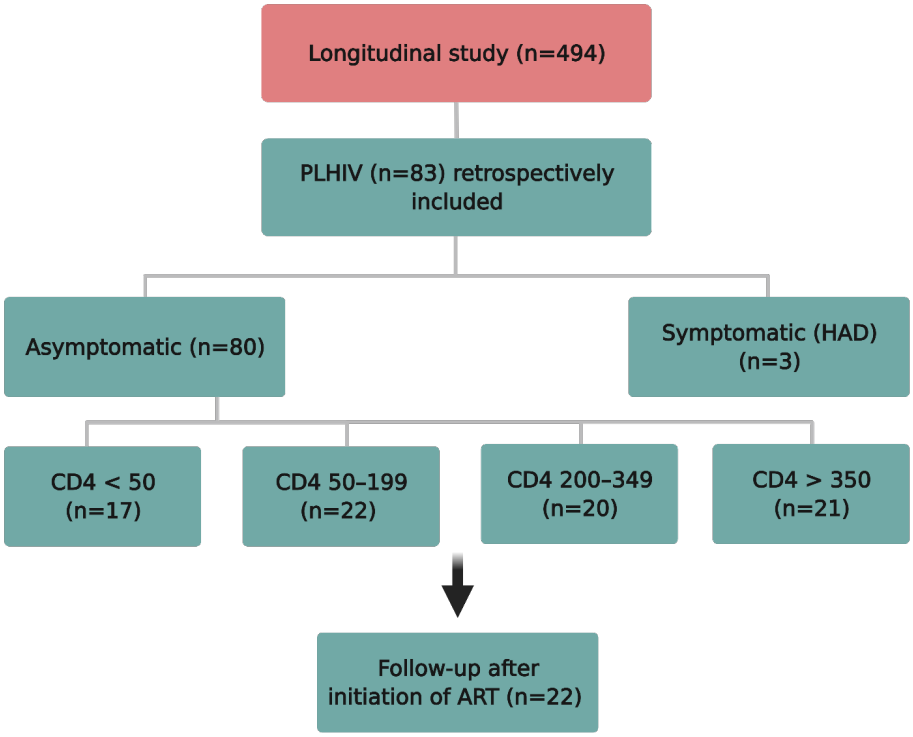


Figure 11. Study design of the retrospective study.[51]

3.3 PAPER IV

To further explore the association between P-homocysteine and NfL found in paper III we designed an open randomised controlled trial to study the effect of vitamin B supplementation on NfL, as a marker of neuronal injury. The inclusion criteria were stable ART for > 12 months, HIV RNA < 50 copies/ml, and age ≥ 18 years. The exclusion criteria were; treatment with trimethoprim-sulfamethoxazole or methotrexate, ongoing vitamin B_{12}, B_6 or folic acid substitution, antiepileptic treatment, small intestine or ventricular resection, disturbed absorption in the small intestine, ongoing neurological or severe psychiatric disease, any malignant tumor in the history, severe ongoing infection or opportunistic infection, alcohol abuse, clinical depression, pregnancy, and significant vitamin B_{12} or folate deficiency that require higher treatment doses. Individuals who met the criteria were screened, and those with P-homocysteine ≥ 12 μmol/L were enrolled in the trial. The participants were randomised to either active treatment arm or control arm, with cross over from the control arm to the active treatment arm after 12 months. The trial continued for in total 24 months. The active treatment consisted of 1 tablet Triobe (cyanocobalamin 0.5 mg, folic acid 0.8 mg, and pyridoxine 3.0 mg) q.d. Based on power calculations it was decided to include 30 PLHIV in each arm.

All study participants were included at the department of Infectious diseases at Sahlgrenska University hospital in Gothenburg from April 2016 to June 2017. The last study visit was performed in June 2019.

