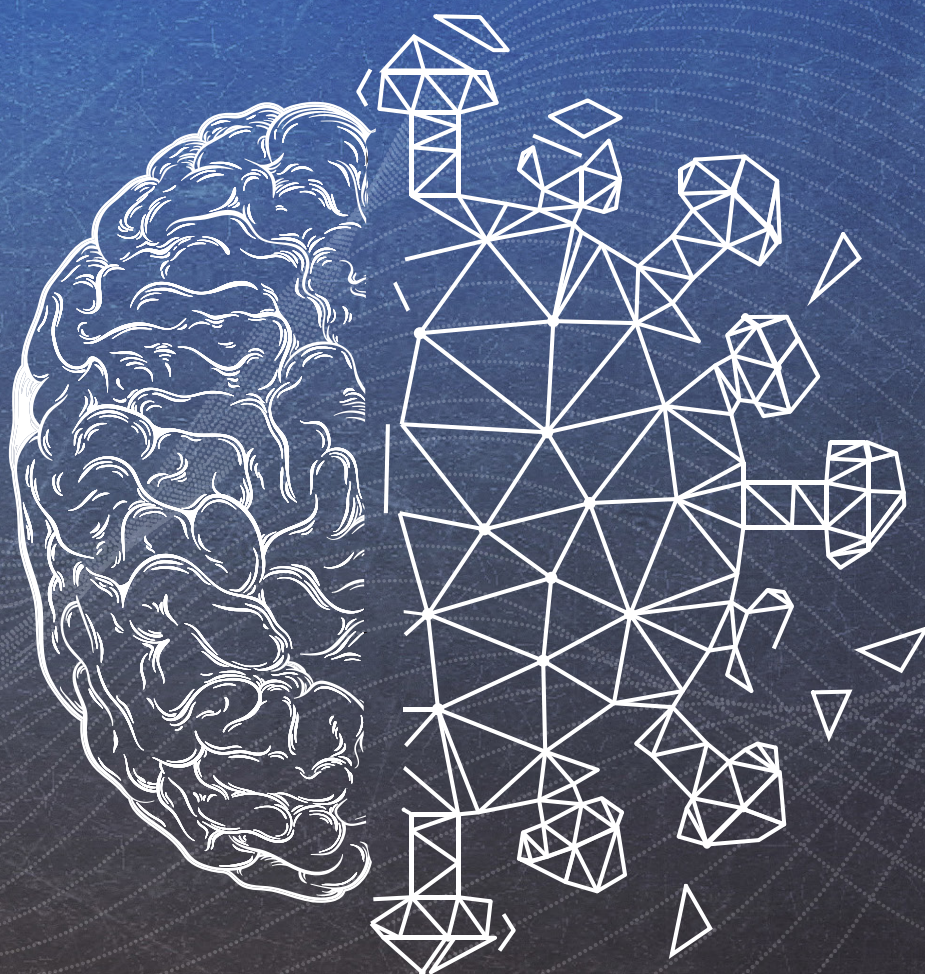# HIV and the Brain

## COGNITIVE COMORBIDITIES AND COMPLICATIONS IN HIV INFECTION AND TREATMENT

Charlotte Hakkers
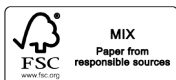
# HIV and the Brain

## COGNITIVE COMORBIDITIES AND COMPLICATIONS IN HIV INFECTION AND TREATMENT

PROEFSCHRIFT

C.S. Hakkers

# HIV AND THE BRAIN
## Cognitive comorbidities and complications in HIV infection and treatment

**HIV en het Brein**

Cognitieve comorbiditeiten en complicaties bij HIV-infectie en -behandeling
(met een samenvatting in het Nederlands)

**Proefschrift**

ter verkrijging van de graad van doctor aan de
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ingevolge het besluit van het college voor promoties
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door

**Charlotte Susanne Hakkers**

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Dr. J.E. Arends

# CONTENTS

# CHAPTER 1

Introduction

# HIV INFECTION: ACUTE TO CHRONIC

The Human Immunodeficiency Virus (HIV) was first discovered in 1983 after physicians worldwide were being faced with an inexplicable severe illness resulting in deaths of countless young, previously healthy patients.[1-4] The little time it took to develop the first line of therapy for this unknown virus after its discovery, was unprecedented.[5] The subsequent development of combination antiretroviral therapy (cART) in 1995 meant a breakthrough in HIV care, finally giving patients something that, although it didn't cure the infection, could protect them from developing the deadly Acquired Immune Deficiency Syndrome (AIDS).[6] Further development of more potent antiretrovirals with less side effects improved HIV treatment so that as of now, HIV-infected patients have a similar life expectancy compared to non-HIV-infected individuals.[7] However, this doesn't mean the problems are over. HIV-infected patients have a higher risk of suffering from comorbidities like cardiovascular disease, osteoporosis, cognitive impairment and certain forms of cancer. These long-term comorbidities are called non-AIDS events. Moreover, in order to suppress the virus, patients are required to take daily medication for the rest of their lives, with its corresponding side effects and long-term toxicity. Currently, it is estimated that 36.7 million people suffer from HIV globally, making it a substantial burden on society and healthcare worldwide.[8]

With the transition from an acute threat to a chronic condition, the focus of HIV care has now shifted from treating life-threatening opportunistic infections to helping patients achieve a high age with little to no comorbidities. This is illustrated by, for example, the latest proposed addition to the 90-90-90 target of the WHO. The initial target aimed to diagnose 90% of all HIV patients, treat 90% of those diagnosed, and obtain viral suppresion in 90% of those treated.[9] In 2016, Lazarus et al suggested a fourth target; that 90% of those with viral suppression have good health-related quality of life.[10] An important factor in achieving that goal is to minimize the long-term effects of the virus and the medication used to treat it. Moreover, as the HIV-population is aging, comorbidities associated with -or worsened by - age are becoming particularly relevant for physicians and patients alike. One of the most important comorbidities in the aging HIV population is increased neurocognitive decline.[11] Signs of cognitive impairment in HIV have been reported since the beginning of the HIV epidemic, but as patients nowadays live longer, understanding and treating these impairments becomes more and more important. Unfortunately, adequate diagnosis and clear etiology of neurocognitive impairments seen in HIV is still lacking. For instance, there is still a lot of debate on the preferred gold standard for diagnosing and classifying cognitive decline in HIV patients. Current methods that are being used to diagnose HIV-Associated Neurocognitive Disorder (HAND) are time-consuming and costly, and may not be sensitive enough to reliably detect mild neurocognitive impairment. Furthermore, it is not clear why HIV

patients develop neurocognitive impairment. Especially the role of cART toxicity has been poorly investigated.

The aim of this thesis is to explore the shape and size of neurocognitive impairment as comorbidity to HIV infection, investigate existing and novel diagnostic options, and to look into the role of cART toxicity.

## HAND: HIV-ASSOCIATED NEUROCOGNITIVE DISORDER

Evidence that HIV travels to the brain quickly after initial infection was already given in 1992 when a patient was accidentally infected with HIV and researchers were able to follow the course of the first days of the infection meticulously.[12] A large amount of young AIDS patients developing full-blown dementia in the pre-cART era, was the first sign HIV has an effect on the brain.[13] Since the development of cART in the mid-1990s, the amount of HIV Dementia cases declined rapidly.[14] However, research has shown that a large number of HIV-infected patients still suffer from a form of neurocognitive decline associated with HIV infection.[11,15,16] A recent study in stable treated HIV-positive patients demonstrated that around 50% suffer from some form of HAND, with most of them showing milder forms of cognitive impairment.[11] Many studies have reported cognitive impairments, but the estimated proportion of patients affected differs greatly across studies.

Cognitive impairment, even in its milder forms, poses sincere complications for patients. HIV-infected individuals suffer mostly from impairments in attention, memory, motor skills, and executive functioning.[17–19] These impairments have a negative effect on quality of life, participation in society and the ability to keep a job, making them a substantial burden on society as well as on the individual patient.[20,21] Moreover, studies have shown that cognitive impairment can decrease treatment adherence, which can result in virological failure and resistance of the virus.[22]

Previously, the different forms of cognitive decline were named Minor Cognitive Motor Disorder (MCMD) and HIV-Associated Dementia (HAD). In 2007, a new consensus about the terminology was made in Frascati, Italy, summarizing different diagnostic classifications in the term HIVAssociated Neurocognitive Disorder (HAND).[19] According to the new Frascati criteria, HAND can be divided into Asymptomatic Neurocognitive Impairment (ANI), Mild Neurocognitive Disorder (MND) and HIV Dementia (HD). These different forms are diagnosed by means of a neuropsychological assessment (NPA).[19] See table 1 for an explanation of the Frascati criteria.

**TABLE 1.** Frascati criteria

|  | NPA outcome | Functional complaints |
|---|---|---|
| Asymptomatic Neurocognitive Impairment (ANI) | At least 1 SD below the norm on at least 2 domains | No functional complaints |
| Mild Neurocognitive Disorder (MND) | At least 1 SD below the norm on at least 2 domains | (mild) functional complaints |
| HIV Dementia (HD) | At least 2 SD below the norm on at least 2 domains | (severe) Functional complaints |

Functional complaints: interference of cognitive impairment with everyday life, SD = standard deviation

An NPA uses different subtests, that each investigate one or two separate domains of cognition, such as attention, working memory, executive functioning or speed of information processing.[23] The combination of multiple subtests results in a performance-based test battery that maps the cognitive abilities of an individual on multiple domains. Because it can be constructed using different subtests, the shape and size of an NPA can differ greatly depending on the reason for testing. The Frascati criteria have specified that, in order to diagnose HAND, at least 5 domains should be tested; preferably at least 2 subtests per domain.[19]

Outcomes of neuropsychological tests are usually given as Z-scores, calculated by using the mean and standard deviation of demographically corrected normative data. This is common practice for several reasons; first because raw outcome measurements of neuropsychological tests differ greatly; sometimes the outcome represents the number of seconds needed to complete the task, other times it can mean the number of properly recalled words. By transferring the outcomes to Z-scores, the outcomes of different tests can be combined, and a composite Z-score can be calculated for interpretation and classification. Another important reason to first convert raw scores to Z-scores is that in this matter, effects of age, education level and/or gender can be corrected by using stratified normative data. In this light, it is sensible to assume that using the appropriate normative data is important, thus data fitting to the population being tested.