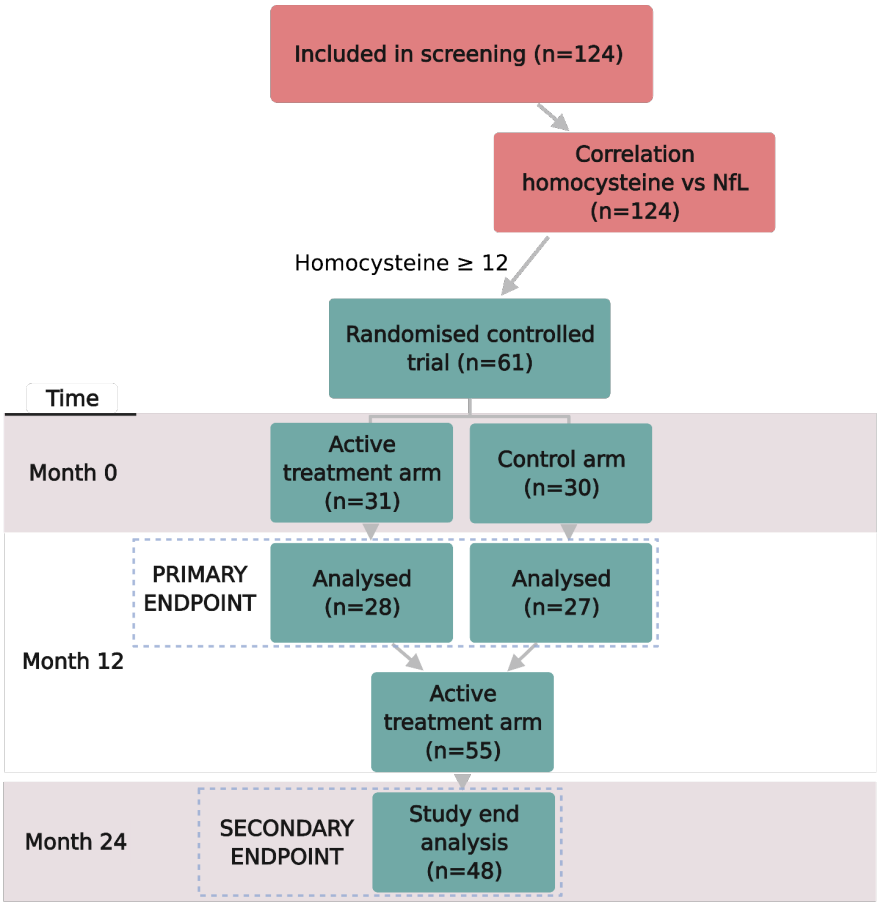


Figure 12. Study design of the randomised controlled trial.[51]

Methods

4 METHODS

4.1 LABORATORY ASSAYS

Blood samples analysed for drug concentrations (paper I), markers of inflammation (paper II) and P-NfL (paper IV) were frozen to –70 degrees directly after sampling until analysis. The additional blood tests analysed in paper IV were analysed directly after sampling. In paper III thawed CSF and blood samples were analysed.

If not otherwise stated the blood sample analyses were performed at the local laboratories according to local standards.

4.1.1 DRUG CONCENTRATIONS

Steady-state drug levels of ATV, DRV, and EFV were analysed at the pharmacology analytical laboratory at Karolinska University Hospital, Huddinge in Stockholm, Sweden. The method used was a reverse-phase High Pressure Liquid Chromatography with ultraviolet detection.

4.1.2 MARKERS OF INFLAMMATION

The following analyses were performed at the department of Clinical Microbiology at Sahlgrenska University Hospital, Gothenburg, Sweden.

Plasma concentrations of sCD14, sCD27, sCD163, sgp130, IL-6, sTNFRII, CXCL-10, sICAM-1, and MMP-3 were measured using the premixed Magnetic Luminex Assay (R&D Systems, Minneapolis, Minnesota, USA) according to the manufacturer's instructions.

The Human high sensitivity C-reactive protein (hs-CRP) ELISA kit (Cusabio, Houston, Texas, USA) was used to analyse hsCRP levels.

4.1.3 NEUROFILAMENT LIGHT PROTEIN

In paper III NfL levels were measured in CSF using a commercial Enzyme-linked immunosorbent assay (ELISA) (Uman Diagnostics, Umeå, Sweden). The age-related cut off levels for CSF NfL used were: 18–30 years: < 380 ng/L; 30–39 years: < 560 ng/L; 40–59 years: < 890 ng/L; > 59 years: < 1850 ng/L.

Initially, only methods for the analysis of NfL in CSF were available, but a sensitive method for analysis in plasma has been developed.[161] This enabled us to use P-NfL in paper IV. P-NfL levels were analysed using an in-house Single molecule array (Simoa) method on an HD-1 Analyzer (Quanterix, Billerica, MA). The clinical age-related cut off levels for P-NfL used were: < 18 years: < 7 pg/mL; 18–50 years: < 10 pg/mL; 51–60 years: < 15 pg/mL; 61–70 years: < 20 pg/mL; and > 70 years: < 35 pg/mL.

All NfL analyses were performed at the Clinical Neurochemistry Laboratory at Sahlgrenska University Hospital, Mölndal, Sweden.

4.1.4 HOMOCYSTEINE AND B VITAMINS

In paper III P-homocysteine was measured using a stable isotope dilution liquid chromatography tandem mass spectrometry (LC-MS/MS) using a Quattro micro instrument (Waters Corporation, Milford, MA, USA). In paper IV the Roche Homocysteine Enzymatic Assay on a Cobas c501 instrument was used (Roche Diagnostics, Rotkreutz, Switzerland).

P-B_{12} and S/P-folate (paper III and IV) were analysed on a Cobas e instrument (Roche Diagnostics, Rotkreutz, Switzerland).

4.2 DRUG-DRUG INTERACTIONS

The Liverpool University HIV drug interactions database[207] was used for analysis of PDDIs between ART and concomitant medications. The database divides interactions into four classes: green (no interaction expected), yellow (potential interaction likely to be of weak intensity, additional monitoring or dose adjustment is unlikely to be required), orange (potential interaction that may require dose monitoring, alteration of drug dosage or timing of administration), and red (do not co-administer). If a specific comedication was not found in the Liverpool University HIV drug database the Swedish drug interactions database Janusmed[208] was used. PDDIs from the orange and red classes were included in the analysis in paper I.

4.3 NEUROCOGNITIVE TESTING

In paper IV neurocognitive testing was performed with Cogstate (Cogstate Ltd, Melbourne, Australia). Cogstate is a computerised neuropsychological screening test that have been validated for HIV-associated neurocognitive impairment.[209, 210] In paper IV the testing consisted of five tasks testing five different cognitive domains: Detection (psychomotor function), Identification (attention), One card

learning (visual learning), One back test (working memory) and Groton Maze learning test (executive function). The five test results were combined to one total score (Cogstate combined z-score), which was used in the analysis.

4.4 STATISTICAL METHODS

Correlations were calculated using Pearson correlation coefficient (paper III & IV). Differences in continuous variables between groups were analysed using either parametric tests (independent T-sample, ANOVA) or nonparametric tests (Mann Whitney U-test, Kruskal Wallis test). In paper I differences in drug levels between groups were adjusted for time, using ANCOVA analysis. When differences were analysed within a group at different timepoints Paired T-test was used. Group comparisons of categorical variables were analysed with Chi-square test and Fisher's exact test. In paper III predictors of homocysteine and NfL were investigated with multiple linear regression analysis with forward selection.

Power analysis was performed in the cross-sectional study (paper I and II) and the randomised controlled trial (paper IV).

Variables were log-transformed when suitable to reduce skewness throughout the thesis.

A p-value < 0.05 was considered significant throughout the thesis. All statistical analyses were performed using SPSS version 21, 25, or 27 (IBM SPSS Statistics, Armonk NY, USA) or Prism version 6.0, 8.0 or 9 (Graph-pad Software Inc., La Jolla, CA, USA).

4.5 ETHICS

All studies in this thesis were granted ethical approval by the Research Ethics Committee at Gothenburg University (paper I & II: Dnr: 642-13; paper III: Dnr: 82-88, and Ö588-10; paper IV: Dnr: 029-16). In addition, the randomised controlled trial described in paper III was approved by the national Swedish Medical Products Agency, and registered at clinicaltrials.gov (NCT number: NCT02773147), and in the European Union Drug Regulation Authorities Clinical Trials Database (EudraCT number: 2015-004311-20).

All studies were performed according to the Helsinki declaration and all participants gave their written informed consent.

HIV in the elderly

5 HIV IN THE ELDERLY

The aim of the cross-sectional study was to describe different aspects of HIV in the elderly. The rationale to conduct the study was a general lack of studies focusing on elderly PLHIV. The number of PLHIV ≥ 65 years of age is increasing, due to a higher number of survivors reaching higher ages but also incident cases in this age group. In many studies still PLHIV over the age of 50 is regarded elderly. Notably, the lack of studies in the old ages is not specific for the HIV research field.[211]

In our study, the term elderly was used to describe those over the age of 65 and it is in this meaning it will be used throughout this thesis. When there is no data available on elderly, data on those ≥ 50 years of age may be discussed.