## SCREENING FOR HAND AND MANAGEMENT OF OUTCOMES

Although an NPA is the current gold standard for diagnosing cognitive impairment in HIV, the diagnostic itself has several drawbacks. Firstly, an extensive NPA incorporating enough subtests and domains can easily take up to several hours, demanding a substantial time investment from the patient. Moreover, an NPA can only be performed by a trained professional (neuropsychologist), making it costly and impractical. This is especially an issue in settings with limited resources, such as low-income countries. The diversity in culture, language and education in exactly these settings poses an extra difficulty with

conducting an NPA, namely finding a fitting normative group. Seeing as the majority of people living with HIV resides in limited-resource environments (i.e. sub-Saharan Africa), there is a need for short and easy screening tools. And even though there are several screening tools available for cognitive decline, they were mostly developed for the Western world, and it is not certain if they are applicable for use in resource-limited settings.

In the Western world, on the other hand, cognitive screening tests are often used, and screening for cognitive impairment in HIV-infected patients is even recommended by several international guidelines. Nevertheless, consensus is lacking on the type of screening test. Some institutions use subjective tests, others use objective tests, or a combination of the two. Furthermore, it is still not clear what the next steps are after a patient fails a screening test, and guidelines do not offer recommendations for the management of an HIV patient with a screening test score below the cut-off. Several comorbidities or other illnesses can underlie cognitive impairment and should be investigated and, when possible, properly treated. These could be conditions that can be dealt with relatively simple, but with great improvement to the patients' quality of life. And if a treatable cause can't be found, physicians should have something to offer their affected patients so that they can learn to manage their symptoms in their everyday life.

## HAND, cART TOXICITY AND EFAVIRENZ

The etiology of neurocognitive impairment in HIV, or HAND, is as of yet not completely clear. Based on the dramatic decline in HIV dementia cases after the implementation of cART as primary treatment, it has been proposed that an active infection and/or viremia is the causative agent in HAND. Evidence of a direct toxic effect of the virus on the brain comes from autopsy data showing large quantities of HIV particles in the brain, especially in the basal ganglia and hippocampus in untreated AIDS-patients with dementia.[24,25] However, a large proportion of patients who are being properly treated with cART also suffer from HAND. This leads to the hypothesis that in addition to virus toxicity itself, the medication used to suppress the virus could be a significant contributor to HAND. To test this hypothesis, Robertson et al. performed a large study in 2010 during which they interrupted antiretroviral therapy in a group of stable HIV patients.[26] Indeed, patients showed improved cognitive functioning after cART discontinuation, providing evidence that HIV medication has a deteriorating effect on cognition.[27] This detrimental effect of medication was most present in cART regimes containing Efavirenz. Efavirenz is commonly known to cause emotional and cognitive side effects. For example, a large proportion of patients starting Efavirenz complain of experiencing bad dreams, sleep disturbances, mood complaints, and cognitive issues like memory loss and trouble concentrating.[28–31]

Subsequent in vitro studies provided additional evidence for a direct toxic effect of Efavirenz on neuronal cell lines and primary neurons.[32] Moreover, there have been reports of people crushing Efavirenz and smoking it for its psychedelic effects.[33] Efavirenz use has also been associated with an increased risk of suicide attempts.[34] More recent trials investigating differences in medication regimes in experienced patients found Efavirenz to be a risk factor for neurocognitive impairment.[35,36] All this taken together means that, when investigating HAND and its etiology, cART toxicity should be considered as well, with Efavirenz as its most notable example. Surprisingly, however, the mechanisms by which Efavirenz impacts the human brain, and hence cognitive functioning, is still poorly understood.

Besides from the etiology question of HAND and the role cART toxicity plays in it, properly investigating the effect on Efavirenz on cognition is important for another reason. Efavirenz is part of the first single tablet regime (STR) Atripla, where the different components of a cART regime are incorporated in one tablet. STR's offer a great deal of convenience for HIV patients, and are therefore currently the first regime of choice. Atripla has therefore been the most-prescribed cART regime for a long time. And although newer STR's like Triumeq and Biktarvy have taken their place in the treatment of HAND in the Western world, many HIV patients in Africa remain on Atripla. Atripla has recently become generically available, making it an attractive and economical choice of regime. This means that Atripla's popularity in resource-limited settings could possibly remain high. Even in the Western world, healthcare costs are exponentially growing, making the costs of medication a topic of discussion and controversy. A recent example of this was the introduction of the very costly Directly Acting Antiretrovirals for Hepatitis C. Objective evidence on (cognitive) side effects is important in these discussions.

Importantly, while Efavirenz is historically the antiretroviral most associated with cognitive side effects, newer medications are also associated with neuropsychological side effects. In 2014, Dolutegravir, a second generation of a new class of antiretroviral drugs called Integrase strand transfer inhibitors (INSTIs) became available for treatment. Dolutegravir was a highly anticipated drug because phase II and III studies showed it had a very favorable toxicity profile.[37,38] Nevertheless, after implementation of the drug, signals arose from clinical practice that many patients developed side effects.[39] Especially neuropsychological side effects were frequently observed. The fact that drugs from different drug classes show neuropsychological and cognitive side effects means cART toxicity is not limited to just one drug, and could therefore be an important factor in the etiology of HAND. This makes it important that cART toxicity is thoroughly investigated. However, it is not clear from anecdotal evidence how big the problem with Dolutegravir is. It is important to investigate the occurrence of side effects in the entire population in a standardized method, preferably with a comparison to a different but comparable drug.

# FUNCTIONAL MRI, A NEW PLAYER IN THE FIELD OF COGNITIVE DIAGNOSTICS

Neuropsychological testing is the gold standard for diagnosing HAND. However, as stated above, this method has its disadvantages; it is time-consuming and costly, and might not be sensitive enough to detect subtle cognitive problems. Blood Oxygenated Level Dependent (BOLD) functional MRI is a relatively new method of investigating cognition, and several studies in other medical fields and diseases have shown BOLD fMRI is more sensitive than NPA to pick up brain abnormalities.[40–42] BOLD fMRI uses the difference in the MRI signal of oxygen-rich and oxygen-poor blood to analyze which parts of the brain are in use when performing a functional cognitive task. Examples of cognitive tasks are: tracking a number of moving balls across a screen, memorizing a series of numbers, or inhibiting a response to a trigger. Mapping the amount of activation in certain brain regions can give information on neurocognitive abilities even before this becomes noticeable in screening tests or everyday life. This means BOLD fMRI can be a powerful screening instrument for the early stages of HAND. BOLD fMRI is a relatively new diagnostic tool, and only a few clinical studies have been done with fMRI in HIV patients. This means there is still a lot of uncertainty about the use of this technique in HAND. Can the BOLD technique even be used in HIV patients? On what brain regions should research be focused? Are there specific tasks that can be preferably used for this specific question? Not only is, as always, more research needed, a systematically presented summary of all the research done so far is called for as well.

# OUTLINE OF THIS THESIS

The main topic of this thesis is HIV-Associated Neurocognitive Disorder, with an emphasis on diagnostics (screening and fMRI) and the role of combination antiretroviral cART toxicity in its Etiology.

Part one focusses on the role of cART toxicity in the development of HAND. In **Chapter 2**, the ESCAPE trial is described, where patients switched from an Efavirenz-containing regime to another regime without Efavirenz, and the effect of the switch on NPA is examined. In **Chapter 3**, the correlation between Efavirenz drug levels and neurocognitive functioning is assessed. In the same study, an analysis is done on the usefulness of a novel biomarker; plasma neurofilament light. Finally, in **Chapter 4**, the number of discontinuations of the newest antiretroviral class (Integrase Inhibitors) is reported, with a special focus on neurotoxicity.

Where part one focusses more on screening for HAND, part two investigates the

occurrence and detection of neurocognitive decline in HIV patients. As mentioned before, numbers on the prevalence of neurocognitive decline or HAND vary greatly. There is also some debate about the difference between objective cognitive decline, and subjective complaints patients report.[43,44] In **Chapter 5**, the prevalence of objective and subjective cognitive issues in the HIV-positive out-patient population of the University Medical Centre Utrecht, together with a step-by-step management protocol, is described. Because of the difficulties associated with an NPA, such as costs and duration, cognitive screening tools were used. **Chapter 6** describes a trial investigating the usefulness of such a screening tool in sub-Saharan Africa, where the majority of people living with HIV reside. Additionally, formal NPA testing of the HIV-positive cohort of this trial gave insight in the prevalence of cognitive impairment in resource-limited settings.

Part 3 of this thesis focusses more on the value of functional MRI in diagnosing HAND or cognitive decline. In **Chapter 7**, a systematic review is given of all previously published literature on the subject of fMRI in HIV-positive patients.

Finally, in **Chapter 8**, the main findings of this thesis are summarized and discussed together with perspectives for the future.

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# PART 1

effect of cART on developing
neurocognitive impairment

# CHAPTER 2

Objective and Subjective Improvement of Cognition
after Discontinuing Efavirenz:
A Randomized Controlled Trial

Objective and Subjective Improvement of Cognition after Discontinuing Efavirenz

# ABSTRACT

**Background**
Efavirenz is well known for its clinical cognitive side-effects. Even asymptomatic patients who switch for other reasons than neurocognitive complaints have reported a subjective improvement in cognitive functioning after discontinuing Efavirenz. The aim of this study was to assess the effect on cognition of switching Atripla (TDF/FTC/EFV) to Eviplera (TDF/FTC/RPV), hypothesizing an improvement when discontinuing Efavirenz.

**Setting**
A randomized controlled design with a highly comparable comparator drug was used to minimize bias and to differentiate drug- versus learning effects. An extensive sensitive neuropsychological assessment (NPA) was used to detect subtle changes.

**Methods**
Virologically suppressed, cognitively asymptomatic male HIV-infected patients on Atripla were included and randomized (2:1) to switch to Eviplera (switch group) or continue on Atripla (control group) for 12 weeks. At baseline and week 12, patients underwent an extensive NPA.

**Results**
14 control and 34 switch subjects completed the study. There were no differences at baseline. Group-analysis demonstrated a significantly better improvement for the switch group on the domains attention (p=0.041) and speed of information processing (p=0.014). Normative comparison analyses showed that 5 out of the 34 patients who switched (15%) improved on NPA-score as compared to the control group. Interestingly, subjective improvement after discontinuing Efavirenz made 74% of the switch group chose for a regime without Efavirenz after study completion.

**Conclusion**
Switching from Atripla to Eviplera resulted in objective cognitive improvement on group level in cognitively asymptomatic patients. Discrepancies in objective and subjective cognitive complaints make it challenging to identify patients that would benefit from discontinuing Efavirenz.