There are specific aspects and challenges related to the care of elderly PLHIV, and one of them is testing. Older PLHIV are more often late presenters, i.e. diagnosed at a more advanced stage.[212-214] In many cases there has been at least one contact with healthcare prior to diagnosis where a missed opportunity for testing has occured.[214] Testing is uncommon in the elderly, also in those with a higher risk of transmission.[215] One reason is supposedly a reluctance of healthcare providers to discuss HIV testing and sexual health with older individuals.[216, 217]

Older age at seroconversion is associated with an increased risk of progress to AIDS and mortality in untreated HIV infection.[218, 219] Also, in PLHIV on ART older age is related to poorer $CD4^+$ cell count recovery and disease progression.[220-223] In addition, late presenters have an increased risk of AIDS and mortality,[220, 224] further pointing out the importance of testing and early diagnosis in the elderly.

Another challenge is the physiologic changes related to aging, which may influence the pharmacokinetics and pharmacodynamics of drugs, described in section 1.7.2. To investigate the role of older age on drug levels we analysed the levels of EFV, ATV and DRV, at the time recommended as first line alternatives. We found significantly higher levels of DRV in the elderly compared to the controls. In addition, we also found higher ATV levels in the elderly, with a trend towards significance indicating that it might be a drug class effect. In contrast, we found no difference in EFV levels. In concert with our finding two studies reported higher concentrations of DRV in PLHIV aged > 60 and > 65 years respectively.[225, 226] To my knowledge no other study has compared levels of ATV in the elderly compared to a younger control group. Yet several studies,[227, 228] but

not all,[229, 230] have found an association between ATV concentrations and age. Studies specifically studying EFV in the elderly are also lacking, but in line with our finding studies have not found an association between EFV concentrations and age.[228, 230]

A recent study used mathematical modelling to calculate the effect of aging on the pharmacokinetics of 10 commonly used ART drugs. They found an increased exposure to drugs with age, but it was not considered clinically significant due to the wide therapeutic window of the study drugs.[231] Furthermore, the effect of age on ART pharmacokinetics was recently reviewed by Calcagno et al, who conclude that data in the elderly is scarce, and although exposure of ART may increase with age clinically relevant alterations are rare based on available data.[120] However, the elderly PLHIV may be more vulnerable to the specific toxicities and risks of different ART, such as the renal and bone effects associated with TDF and the possible increased risk of cardiovascular disease associated with PIs and ABC.

Supported by others,[112, 232] we found a higher frequency of concomitant medications and PDDIs in the elderly. PDDIs were common in the elderly in our study, with 59% having one or more amber or red flag PDDIs (data not presented in paper I). The same finding is described by others.[233, 234] Comparable to other studies,[235] we found red flag PDDIs in 8% of the elderly. All of them were related to PI use, not surprisingly since the PIs are notorious for PDDIs. At the time when the study was executed, DRV and ATV were the options at hand when a robust ART was preferred and in the presence of resistance. Today, with the arrival of dolutegravir and bictegravir as robust alternatives and with fewer drug interactions, these might be alternatives.

In our study 68% of the elderly met the criteria of polypharmacy, defined as 5 drugs or more including ART but not booster (data not reported in paper I). This is in concert with a previous finding of 70%, but somewhat lower than another study which reported 93%.[233, 236] Polypharmacy is associated with several disadvantageous events, e.g. increased risk of adverse events,[237] drug-drug interactions,[237] non-adherence,[237, 238] hospitalization,[237] and quality of life.[239] This highlights the need for proper prescription in the elderly. As a result, tools have been established to help reduce the use of inappropriate medicines, such as the screening tool of older people's prescriptions and screening tool to alert to right treatment (STOPP/START) and Beer's criteria.[240, 241] In our study 25% of the elderly fulfilled a STOPP criterion (data not presented in paper I). Indeed substantial, but lower than previous studies in elderly PLHIV who reported frequencies of 63% and 71%.[234, 236] Our result may underestimate the prevalence of STOPP criteria since the study was not designed for this and some data is lacking.

A pillar of successful treatment with virologic control and beneficial effects on morbidity and mortality, is good adherence to ART. Older PLHIV are in general more adherent than younger.[242, 243] Our study showed no difference in adherence between the elderly and the controls. Of note, the median age in the control groups were 43–46 and thus not very young. However, adherence may decline with more advanced age with the possible development of dementia and neurocognitive decline.[242-244] In addition, as described above, polypharmacy might have a deteriorating effect. Insufficient data exist on how to improve adherence in elderly PLHIV.[245] Nonetheless, several issues that are a concern for the elderly have been related to non-adherence, such as loneliness, poor social support, and depression.[5] Furthermore, pill burden and multiple daily doses have been related to poorer adherence.[246] On the other hand, treatment simplification with fixed-dose combinations and once-daily dosing has been related to better adherence, but notably with uncertain effect on clinical outcome.[247]

Elderly have an increased risk of adverse events. Although, we did not find any difference in self-reported side effects between the elderly and the controls. When analysing the elderly separately, we found a higher frequency of side effects in the DRV arm compared to ATV, and the EFV arm compared to ATV. Interestingly, we did not find a higher frequency of CNS side effects caused by EFV in the elderly, which has been reported to be related to age, comorbidities, and concomitant medications.[248] The use of self-reporting of side effects is both easy and may reveal side effects that are important to the patient. However, the result may have been influenced by side effects related to the ART backbone or other concomitant medications. A study focusing on side effects after the initiation of ART or after ART-switch would be interesting to investigate how side effects affect the elderly.

As described in chapter 1 PLHIV are at higher risk for comorbidities compared to HIV negative individuals. In addition, many comorbidities are related to aging. We found, as expected, a higher number of comorbidities in the elderly group compared to controls, and only 2 (2%) of the elderly had no comorbidity. In table 4 comorbidities affecting the elderly in our study are presented (data not shown in paper I). The three most common comorbidities were all related to aging; hypertension, dyslipidemia, and cardiovascular disease, in line with the findings of others.[93, 94, 249]

Table 4. Comorbidities in PLHIV ≥ 65 years of age included in our study.

Comorbidity	Number of individuals
Cardiovascular disease	20
Hypertension	47
Hyperlipidemia*	45
Diabetes mellitus	15
Malignancy+	16
Sleep disorder	13
Psychiatric disorder	11
Nervous system disease	10
Pulmonary disease	8
Chronic kidney disease	2
Inflammatory disease	1
Other	71

Other diseases with ≥ 2 individuals include: allergy, arthrosis, cardiac arrhythmia, chronic pain, chronic constipation, diverticulosis, eye diseases, gastro-esophageal reflux disease or gastritis, gout, haemophilia, harmful use of alcohol, hearing impairment, hyperplasia of prostate, irritable bowel syndrome, kidney stone, liver diseases, osteoporosis, polyp of colon, recurrent cystitis, recurrent herpes simplex, skin diseases, vitamin deficiencies

*As diagnosis or on lipid lowering treatment

+current or in personal history

In addition to the increased risk of comorbidities related to natural aging, PLHIV have an increased risk independent of age. Possibly adding further to the risk, elderly PLHIV more often have been exposed to the early ART drugs with more toxicities.[249, 250]

As mentioned in section 1.7.3, PLHIV have, despite ART, a persisting low-level inflammation and immune activation.[121-123, 126] This has been proposed as one of the possible mechanisms underlying the elevated risk of comorbidities.[104] In our study we compared the influence of ART regimen on levels of inflammation markers in the elderly group. Among the ten analysed markers we found differences between the arms in IL-6, sICAM-1, and CXCL10 levels. The ATV group had lower IL-6 levels compared to the other arms. On the other hand, the EFV group had lower levels of sICAM-1. The DRV group had higher CXCL10 levels, but significantly different only when compared to the EFV arm. A potential possibility of mitigating the residual inflammation with the choice of ART is interesting. However, it is not possible to draw any conclusion of causality based

on these results and the clinical relevance is uncertain. Earlier studies have not focused on elderly PLHIV which reduces the comparability of the studies.[251-253] In addition, intervariability between methods and within the Luminex method further complicates comparison.

When analysing and evaluating data concerning elderly PLHIV, it is important to remember that the group is not homogenous. The group consist of on the one hand the survivors, who probably experienced periods of immunodeficiency and were exposed to early ART drugs with toxicities. On the other hand, the group includes those who were diagnosed at an older age and may have started ART early during the course of infection. In addition, studies performed at different times during the course of the pandemic have included untreated PLHIV and those who started ART at different times during infection due to the recommendations at the time, and often a combination of these groups. These differences need to be considered, and may limit the comparability between studies. The future will come with new aspects and the composition of the elderly group will continue to evolve over time.