# INTRODUCTION

Neurocognitive impairment (NCI) is a frequently occurring complication of HIV-infection, with a negative effect on quality of life, participation, and drug adherence.[1–6] The different presentations of NCI in HIV-infection are summarized in the term HIV Associated Neurocognitive Disorders (HAND), which is conventionally diagnosed with a neuropsychological assessment (NPA). The etiology of NCI in HIV is currently not yet fully elucidated. Evidence has pointed in the direction of an effect of the virus itself or an effect of immune-activation, but this doesn't explain the fact that HIV-patients who are being adequately treated suffer from NCI as well.[6] Even in the era of combination antiretroviral therapy (cART), HAND prevalence remains as high as 50%, and around 40% of patients in out-patient settings report subjective neurocognitive complaints.[4,7] Recently, a study found a beneficial effect of discontinuing cART on cognition, and additionally, several studies have suggested a direct negative effect of antiretroviral treatment on cognitive function.[8–10]

When investigating neurocognitive toxicity of cART, a good example is Efavirenz, a drug that, although being one of the most frequently used antiretrovirals world-wide, has been associated with considerable neurocognitive complaints.[11–14] This is mostly visible in patients who experience evident side-effects and subsequently quickly change their regimen.[9] This toxicity profile, along with the emergence of newer antiretrovirals, has resulted in a decline in Efavirenz' popularity in the Western world. However, Efavirenz is still very much the drug of first choice in low income countries.[15,16] Furthermore, the most popular combination of Efavirenz with other antiretrovirals in a single tablet regime (STR), namely Atripla, has recently become generically available, making it a very attractive regimen for economical regions. Moreover, a substantial group of patients on Efavirenz do not report cognitive complaints, i.e. cognitively asymptomatic patients. Anecdotal evidence has suggested that the negative effect on cognition even in these 'asymptomatic' patients seems reversible. That is, studies in asymptomatic patients did show an improvement in cognition, the amount of adverse events, and drug adherence after discontinuing Efavirenz.[10,17] Additionally, from clinical experience in our out-patient clinic, we have learned that patients often develop a subjective cognitive improvement after switching Efavirenz for other reasons than cognitive side-effects. Nevertheless, the effect of Efavirenz in asymptomatic patients has not yet been properly studied. There are only two studies in asymptomatic patients investigating the effect of switching from an Efavirenz-containing regime by means of an NPA.[18,19] These studies are however limited by their design (i.e. observational or retrospective and no comparative control group), sample sizes and/or measurements of cognition.

When studying cognition, the choice of the NPA used is very important. For example, HAND is diagnosed by an NPA measuring at least five cognitive domains with preferably

at least two subtests per domain.[20] Because the current study focusses on asymptomatic patients, it is important to use a sensitive NPA which is not so much focused on impairment, but on performance.[21] This can be accomplished with subtests designed to pick up subtle changes within the higher ranges of measured cognition. This is especially important when investigating (long term) cART toxicity, seeing as patients with subtle complaints usually stay on their regime, rather than switching to a different regime altogether.

Given the attributed effects of Efavirenz on cognition, the hypothesis of this study is that discontinuation of Efavirenz leads to measurable improvement in neurocognitive functioning. The aim of this study is to investigate the effect of discontinuing Efavirenz in stable, asymptomatic HIV patients, using an extensive and sensitive NPA together with inclusion of a control group.

## METHODS

**Participants**

The ESCAPE (Effect of SwitChing AtriPla to Eviplera on neurocognitive and emotional functioning) study ran from May 2015 till December 2016. Stable HIV-infected patients on Atripla® were recruited from the outpatient department of a large academic HIV treatment center (UMC Utrecht) and a large peripheral HIV treatment center (OLVG) in the Netherlands. In order to ensure a homogeneous study group, only patients on Tenofovir/Emtricitabine/Efavirenz (Atripla®) at time of inclusion were asked to participate. Patients were eligible if they were male, between 25 and 50 years old, were on Atripla for at least 6 months with an undetectable viral load on the last visit before inclusion, were fluent in Dutch, and without any subjective cognitive complaints in the last year. Exclusion criteria were: having active or past CNS opportunistic infections, active psychiatric of neurologic disorders,  a history or evidence of alcohol or drug abuse, assessed with the Drug Abuse Screening Test (DAST-10), and/or anatomical abnormalities on an MRI-scan of the brain.[22] After being screened for in- and exclusion criteria and receiving complete information on the study procedures, all patients signed written informed consent. The study was performed according to the Declaration of Helsinki[23], was reviewed and approved by the medical ethical board of the University Medical Center Utrecht, and was registered at clinicaltrials.gov under number NCT02308332.

**Study design**

Patients on Atripla® were randomized to the switch group (S; Eviplera®, i.e. Tenofovir/Emtricitabine/Rilpivirine) or the control group(C; continuing on Atripla®) with an S:C ratio of 2:1. We chose to put the switch group on Eviplera® because it is a single tablet

regime (STR) like Atripla®, with the same backbone (Emtricitabine/Tenofovir) and with a third agent within the same drug class of non-nucleoside reverse transcriptase inhibitors (NNRTI). One of the differences between the regimes is the dietary instructions of Eviplera® which has to be taken with a substantial meal (390 kcal and 12 grams of fat). This, however, means patients experience more stringent lifestyle rules with Eviplera®, with dietary consequences and a more strict regularity in timing and size of the main meal of the day.

Patients in both groups underwent study-related procedures at baseline and week 12 including an extensive NPA together with measurements of HIV-RNA loads and CD4 cell counts, and an MRI scan of the brain. Additionally, plasma concentrations of Efavirenz for all patients at baseline and either Efavirenz or Rilpivirine concentrations on week 12 depending on the switch or control group were determined. Finally, patients filled out questionnaires on quality of life, participation and mood. As planned, patients in the switch group were seen at week 2 and 4 after the switch, to check for Eviplera side effects and to perform routine laboratory tests. After completion of the study, patients were given the explicit choice to either go back to their pre-study regime, remain on Eviplera, or switch to a different cART regime all together.

**NPA**
The NPA was conducted by a trained neuropsychologist (ME), and interpreted by a senior neuropsychologist (MvZ), who were both blinded for treatment allocation. Afterwards, data was further analyzed by the trial physician (CH).

The NPA comprised of 16 subtests, testing for seven cognitive domains. Since the ESCAPE study group consisted of asymptomatic patients, we specifically chose NPA tasks that can detect subtle changes. This means we focused on those tasks that are not limited to detect cognitive impairment, but that can also chart performance without a ceiling effect; i.e. tests without a predefined maximum, or timed tests. The domains tested were language, learning and memory, executive functioning, attention/working memory, speed of information processing, and psychomotor speed. The subtests and their accompanying domains are given as supplemental data. In order to control for test-retest effects alternate test versions were used for the week 12 NPA. Dutch age- and education level adjusted normative data were used to transform raw test scores into standardized z-scores to allow further comparison between tests and domains. Cognitive domain scores were calculated by averaging the Z-scores of the different tests per domain, and a composite Z-score was calculated by averaging all tests' Z-score. The change (improvement or worsening) of these domain- and composite Z-scores in both groups were used to assess the effects of discontinuing Efavirenz.

**Questionnaires**

Patients filled out questionnaires at baseline and at follow-up including the Short Form Health Survey (SF-36) to investigate Quality of life. The SF-36 is a survey constructed for self-administration made up by 36 items on quality of life, divided into 8 sections. It has been proven to be a practical, reliable and valid instrument for use in chronically ill patients.[24,25] An important aspect of quality of life is how well an individual can function in society, e.g. having a payed job, being able to interact with others, the ability to run a household and practice self-care. This concept is also called Participation. In this study, participation was measured using the Utrecht Scale for Evaluation of clinical Revalidation – Participation (USER-P), a brief instrument to rate objective and subjective participation consisting of 31 items on three scales: Frequency, Restrictions and Satisfaction. Outcomes were measured on a scale from 0 to 100 (most positive outcome). The Hospital Anxiety and Depression Scale (HADS) was filled out to examine mood complaints. The HADS is a self-report screening scale developed to indicate presence of anxiety and depressive states. It comprises a 7-item scale with a maximum of 21 points. A score of 11 points or more indicates a probable mood disorder. Furthermore, four short forms from the Patient Reported Outcome Measurement Information System (PROMIS) were used (see http://www.assessmentcenter.net and http://www.dutchflemishpromis.nl). PROMIS questionnaires, or short forms, are a valid and reliable measurement system to measure patient reported health outcomes. By using a dynamic system of item banks, multiple aspects of health and wellbeing can be tested. In this study, the short forms Anxiety, Depression, Sleep disturbances and Satisfaction with Social Roles and Activities were used. The raw scores of the PROMIS short forms were transformed into T-scores with a mean of 50 and a standard deviation of 10.

**Statistical analyses**

Differences in baseline characteristics were evaluated using a chi-square test in case of categorical variables, and an independent samples T-test (for normal distribution) or Mann-Whitney U test (for skewed distribution) in case of continuous variables.

The main outcome measure was the change in NPA composite Z-score at 12 weeks after the switch compared to the control group. Effects of the switch were analyzed at group level using a linear mixed effects model with composite and domain Z-scores as outcome measurement. The interaction between time (baseline or week 12) and group (switch or control) was used to assess the group effect (estimated difference) on  Z-score. Missing data were accounted for by the model using maximum likelihood estimation. Models with random effects for intercept (per individual) and slope (for time) were evaluated for each score. Model diagnostics were performed by checking standardized residuals versus fitted values, residuals per subgroup and per individual and by checking homoscedasticity of random intercepts. A two-sided alpha level of

0.05 was used, and 95% Confidence Intervals (CI) were calculated. Moreover, to assess differences in improvement in the switch group compared to the control group at individual level, analysis was done on the delta (difference) of the Z-scores per individual using a corrected normative comparison (NC) developed by Huizenga et al.[26] This method, used for evaluating multiple neuropsychological tests by comparing them to a control group performing the same tests, uses step-down resampling as a correction for multiple comparisons. Questionnaires outcomes were evaluated on a group level with a repeated-measures GLM.

Mixed model analyses were performed using R Statistical Software version 3.3.2, for the remaining analyses, IBM SPSS version 21 was used.

## RESULTS

From all patients eligible for inclusion, 59 were willing to participate and were screened for inclusion (figure 1). The main reasons patients did not want to participate was because of time investment, and reluctance to switch to a regime that had a dietary restriction. Four patients were excluded after screening, leaving 55 patients to complete the baseline visit. Subsequently, due to a detected lesion in the brain on the anatomical MRI, an additional patient was excluded leading to randomization of 54 patients; 16 patients in the control and 38 patients in the switch group. Six patients did not have a week 12 visit because of side effects (1 control and 1 intervention), technical issues with the MRI scanner or inability of the patient to comply with the time window of 12 weeks (figure 1). There was no significant difference between the switch and the control group at baseline with respect to age, years of education and mean composite z-score (table 1). The mean age was 41.6 (SD 6.1) for the control group and 41.3 (SD 6.7) for the switch group. Mean years of education was 16.8 (SD 0.8) for the control group and 16.2 (SD 1.7) for the switch group. Furthermore, patients had a high mean level of education according to the Verhage scale[27] (5.8 control and 5.5 switch), and scored well on subjective measurements of quality of life and participation. Moreover, unemployment rate was low (12% and 3%).

### NPA group analysis

The main study outcome was the change in NPA composite Z-score for the switch group compared to the control group. At baseline, there was no significant difference in the mean composite Z-score between the control group 0.36 (IQR 1.14) and the switch group 0.21 (IQR 1.04; p=0.40). Additionally, no significant difference was found at baseline between the groups on the seven cognitive domain-Z-scores (table 2a). After 12 weeks, the mean composite Z-score for the control group improved to 0.67 (IQR 1.16) and for the switch group to 0.52 (IQR 0.83; p=0.40). Both groups improved, although not significantly,

on all domains except on the domain memory. Subsequently, the difference in change at week 12 between the two groups was assessed using a mixed model (Table 2b). No significant improvement was found on composite Z-score for the switch group compared to the control group (estimated Z-score difference = 0.15; p-value=0.10). However, the switch group improved significantly more than the control group on the domains attention (estimated Z-score difference = 0.37, p=0.04) and speed of information processing (estimated Z-score difference = 0.37, p=0.01) (figure 2a and 2b).
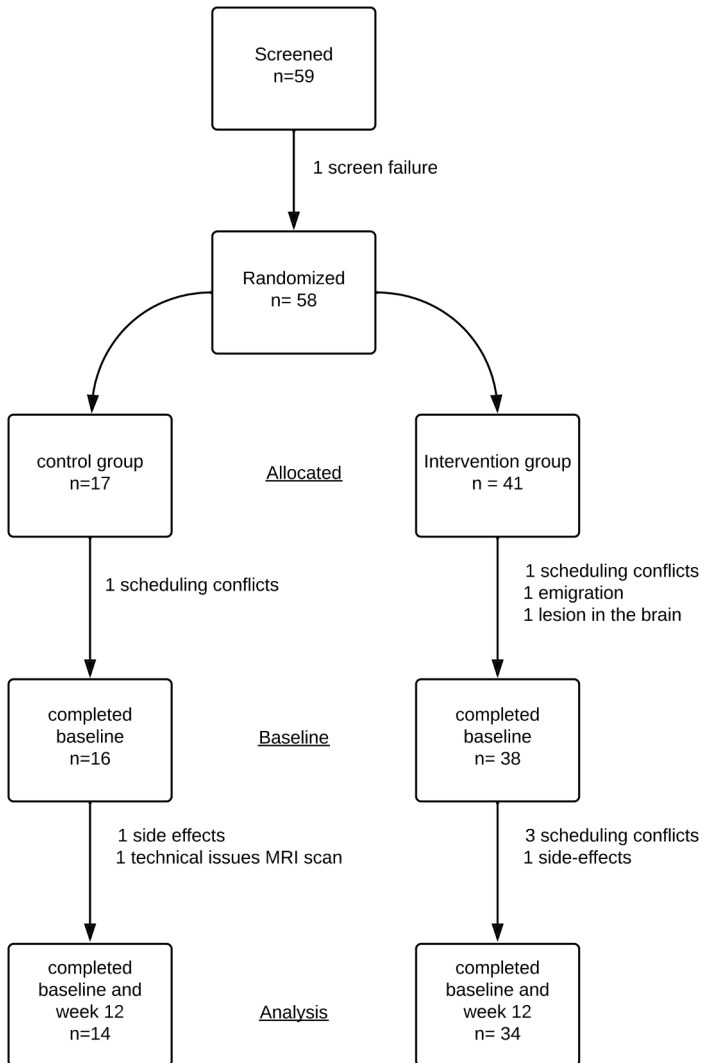
FIGURE 1. Inclusion flow-chart

**TABLE 1.** Table of baseline patient characteristics

|  | Control (16) | Intervention (38) | p-value |
|---|---|---|---|
| Age | 41.6 (6.1) | 41.3 (6.7) | 0.76 |
| CD4 | 699.3 (200.4) | 665.4 (238.8) | 0.91 |
| Years of education | 16.8 (0.8) | 16.2 (1.7) | 0.18 |
| Education level Verhage* | 5.8 (0.8) | 5.5 (0.86) | 0.95 |
| CD4 nadir | 263.3 (118.6) | 293.4 (157.0) | 0.38 |
| Time since HIV diagnosis (months) | 108.4 (50.9) | 89.2 (58.5) | 0.29 |
| Employed (in %) | 88 | 97 | 0.21 |
| Time on cART (months) | 63.9 (32.8) | 62.7 (40.8) | 0.99 |
| Time on Efavirenz (months) | 59.6 (25.7) | 57.1 (30.2) | 0.86 |
| Comedication (in %) |  |  | 0.77 |
| 0 | 53 | 63 |  |
| 1 | 37 | 31 |  |
| 2 or more | 10 | 6 |  |
| Previous cART regimes (in %) |  |  | 0.35 |
| 0 | 81 | 87 |  |
| 1 | 19 | 8 |  |
| 2 or more | 0 | 5 |  |
| User-P satisfaction score (0-100) | 80.8 (14.1) | 74.3 (16.6) | 0.21 |
| User-P restrictions score (0-100) | 99.1 (1.9) | 97.9 (5.5) | 0.42 |
| SF-36 general health score (0-100) | 72.2 (11.0) | 77.5 (12.3) | 0.30 |
| SF-36 total score –physical | 53.2 (5.0) | 53.2 (7.2) | 0.98 |
| SF-36 total score – mental | 53.2 (4.7) | 51.1 (7.7) | 0.33 |

All outcomes are shown in mean (SD), unless otherwise specified
cART = combination AntiRetroviral Therapy, SF-36: short form 36, User-P: Utrecht Scale for Evaluation of Revalidation – Participation
*Verhage education level: Dutch classification system including 7 categories from 1 (did not finish primary school) to 7 (university degree)

**TABLE 2. Change in composite and domain Z-scores on baseline and week 12**
A: Composite and domain Z-scores on baseline and week 12

|  | baseline | | Week 12 | |
|---|---|---|---|---|
| Median z-score | Control (16) | Intervention (38) | Control (14) | Intervention (34) |
| Composite | 0.358(1.14) | 0.186(1.04) | 0.670(1.16) | 0.520(0.83) |
| Domain verbal | 0.370(1.67) | 0.150(1.45) | 0.990(1.90) | 0.364(1.18) |
| Domain memory | 0.005(0.66) | -0.015(0.66) | -0.092(1.52) | -0.227(0.82) |
| Domain executive functioning | 0.568(1.66) | 0.183(1.23) | 0.958(1.29) | 0.713(0.80) |
| Domain attention | -0.298(1.53) | -0.348(1.81) | 0.003(1.08) | 0.370(1.79) |
| Domain speed | 0.193(2.01) | -0.122(1.50) | 0.345(2.01) | 0.307(1.02) |
| Domain motor | 0.395(1.05) | -0.120(1.79) | 0.528(1.26) | 0.428(1.19) |
| Domain learning | 0.340(1.15) | 0.369(1.12) | 0.778(1.44) | 0.867(1.34) |

B: Corrected estimated difference in composite and domain Z-scores

| Type of Z-score | Z-score difference estimate | 95% CI | p-value |
|---|---|---|---|
| Composite | 0.152 | -0.028 - 0.322 | 0.103 |
| Domain verbal | 0.118 | -0.394 – 0.626 | 0.652 |
| Domain Memory | -0.149 | -0.149 – 0.208 | 0.414 |
| Domain Executive Functioning | -0.054 | -0.363 – 0.256 | 0.735 |
| Domain attention | 0.368 | 0.023 – 0.714 | 0.041 |
| Domain Speed | 0.371 | 0.084 – 0.657 | 0.014 |
| Domain Motor | 0.364 | -0.099 – 0.827 | 0.128 |
| Domain Learning | 0.049 | -0.504 – 0.603 | 0.859 |

CI: Confidence Interval
All outcomes are shown as median (interquartile range (IQR))

A.

B.
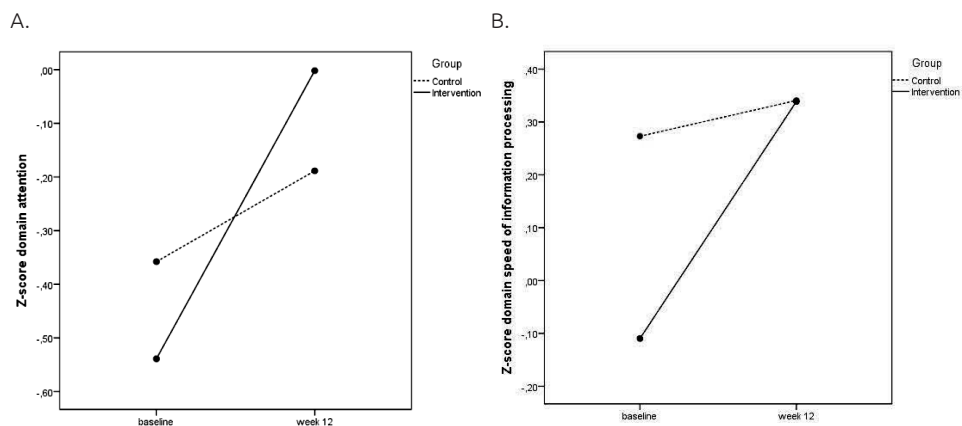


**FIGURE 2. Change in NPA Z-score on domains attention and speed of information processing**
A: Domain attention
B: Domain speed of information processing

### NPA NC analysis

As an addition to the group analysis, an analysis at individual level was conducted with the NPA scores of the control group as a NC-group. Five out of 34 patients (15%) in the switch group were found to improve significantly more on their NPA as compared to the control subjects. These five patients differed from the rest of the group on age (44.6 vs 40.8, p=0.049) and BMI (27.8 vs 24.1, p=0.02).