When caring for the increasing number of elderly PLHIV we need to consider certain aspects that are particularly important in this group. The choice of ART regimen is a cornerstone in all HIV care, but the need of individually tailored drug regimens are particularly important in the elderly. In our choice of ART, we need to consider how to reduce the disadvantageous effects of polypharmacy and PDDIs. Also, we need to consider how to ensure adherence, including social aspects. Lastly, we need to diagnose comorbidities early and treat them correctly.

Vitamin B metabolism in HIV infection

6 VITAMIN B METABOLISM IN HIV INFECTION

An article in Läkartidningen[254] discussing and summarizing a trial of the effect of vitamin B therapy on the rate of brain shrinkage attracted our interest to the field of homocysteine, vitamin B metabolism, and neurocognition.

In 1985 Petito et al reported that the myelopathy of AIDS had a pathological resemblance to the myelopathy found in vitamin B deficiency.[255] Several researchers hypothesised and investigated disturbances of the methylation cycle as a possible mechanism in HIV-related neurodegeneration.[256, 257] Vitamin B_{12} deficiency was reported as a common finding before the introduction of effective ART.[258, 259] However, studies investigating the association of B_{12} levels and cognition yielded contradictory results.[258, 260] Homocysteine levels are higher in untreated PLHIV compared to HIV-negative individuals, a finding that is consistent in those on ART.[261]

In the retrospective study, presented in paper III, we report a novel finding of a correlation between P-homocysteine and CSF NfL in 83 individuals with untreated HIV infection. The correlation remained significant also after the initiation of ART (in a second sample of 22 individuals). The result was reproduced in paper IV where a correlation was found between P-homocysteine and P-NfL in 124 PLHIV on ART.

As described in section 1.9, elevated homocysteine is a marker of vitamin B_{12} and/or folate deficiency. In the screening-cohort (n = 124) of the clinical trial one individual had vitamin B_{12} deficiency and five had folate deficiency, (diagnosed as P-homocysteine > 15 μmol/L and P-B_{12} < 140 pmol/L or P-folate < 7 nmol/L), and thus deficiency was rare. However, vitamin levels in the low-normal spectrum were frequent, 40% for P-B_{12} (< 300) and 35% for P-folate (< 10). Furthermore, 27% had elevated homocysteine levels, suggesting that suboptimal vitamin levels may be common in this group.

From the screening cohort those with P-homocysteine ≥ 12 μmol/L were included in the randomised trial, as described in section 3.3. After 12 months of treatment with vitamin B_{12}, folate, and B_6 the P-homocysteine levels had decreased, and the P-B_{12} and P-folate levels had increased in the active treatment arm. However, this was not accompanied by a decrease in P-NfL when compared to the control group. Neither after 24 months of vitamin B supplementation a

P-NfL lowering effect was noted. This result speaks against suboptimal vitamin B_{12} and folate as the cause of the association between homocysteine and NfL.

Both homocysteine and NfL increase with age.[177, 262] However, age was accounted for in the multiple regression analysis in paper III. Homocysteine inversely correlates to kidney function.[177, 263, 264] An earlier study of PLHIV found no correlation between kidney function measured by creatinine and P-NfL, but some later studies in PLHIV and non-HIV-infected individuals have found an association between P-NfL and creatinine.[265-268] However, it would not be probable that kidney function in the normal range, as is the case in paper III, would influence CSF NfL levels, speaking against kidney function as the cause of the association.

Immune activation is considered a pathogenic mechanism of HIV infection both systemically and in the CNS.[50, 153, 171] Despite continuous ART for several years signs of immune activation in the CNS, measured as neopterin, persist.[170, 269] Interestingly, in HIV-negative individuals serum neopterin levels have been associated with homocysteine levels.[270] In paper III we found a moderate correlation between neopterin and homocysteine in bivariate correlation but not in the multiple regression analysis, and thus did not support this as a common pathologic mechanism.

The metabolic syndrome has been connected with cognitive impairment both in the general population and in PLHIV.[271-273] Elevated homocysteine levels have in turn been related to the metabolic syndrome.[274, 275] In addition inflammation has been related to dementia and cognitive decline.[276, 277] As described above PLHIV have higher homocysteine levels and signs of inflammation compared to HIV negative individuals.[121, 123, 261] Furthermore, the metabolic syndrome is common among PLHIV, studies having reported a prevalence of 20–40%.[272, 278] We did not study the metabolic syndrome in the present study and hence a conclusion cannot be drawn from our data, but hypothetically the interactions of these factors may be a plausible explanation for the association between homocysteine levels and signs of neuroaxonal injury in PLHIV.