### Questionnaires

In order to investigate subjective difference, outcomes of the questionnaires were examined (table 1). As shown in table 1, according to the USER-P, a tool that measures a persons amount of participation in society, patients in both groups had little to no restrictions in participation (switch: 98.5/100, control: 97.4/100, p=0.45), and a high satisfaction with their ability to participate (switch: 76.3/100, control: 71.4/100, p=0.31). After 12 weeks, the score for restrictions did not change significantly (switch: 97.4, control: 96.2 p=0.68), and the score for satisfaction stayed high as well (switch: 78.2, control: 72.1, p=0.23). The USER-P also measures the frequency of participation, which remained virtually the same from baseline to week 12 (switch: 41.5 to 42.4, control: 41.0 to 41.0, p>0.05). A similar pattern was seen while analyzing the SF-36, measuring different aspects of quality of life. The mean score was above 70/100 on all subdomains with no significant difference between groups or between baseline and week 12 (table 1). Finally, patients did not show signs of depression or anxiety disorders according to the HADS at baseline (mean score 5/21). This was not significantly different between groups (switch: 5, control: 7), and did not significantly change at week 12 (switch: 4, control: 6).

At baseline, patients had a T-score on the PROMIS short forms for anxiety, depression, and sleep disorders of just below 50 (48, 46, and 47 respectively), and just above 50 on the short form for satisfaction with social roles (53). At week 12, the scores remained virtually the same (47, 46, 47, and 53) without a significant difference between switch and control group at week 12.

**Choice of regime**

After completion of the study, patients were asked whether they wished to switch back to their original Atripla® (figure 3). Three months after discontinuation of the study, 74% (25/34) of patients in the switch group were on a non-Efavirenz-containing regime versus 14% (2/14) of the controls (p<0.01). This was mostly due to a subjective improvement in everyday life after switching from Efavirenz noted by the treating physician in the patient file. Patients who did not experience a subjective change, switched back to Atripla (n=9) because it is a more convenient STR without a dietary restriction.
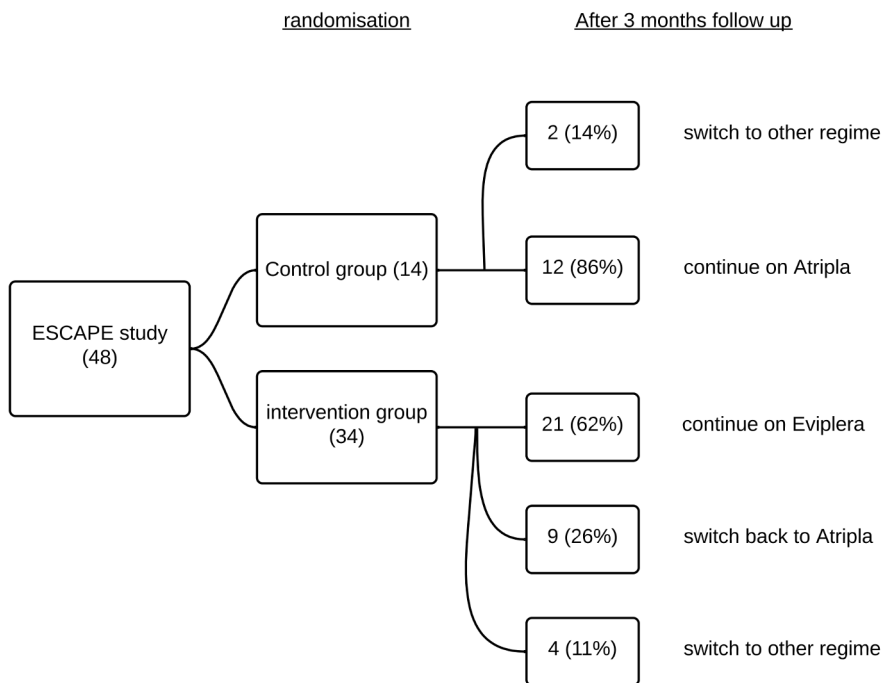


**FIGURE 3. Choice of regime after 3 months follow up**

# DISCUSSION

In this study we aimed to investigate the effects of Efavirenz use in cognitively asymptomatic high educated HIV-infected patients. We found an objective improvement in two domains of cognitive functioning on group level in those patients switching from Atripla® to Eviplera®. At an individual level, in 15% of the patients, performance on NPA was significantly improved after switching. Moreover, 74% of patients experienced a subjective improvement, represented in their choice of regime after study completion. The objective improvement on switching to Efavirenz was significant in the cognitive domains attention and speed of information processing. This is consistent with previous literature investigating the effect of HIV on the brain.[28,29] An explanation for the fact that the most apparent effect was seen in these domains, is that the sub-tests used for these two domains were the most challenging tests or timed tests. These tests do not have ceiling effects and, as such, are more sensitive to changes at the level of performance more than impairment. It is therefore likely that these tests are the most sensitive for detecting cognitive issues, and subsequently, the tests that show the first signs of cognitive decline.  It is conceivable that patients who already struggle with cognitive complaints are the first to report negative cognitive effects of ART. Therefore, cognitive decline associated with Efavirenz might be underreported  when they are compensable in patients with sufficient cognitive reserve capacity, as might well be the case in our study sample. Previous studies have suggested a brain reserve theory for cognitive decline in HIV-patients, proposing a negative effect on the brains' reserve capacity that is addressed when handling complex or challenging cognitive demands.[30,31] In this study, the effect of switching Efavirenz was largest on the more complex tests, which is in line with the brain reserve theory as well.

The next question is then how to distinguish within this total group of asymptomatic patients those who would cognitively benefit from switching versus those who wouldn't. The fact that patients don't always report cognitive complaints poses a challenge for physicians treating HIV-patients, since they can't trust on the patients' clinical presentation to identify which group of patients would benefit. Importantly, individual analyses using normative comparison identified a small but significant subgroup of 15% of the patients (5/34) that would particularly benefit from discontinuing Efavirenz. Additionally, no discriminatory clinical parameters were found that could identify these patients beforehand. There was also a discrepancy between the amount of patients with a subjective improvement and with an objective improvement. Even with Eviplera's dietary inconvenience, the majority of patients in the switch group chose to stay on Eviplera rather than switch back to Atripla as was reported by their treating physician in the patients' hospital file in the 0-3 months after completion of the study. It is important for future research to identify those patients at risk for Efavirenz-induced negative effects

on cognition since Atripla is currently generically available and therefore less expensive compared to non-generic cART in Western countries. More importantly, it is the drug of choice in most resource-limited settings, where the greater part of the HIV-infected population lives. Also, recent evidence suggests that Efavirenz is not the only ART with negative neurocognitive effects. For instance, several studies have now demonstrated that neuropsychological and cognitive complaints appear frequently in Dolutegravir and other members of the group of integrase strand transfer inhibitors as well.[32–34] This, together with the fact that the present study showed that a negative effect can exist even in asymptomatic patients with assumed large cognitive reserve capacities, makes it sensible to investigate the matter of cART neurotoxicity further, to see if it is justified to empirically switch patients off ART with known neurocognitive side-effects.

Studies on the effect of Efavirenz on neurocognition using an NPA are hampered by methodological issues in particular to the extent of the used NPA. For example, in the earlier mentioned studies in cognitively asymptomatic HIV-infected patients on Efavirenz, Payne et al. used only six subtests and Tiraboschi et al. only three.[18,19] The current study used 14 internationally established neuropsychological tasks. Furthermore, this study uses strong methodological criteria to keep bias to a minimum. First, unlike previous studies, this study used a control group of patients remaining on Atripla. In this study, the control group improved on the NPA as well, albeit less than the switch group. This can be explained by a learning effect from performing an NPA for the second time. Previous studies who did not use a control group could have mistaken this learning effect for an improvement. Second, this study used a highly comparative antiretroviral drug as comparator for the switch group, namely Eviplera. Eviplera was chosen because it has the exact same backbone as Atripla, and a third agent in the same class as Efavirenz. And although Eviplera has a dietary restriction and Atripla does not, the alternative, switching to a different drug class, will lead to differences in metabolism, the necessity for a booster, or multiple drug intake moments a day, creating an even bigger bias for the study. There are several limitations to this study. First, we studied patients who had been stable on Efavirenz for a mean of 57.76 months, representing a group of patients that appear to tolerate the drug for long periods. The results in this group might henceforth be an underestimation of the degree or nature of neurocognitive decline of all patients on Efavirenz. Secondly, the main outcome variable used in this study was a composite Z-score. While this is a sensitive and broadly used outcome measurement, it does not take into account any sum to zero effects where negative and positive results cancel eacht other out, unlike for instance the global deficit approach. However, by also doing sub-analyses on domain scores, and a Normative Comparison analysis that uses a multivariate approach to NPA outcomes, this study sufficiently tackles this statistical pitfall.

In conclusion, discontinuing Efavirenz resulted in a subjective and objective improvement in neurocognitive functioning in a group of asymptomatic, high-functioning HIV-patients. Discrepancies in subjective and objective results make it difficult to select patients who would benefit from a switch.

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**2**

Objective and Subjective Improvement of Cognition after Discontinuing Efavirenz

CHAPTER **3**

High Efavirenz drug levels but not neurofilament light plasma levels are associated with poor neurocognitive functioning in asymptomatic HIV-patients

# ABSTRACT

**Aim**
To assess the effect of efavirenz exposure on neurocognitive functioning, and investigate plasma neurofilament light (Nfl) as a biomarker for neurocognitive damage.

**Methods**
Sub-analysis of the ESCAPE-study, a randomised controlled trial where virologically suppressed, cognitively asymptomatic HIV patients were randomised (2:1) to switch to rilpivirine or continue on efavirenz. At baseline and week 12, patients underwent an extensive neuropsychological assessment (NPA), and serum efavirenz concentration and plasma Nfl levels were measured. Subgroups of elevated (≥ 4.0 mg/L) and therapeutic (0.74 to< 4.0 mg/L) baseline efavirenz concentration were made. Differences between these groups in baseline NPA Z-scores and in delta scores after efavirenz discontinuation were assessed. Nfl level was measured using an ELISA analysis using single molecular array (Simoa) technology. Correlation of plasma NFL with NPA Z-scores was evaluated using a linear mixed model.

**Results**
The elevated group consisted of 6 patients, the therapeutic group of 48. At baseline, the elevated group showed lower composite Z-scores (median -1.03; IQR 0.87 versus 0.27; 0.79. p 0.02). This effect was also seen on the subdomains verbal (p 0.01), executive functioning (p 0.02), attention (p <0.01) and speed (p 0.01). In the switch group, the elevated group improved more on composite scores after discontinuing efavirenz (mean 0.58; SD 0.32 versus 0.22; 0.54, p 0.15). No association between plasma Nfl and composite Z-score was found.

**Conclusion**
High efavirenz exposure is associated with worse cognitive functioning compared to patients with therapeutic concentrations. Plasma Nfl is not a suitable biomarker to measure cognitive damage in this group.

# INTRODUCTION

Antiretroviral agents used to treat infection with human immunodeficiency virus-1 (HIV) have been associated with Neurocognitive Impairment (NCI).[1,2] Especially efavirenz, a non-nucleoside reverse transcriptase inhibitor, is known for its neurological and psychiatric side effects, and has been associated with higher rates of NCI.[3–8] Even in patients without clinically manifest cognitive complaints (cognitively asymptomatic patients), a negative effect of efavirenz on cognition has been shown.[2] In the ESCAPE study, we found that discontinuing efavirenz led to an objective improvement in neurocognitive functioning in a group of asymptomatic people with HIV.[9] However, Efavirenz remains a populair choice in antiretroviral therapy, mainly in resource-limited settings, mainly because is it part of Atripla, a single-tablet regime that is relatively cheap and has a convenient once a day dosage.

Multiple mechanisms on how efavirenz causes neurotoxicity have been described through in-vitro and in-vivo studies. For instance, a neurotoxic effect of efavirenz and its major metabolite 8-hydroxy-efavirenz was found in neuronal cultures, affecting dendrites and dendritic processes.[10–12] Moreover, studies have shown a detrimental effect of efavirenz on the blood-brain barrier, and on neuronal action potential thresholds.[12,13] Effects on other mechanisms such as calcium homeostasis or creatine kinase metabolism have also been shown.[11,14] Most studies show a concentration-dependent effect of efavirenz on CNS side effects such as impaired concentration, this is most notable from serum concentrations above > 4 mg/L.[15,16] It might therefore be interesting to investigate the effect of high serum efavirenz concentrations on cognition.

Furthermore, there is a need for fast and patient-friendly diagnostic tools (biomarkers) for diagnosing neurocognitive damage, because the current gold standard, a neuropyschological assesment (NPA), is timely and expensive. Recent interest has emerged in a protein called neurofilament light (Nfl) which is a major structural component of axons and is released into the cerebrospinal fluid (CSF) and blood upon axonal damage and neuronal death.[17] CSF Nfl is elevated in patients suffering from HIV-associated dementia.[18] The recent development of an ultra-sensitive immunoassay for plasma Nfl using Single molecule array (Simoa) technology, allows testing for neurocognitive injury in plasma instead of CSF.[19] Several studies have established that there is a strong correlation between plasma Nfl and CSF Nfl.[20–24] Besides HIV infection, plasma Nfl has been investigated in neurological conditions such as frontotemporal dementia, multiple sclerosis and Creutzfeldt disease, and proven to be useful as a biomarker of neurodegeneration.[25–27] The results from two studies suggest that plasma Nfl may provide an almost equally good indicator of active CNS injury compared to CSF Nfl in people with HIV.[28,29]

Given the demonstrated improvement in neurocognitive functioning in earlier studies after discontinuing efavirenz, the main hypothesis of this study is that this observed effect is related to efavirenz exposure, measured by elevated serum drug levels. Furthermore, we investigate the hypothesis that a high exposure to efavirenz leads to axonal damage and/or neuronal cell death, which can be measured by plasma Nfl.

## METHODS

### Participants

This study is a sub-analysis of the ESCAPE-trial (Effect of SwitChing AtriPla to Eviplera on neurocognitive and emotional functioning) which was previously published.[9] In short, this randomised controlled trial included neurologically asymptomatic, stable (i.e. undetectable viral load), HIV-infected male patients on efavirenz/emtricitabine/tenofovir (Atripla) for at least 6 months, aged from 25 to 50 years old. Participants were excluded if they had active or past CNS opportunistic infections, active psychiatric of neurologic disorders, and/or a history or evidence of alcohol or drug abuse. The study was performed according to the declaration of Helsinki, and was reviewed and approved by the medical ethical board of the University Medical Center Utrecht. All participants signed written informed consent.[9]

### Study design

In the ESCAPE study, participants were randomised to the switch group, where they would switch to rilpivirine/emtricitabine/tenofovir (Eviplera), or the control group (continuing on Atripla) with a randomisation ratio of 2:1. At baseline and study week 12, blood was collected for serum measurement of efavirenz concentration and plasma measurement of NFL concentrations, as well as HIV-RNA and CD4 cell count. Also, a comprehensive NPA was performed. Seven cognitive domains were tested by the NPA: language, learning and memory, executive functioning, attention/working memory, speed of information processing, and psychomotor speed.[9] The different subtests used were: Controlled Oral Word Association Test[30], category fluency[31], Rey Auditory Verbal Learning Test, Rey complex figure Test[32], Trailmaking Test part A&B[33], Brixton Spatial Anticipation Test[34], Visual Elevator[35], Paced Auditory Serial Addition Test[36], Letter-Number-Sequencing WAIS-IV NL, Digit Symbol WAIS-IV NL, Symbol Search WAIS-IV NL[37], Grooved Pegboard (dominant and non-dominant).[38] When possible, different test versions were used on baseline and for week 12, in order to minimize repeated testing effects. By using Dutch norm data, domain Z-scores were calculated, and a composite Z-score was calculated taking all different domains into account. A Z-score correlates to the amount of standard deviations a person deviates from the mean of the norm group; so a higher Z-score means a better performance.

### Efavirenz concentration analysis

For the analysis of efavirenz serum concentrations, an aliquot of 50 µL serum was diluted with 200 µL 0,1 M zinc sulphate and 500 µL internal standard solution. The vials were vortexed for one minute and centrifuged at 13000 rpm for 5 minutes and 25 µL was injected on the LC-MS/MS system, a Thermo Fisher Scientific (Waltham, MA, USA) triple quadrupole Quantum Access LC–MS/MS system with a Surveyor MS pump and a surveyor Plus autosampler with an integrated column oven. The Quantum Access mass selective detector was set in electrospray-positive ionisation mode and performed selected reaction monitoring. Data acquisition and data processing were performed using Xcalibur software version 2.10. The analytical column was a HyPurity C18 50 mm x 2.1 mm column with 3 µm particle size (Thermo Scientific). Analytes were detected by a Thermo Fisher Scientific (Waltham, MA) triple quadrupole Quantum Access detector using heated electrospray ionisation (HESI). Ions monitored in the selected reaction monitoring (SRM) mode Regression coefficient ($R^2$) was 0.98. The lower limit of quantification (LLQ) was 0.1 mg/L. Accuracy and precision were within the maximum tolerated bias and coefficient of variation; 20% for LLQ and 15% for medium and high quality controls.

### Plasma NFL analysis

Plasma NfL concentrations were quantified in blood by the Neurochemistry Laboratory, Amsterdam UMC, location VUmc, using an in-house developed Homebrew Simoa assay, validated according to standardised international protocols and described in detail elsewhere.[19,39] The monoclonal NfL capture antibody (Anti NfL mAb 47:3; UmanDiagnostics, Umeå, Sweden) was titrated to 0.3 mg/mL and chemically coupled to paramagnetic carboxylated beads (Quanterix, Lexington, USA). The assay had a lower limit of quantification of 1.54 pg/mL. All samples were measured in duplicate.

### Statistical analysis

Initially, all participants were divided into two groups: elevated baseline concentration of efavirenz, meaning a concentration of ≥ 4.0 mg/L, and therapeutic baseline concentration of efavirenz, meaning a concentration of < 4.0 mg/L. This division was made at baseline in the entire population to assess concentration effects on NPA Z-score in a cross-sectional manner, and again at baseline in only the switch group to investigate longitudinal effects. Subsequently, differences in baseline characteristics (age, education, employment status, BMI, duration of HIV infection and cART, current and nadir CD4) between elevated and therapeutic concentration groups were investigated. For categorical variables, either a chi-square test or Fisher's exact test (if values were expected to be below five) was used, and for continuous variables either an independent samples T-test for normal distribution or a Mann-Whitney U test for skewed distribution was used. Level of education according to the Verhage scale [40] was divided in high (group six and seven) and low (< group six). Differences between NPA Z-scores of the two groups at baseline were analysed using an

independent samples T-test for normal distribution or a Mann-Whitney U test for skewed distribution. With the same approach, difference ('delta') scores of NPA Z-scores were assessed in the switch group.

Then, to evaluate the effect of plasma NFL on composite Z-score, a linear mixed model with random intercept was built combining measurements at baseline and end of study, resulting in 108 measurements. The variable concentration of efavirenz was transformed to a log variable to ensure better fitting of the model. A restricted maximum-likelihood linear mixed model was run to investigate the effect of plasma NfL on composite Z-score, including the following covariates (i.e. fixed effects): age, neuropsychiatric co-medication, months on cART, CD4 count, HIV disease duration, CD4 nadir, concentration of efavirenz and 'time point in study' (marked as a categorical variable). Duration of efavirenz use in terms of months was left out of the analysis because of the by now reached steady state of this drug. Participants were defined as random effects to correct for multiple measurements in one participant. After building a full mixed model, the backward method according to the principle of a linear regression was used to investigate factors of interest (i.e. plasma NfL and other fixed effects) by building a final model. This model was again fit by restricted maximum likelihood.

Finally, to investigate disturbing influences on plasma NfL alone, a univariable and multivariable linear regression was performed. Factors with a p-value <0.20 in the univariable models, or with a scientific rationale, were entered in the multivariable linear regression model. The backward method was used and p < 0.05 was applied as a cut-off level for acceptance. Only five variables were entered, approximately one per every ten participants.

Mixed model analyses were performed using R Statistical Software version 3.3.2; for the remaining analyses, IBM SPSS version 21 was used.  Overall, an alpha of <0.05 was used as a cut-off.