Conclusion

7 CONCLUSION

We found higher steady-state concentrations of PIs in elderly PLHIV compared to younger controls. In addition, polypharmacy was common in the elderly and they had a higher risk of potential drug-drug interactions. Altogether this puts elderly PLHIV in risk of adverse drug events and warrants careful consideration when prescribing both antiviral and non-antiviral drugs.

Despite effective ART PLHIV show a persistent chronic low-grade inflammation. Elderly treated with atazanavir, darunavir, or efavirenz had different levels of IL-6, ICAM-1 and CXCL10. Further studies are needed to confirm if different ART may give rise to different inflammatory profiles.

An association was found between NfL, a marker of ongoing neuroaxonal injury, and homocysteine, a marker of vitamin B deficiency, in PLHIV with and without ART. Supplementation with B vitamins decreased homocysteine levels but had no effect on NfL levels. This suggest that suboptimal B vitamin levels are not the cause of the association. Further studies to investigate possible underlying causes are warranted.

Future perspectives

8 FUTURE PERSPECTIVES

The worldwide recommendation to treat all irrespective of $CD4^+$ cell count as a result of the START study was a turning point in HIV medicine. Early treatment changes the focus from complications related directly to the virus to those of ART, comorbidities, and indirect effects of the virus.

As seen in our studies elderly PLHIV are at risk of drug related unfavorable events. There are today a couple of cohort studies focusing on older and elderly PLHIV,[279] but in the future, studies designed to study ART and the relationship to concomitant medications in the elderly are needed.

We need to learn how to best care for the growing number of elderly PLHIV. In the UK a combined HIV and geriatrics clinic has been developed that may lead the way.[280]

Still in the era of effective ART, PLHIV are at higher risk of developing comorbidities. Further studies are needed that investigate the mechanisms underlying the higher risk of comorbidities and how to mitigate them.

We still do not know all the effects of long-term ART and future studies focusing on organ dysfunction and comorbidities, are needed. Prospective studies are needed to see if residual inflammation is related to ART regimen.

Studies are needed to further elucidate the mechanisms that drive the development of and progression of HAND in those with effective ART. Our finding of a relationship between NfL and homocysteine warrants further investigation into the cause, including the role of inflammation and the metabolic syndrome in neuroaxonal injury in PLHIV.

The ever alluring goal is cure. In the history of HIV two individuals living with HIV have been medically cured. The first one was Timothy Brown, the so called "Berlin patient" who was reported to be cured of HIV in 2006. Timothy Brown received an allogenic stem-cell transplantation due to acute myeloid leukemia with a donor who was homozygous for the CCR5Δ32 mutation. A second transplant was needed after relapse but led to complete remission of the leukemia and no virus could be detected during the follow-up period.[281] Timothy Brown died of leukemia in 2020. He experienced no reoccurrence of HIV during his lifetime.[282] The second case, Adam Castillejo, initially known as "the London patient", was reported at the Conference on Retroviruses and Opportunistic Infections in 2019,

a conference I myself attended. As with Timothy Brown, Adam Castillejo received an allogenic stem-cell transplant with a donor homozygous for the CCR5Δ32 mutation due to treatment of lymphoma. He was transplanted in 2016, stopped ART in 2017 and have experienced no viral rebound.[283] Allogenic stem-cell transplantation is a dangerous treatment and not feasible to perform with the purpose to cure HIV but has rendered some hope and insights. Furthermore, in the case of two elite controllers the analysis of a large number of cells failed to identify any replication competent proviral DNA suggesting naturally achieved cure.[284]

As previously discussed, the latent reservoir constitute an obstacle to HIV cure and subsequently the reservoir has been in focus in the research for a cure. Different modes of action have been suggested and investigated such as; the "shock and kill" strategy which aims to activate latently infected cells who are then killed by cells of the immune system; inactivate or remove proviral DNA through gene-editing such as CRISPR/Cas9 ; or the "lock and block" theory that aims to hinder latently infected cells to reactivate.[285, 286] None of the strategies has of yet been successful but the search for a cure will continue.

Last but not least we cannot forget that a life with HIV today looks very different depending on where you live. Large inequalities in HIV care exist globally and the diagnosis is still often associated with stigma.

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