## RESULTS

In the ESCAPE trial, a total of 54 participants was included. Participants were divided into therapeutic baseline efavirenz concentration (n = 48) and elevated baseline efavirenz concentration (n = 6) (Figure 1). There was no significant difference between patient characteristics of these groups at baseline, except for employment status (p =0.03) (Table 1).
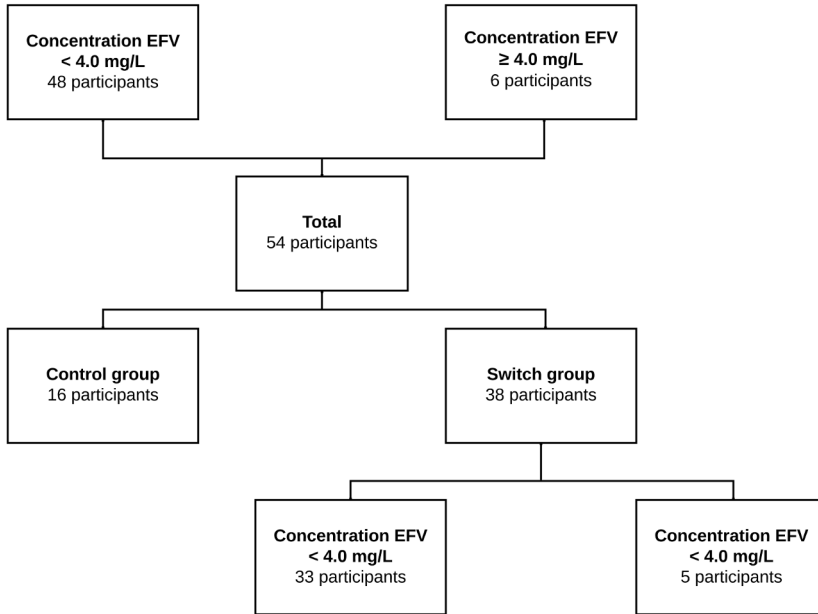
**FIGURE 1.** flowchart
*Abbreviations: EFV = efavirenz*

**TABLE 1.** Baseline characteristics

| Variable | Total<br><br>N = 54 (100%) | Concentration<br>EFV < 4.0 ng/mL<br>N = 48 (88.9%) | Concentration<br>EFV ≥ 4.0 ng/mL<br>N = 6 (11.1%) | p-value |
|---|---|---|---|---|
| Age, in years | 41 (11) | 41 (11) | 43 (11) | 0.81 |
| High educational attainment according to Verhage (group 6 and 7), N (%) | 26 (48.1) | 23 (47.9) | 3 (50.0) | 0.63 |
| Employed  N (%) | 51 (94.4) | 47 (97.9) | 4 (66.7) | 0.03 |
| BMI, in kg/m2<br>mean ± SD | 24.1 ± 3.3 | 24.1 ± 3.5 | 23.4 ± 2.7 | 0.52 |
| Use of neuropsychiatric medication, N (%) | 7 (13.0) | 6 (12.5) | 1 (16.7) | 0.58 |
| cART, in months | 58 (51) | 58 (50) | 58 (85) | 0.99 |
| EFV treatment duration, in months<br>mean ± SD | 56 ± 28 | 56 ± 29 | 56 ± 24 | 0.88 |
| CD4 count, in cell/mm3 | 605 (268) | 620 (255) | 546 (365) | 0.46 |
| CD4 nadir, in cell/mm3 | 295 (138) | 275 (144) | 355 (100) | 0.05 |
| HIV disease duration, in months | 92 (56) | 92 (51) | 85 (140) | 0.85 |
| EFV concentration, in mg/L | 2.16 (1.50) | 1.80 (1.31) | 7.11 (9.67) | <0.01 |
| Plasma NFL, in pg/mL | 21.6 (16.6) | 21.6 (16.8) | 20.7 (24.3) | 0.82 |

Values shown as median (IQR), unless otherwise specified
*Difference considered significant (p-value < 0.05)
*Abbreviations: N = number; EFV = efavirenz; IQR = interquartile range; BMI = body mass index; SD = standard deviation; cART = combination antiretroviral therapy; HIV = Human immunodeficiency virus; NFL = neurofilament light*

High Efavirenz drug levels but not neurofilament light plasma levels are associated with poor neurocognitive functioning in asymptomatic HIV-patients

**Effect of efavirenz concentration on NPA**

Participants with an elevated concentration of efavirenz at baseline had a significantly lower NPA composite Z-score (i.e. decreased cognitive function) at baseline compared to those with a therapeutic efavirenz concentration (-1.03; IQR 0.87 versus 0.27; IQR 0.79, p = 0.02). When analysing the specific domains, elevated efavirenz concentrations were associated with lower NPA scores in the following domains: verbal (-0.66; SD 0.83 versus 0.41; SD 0.93, p = 0.01), executive functioning (-0.59; SD 0.78 versus 0.26; SD 0.83, p = 0.02), attention (-2.05; SD 1.33 versus -0.35; SD 1.04, p <0.01) and speed (-1.08; SD 0.81 versus 0.07; SD 1.00, p = 0.01) (Table 2a).

**TABLE 2A. NPA Z-scores at baseline in normal and high concentration of EFV groups**

| Type of NPA Z-score | Concentration EFV < 4.0 mg/L | Concentration EFV ≥ 4.0 mg/L | p-value |
|---|---|---|---|
| **Composite median (IQR)** | 0.27 (0.79) | -1.03 (0.87) | **0.02** |
| **Domain verbal** | 0.41 ± 0.93 | -0.66 ± 0.83 | **0.01** |
| **Domain memory** | -0.04 ± 0.50 | -0.18 ± 0.46 | **0.52** |
| **Domain executive functioning** | 0.26 ± 0.83 | -0.59 ± 0.78 | **0.02** |
| **Domain attention** | -0.35 ± 1.04 | -2.05 ± 1.33 | **< 0.01** |
| **Domain speed** | 0.07 ± 1.00 | -1.08 ± 0.81 | **0.01** |
| **Domain motor median (IQR)** | 0.18 (1.24) | -1.14 (2.98) | **0.08** |
| **Domain learning** | 0.33 ± 0.68 | 0.12 ± 1.02 | **0.52** |

Values shown as mean ± SD, unless otherwise specified
*Difference between groups considered significant (p-value < 0.05)
*Abbreviations: NPA = neuropsychological assessment; EFV = efavirenz; IQR = interquartile range; SD = standard deviation*

Next, we evaluated only the group of participants that switched from a regimen with efavirenz to a regimen without efavirenz in order to study the effect of efavirenz discontinuation. Differences ('delta-scores') between NPA Z-scores on baseline and end of study were investigated. In the switch group, 5 participants had an elevated concentration and 33 participants had a therapeutic concentration of efavirenz (Figure 1). All participants improved on the second NPA due to a learning effect. Participants with an elevated concentration at baseline had a higher delta, i.e. improved more on composite Z-score (0.58; SD 0.32 versus 0.22; SD 0.54, p = 0.15) compared to those with a therapeutic concentration. When looking at sub-domains, the group with an elevated baseline concentration of efavirenz improved more on the domains verbal (0.47 SD 0.42 versus 0.15 SD 0.64, p = 0.63), attention (0.98 SD 0.67 versus 0.46 SD 0.6 p= 0.33), speed (0.93; SD 0.73; versus 0.42; SD 0.41, p = 0.05), motor (0.80 SD 0.36 versus 0.54 SD 0.85, p = 0.18) and learning (0.80 SD 0.31 versus 0.41 SD 1.00 p = 0.65) (Figure 2; Table 2b). Although none of these improvements were statistically significant, a trend towards significance was seen in the domain speed.

Becauses of the significant difference between the two groups in employment status and the near-significant difference in nadir CD4, we ran an extra GLM including these factors and they had no effect on both outcomes (Z-score or delta Z-score).
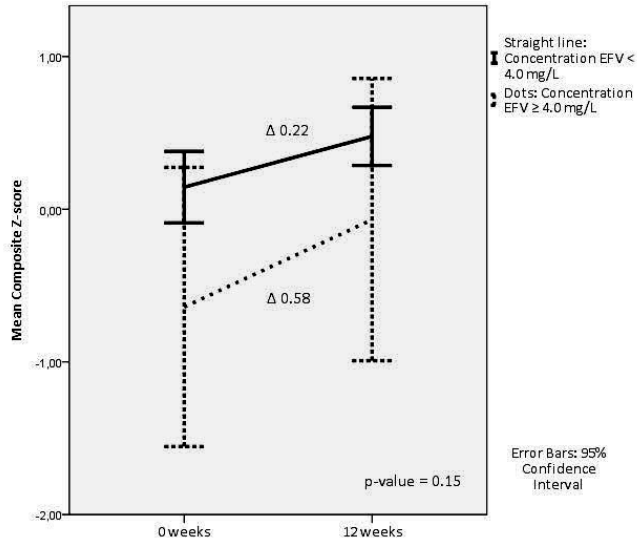


**FIGURE 2. Mean composite Z-scores with confidence intervals in the switch group, divided at baseline in: therapeutic and elevated concentration of efavirenz**

**TABLE 2B. Difference ('delta') scores of NPA Z-scores in normal and high concentration of EFV groups within the switch group**

| Type of NPA Z-score | Concentration EFV < 4.0 mg/L | Concentration EFV ≥ 4.0 mg/L | p-value |
|---|---|---|---|
| Composite median (IQR) | 0.22 (0.54) | 0.58 (0.32) | **0.15** |
| Domain verbal | 0.15 ± 0.64 | 0.47 ± 0.42 | **0.63** |
| Domain memory | -0.24 ± 0.49 | -0.03 ± 0.73 | **0.48** |
| Domain executive functioning | 0.42 ± 0.52 | 0.33 ± 0.30 | **0.82** |
| Domain attention | 0.46 ± 0.60 | 0.98 ± 0.67 | **0.33** |
| Domain speed | 0.42 ± 0.41 | 0.93 ± 0.73 | **0.05** |
| Domain motor | 0.54 ± 0.85 | 0.80 ± 0.36 | **0.18** |
| Domain learning | 0.41 ± 1.00 | 0.80 ± 0.31 | **0.65** |

Values shown as mean ± SD, unless otherwise specified
*Difference between groups considered significant (p-value < 0.05)
*Abbreviations: NPA = neuropsychological assessment; EFV = efavirenz; IQR = interquartile range; SD = standard deviation*

High Efavirenz drug levels but not neurofilament light plasma levels are associated with poor neurocognitive functioning in asymptomatic HIV-patients

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## NFL as a biomarker for neurocognitive impairment

To evaluate whether Nfl plasma concentration is related to neurocognitive impairment, a linear mixed model with random intercept was built. The full model contained 8 covariates (age, use of psychoactive comedication, duration of cART and HIV infection, current and nadir CD4, Efavirenz concentration and time point in study (baseline or week 12)). A significant relation was found between composite Z-score and time point in study indicating that all patients increased their Z-score on the second time point, which can be explained by a learning effect from doing the NPA for the second time. (coefficient = 0.26, standard error = 0.0746, p = <0.01). No other factors had a significant association with composite Z-score (Table 3). A final model was created by using the backward method, containing four variables (use of psychoactive comedication, duration of cART, Efavirenz concentration, time point in study). Plasma NFL still did not show a significant association with the outcome composite Z-score (coefficient = 0.0012, standard error = 0.0030, p = 0.71).

Next, we investigated which variables had an effect on plasma Nfl in order to identify possible disturbing influences in Nfl levels. Univariable linear regression on plasma neurofilament light at baseline showed no significant association, except for HIV disease duration ($\rho = 0.32$; p = 0.02) (supplemental data). For the multivariable linear regression 5 variables were entered in the model; age (p = 0.12), months on cART (p = 0.08), CD4 nadir (p = 0.96), HIV disease duration (p = 0.02) and viral load at baseline (p = 0.14) (supplemental data). When creating a multivariable linear regression model using the backward method, only HIV disease duration remained statistically significant (coefficient 0.090, standard error = 0.037, p = 0.02).

**TABLE 3.** Full linear mixed model on the outcome composite Z-score

| Fixed effect | Coefficient | Standard error | p-value |
|---|---|---|---|
| Intercept | 0.38381 | 0.66321 | 0.57 |
| Age, in years | -0.00065 | 0.01631 | 0.97 |
| Use of neuropsychiatric medication | 0.15100 | 0.33070 | 0.65 |
| cART, in months | -0.00378 | 0.00441 | 0.40 |
| CD4 count, in cell/mm3 | -0.00008 | 0.00030 | 0.78 |
| CD4 nadir, in cell/mm3 | -0.00010 | 0.00072 | 0.89 |
| HIV disease duration, in months | 0.00006 | 0.00345 | 0.99 |
| EFV concentration, in mg/L* | -0.07816 | 0.05470 | 0.17 |
| Plasma NFL, in pg/mL | 0.00117 | 0.00355 | 0.75 |
| Time point in study** | 0.26045 | 0.07457 | <0.01 |

* efavirenz concentration transformed to log variable
**Time is defined as a categorical variable
*Abbreviations: cART = combination antiretroviral therapy; HIV = Human immunodeficiency virus; NFL = neurofilament light*

# DISCUSSION

This is the first study aimed to investigate the effect of efavirenz exposure, measured by plasma concentration, on objectively measured cognitive functioning in cognitively asymptomatic people with HIV. An elevated efavirenz concentration was associated with worse cognitive functioning overall and in different domains (verbal, executive functioning, attention and speed). Furthermore, discontinuing efavirenz resulted in more neurocognitive improvement in those with an elevated efavirenz baseline concentration compared to those with a therapeutic efavirenz baseline concentration. However, this effect was not statistically significant, apart from a trend in the subdomain speed, most likely due to a limited sample size. Moreover, when exploring the use of plasma Nfl as a biomarker in neurocognitive functioning, no association between plasma Nfl and composite Z-score was found.

Patients usually switch to another (efavirenz-sparing) regimen when they experience neurocognitive side effects. However, there is a group of patients that tolerate efavirenz and do not experience a clinically significant effect on cognition. The strength of our study lies in the fact that we analysed the effect of efavirenz concentration in these cognitively asymptomatic people with HIV, as opposed to patients with overt neurocognitive complaints. The domains that showed the largest effect of elevated efavirenz concentration (speed and attention) are also the cognitive domains that were mostly affected by discontinuing efavirenz in the ESCAPE trial.

Although toxicity thresholds from 2.74 to 4.7 mg/L for Efarivenz have been used in studies[41,42], we chose to use a cut-off level of 4.0 mg/L for efavirenz concentration, seeing as this is the most-used threshold in international literature.[16,43–46] In order to measure cognition in a fast and less time-consuming manner, there is a need for biomarkers that can preferably be measured in plasma. Plasma Nfl has been shown to be useful in providing an indication of active CNS injury in HIV infection.[28,29] However, these studies found the most significant results in patients with HIV dementia or in untreated people with HIV. The current study is the first study that aimed to explore the utility of plasma Nfl as a biomarker in treated cognitively asymptomatic patients with HIV. An association between plasma Nfl and composite Z-score was not found. Moreover, the concentration of efavirenz did not have a significant association in this relationship, and therefore the switch in regime was not a contributing factor in explaining the association between plasma Nfl and composite Z-score in this analysis. The hypothesis of this study that efavirenz causes axonal damage, and therefore results in rising plasma Nfl levels, could therefore not be proven in this study. The negative effects of efavirenz on the brain might be explained by other mechanisms than axonal damage. In vitro studies did show a larger effect of efavirenz on dendrite cells than axonal cells.[10–12] Furthermore, most

studies found that neuronal death, which would also be measured by Nfl, was not the major reason for neurotoxicity of efavirenz.[10] Another explanation could be that plasma Nfl is not as sensitive as CSF Nfl, as is suggested in animal studies.[47]

There are some limitations to this study. First, the small number of participants was due to the limited number included in the time-consuming ESCAPE trial. Since no power calculation was done for this sub-analysis, it is possible that the used numbers do not provide sufficient power for the analysis. Moreover, there was a substantial difference between the amount of patients in the two groups (6 versus 48), that further reduced our power. Second, in contrast to previous reports on Nfl[28], in this study plasma Nfl did not correlate with age. This could be explained by the fact that the variation in age was limited in this population due to the study inclusion criteria. Moreover, studies have shown that the correlation between age and Nfl levels is more evident in healthy controls.[48] However, this study found a significant correlation with duration of HIV infection and plasma Nfl levels. This effect might be explained by the hypothesis that HIV infection itself together with the accompanying chronic immune activation have an added neurotoxic effect. Furthermore, people who have been infected with HIV for a longer period have consequently been on more and older cART regimens. Previous cART regimens possibly did cause axonal damage and therefore caused higher levels of plasma NFL.[49–52] Considering the fact that detecting Nfl in plasma is a recent development, an all-encompassing answer explaining all possible factors influencing plasma/serum NfL levels is not yet available. Therefore, it is important to further investigate the applicability and validity of plasma Nfl as a biomarker, preferably in larger cohorts of people with HIV suffering from neurocognitive impairment.

In conclusion, elevated serum efavirenz concentration is associated with worse cognitive functioning, and there are signs that subsequent discontinuation results in improvement of cognitive functioning compared to those with normal concentrations. Plasma Nfl is not suitable as a biomarker for cognitive damage in this group.

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High Efavirenz drug levels but not neurofilament light plasma levels are associated with poor neurocognitive functioning in asymptomatic HIV-patients

# CHAPTER 4

Discontinuation of Dolutegravir- and Elvitegravir-
containing cART for HIV in the Netherlands;
incidence rates and risk factors

Discontinuation of Dolutegravir- and Elvitegravir-containing cART for HIV in the Netherlands; incidence rates and risk factors

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# ABSTRACT

**Background**
Unexpectedly high rates of neuropsychiatric adverse events (NPAEs) and drug discontinuation have been reported with the use of dolutegravir-based combination antiretroviral treatment (cART) for HIV in observational studies compared to randomised controlled trials.

**Methods**
We included all HIV-1 positive adults, enrolled in the AIDS Therapy Evaluation in the Netherlands (ATHENA) national observational HIV cohort, first initiating dolutegravir- or elvitegravir-based cART between December'13 and February'16. cART discontinuation rates for 1) any reason, 2) adverse events (AE), and 3) NPAEs were determined, separately for cART-naïve and –experienced patients. Associations between patient characteristics, the specific integrase-inhibitor used, and time-to-cART discontinuation, were evaluated through multivariable Cox proportional-hazards models.

**Findings**
3,416 patients were included of whom 1051(31%) were cART-naïve and 2,365(69%) were cART–experienced. cART-experienced patients were more likely to discontinue both dolutegravir- and elvitegravir-based cART for any reason and for AE than cART-naïve patients (log-rank; $P<\cdot0001$). Factors associated with discontinuation for AE, independent of the use of dolutegravir- or elvitegravir-based cART, for cART-naives were being of non-Western origin and CD4 cell-count <200 or ≥500 cells/mm$^3$, and for cART-experienced patients; age ≥60 years, ever or current use of psychotropic drugs, and a history of discontinuing prior cART for AE. Discontinuation for NPAEs in cART-experienced patients was associated with age ≥60 years.

**Interpretation**
In the Netherlands, patient characteristics contribute more to the risk for dolutegravir or elvitegravir-based cART discontinuation for AE than the particular integrase-inhibitor used. Especially cART–experienced ageing populations seem susceptible for discontinuation for AE and NPAE after start dolutegravir- or elvitegravir-based cART.

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# INTRODUCTION

The integrase inhibitor (InSTI) dolutegravir became available for treatment of HIV-1 infection in 2014.[1] Phase II and III studies showed that dolutegravir was highly effective in reducing HIV-1 RNA viral load, with rates of drug discontinuation due to adverse events being lower compared to other third agents such as efavirenz and darunavir.[2-6] Moreover, the convenient once daily dosing regimen, no food-intake requirements and few drug-drug interactions, made dolutegravir a highly anticipated and appealing third agent for combination antiretroviral therapy (cART). Within months after its introduction, the drug was first included in international treatment guidelines.[7,8] One year later, dolutegravir was preferred over efavirenz as one of the recommended third agents for initial treatment of HIV-positive patients and hence, within a short time-frame, dolutegravir, became widely used in Europe and the United States.[9-11] Nowadays, low- and middle-income countries are likewise transitioning to the use of dolutegravir-based cART as first-line treatment.[12]

In spite of the overall favourable safety profile shown in randomised controlled clinical trials (RCTs), some, but not all, post-licensing cohort studies have reported higher discontinuation rates for dolutegravir compared to discontinuation rates in RCTs.[13-16] For instance, Dutch colleagues from two HIV-treatment centres reported a discontinuation rate of 13·7% for their cohort, while other European cohort studies found discontinuation rates varying between ~4-11% within the first year after start of dolutegravir-based cART.[16-20] These values are considerably higher than the discontinuation rates of 2-3% after 48 weeks of treatment reported in RCTs including dolutegravir.[2-6,21] Additionally, in some cohort studies particular toxicities resulting in dolutegravir discontinuation were neuropsychiatric adverse events (NPAEs), including depression, insomnia and headache[16,17,19,20,22], and these NPAEs seemed more frequently reported for dolutegravir than for the other InSTIs.[14,15]

Explanations for these discrepancies between RCTs and observational studies on dolutegravir so far are inadequate. Besides, there have been studies suggesting a class-effect of InSTIs, rather than an isolated dolutegravir effect.[23,24] This is supported by results from, for instance, the Swiss HIV Cohort Study, in which dolutegravir and raltegravir resulted in comparable discontinuation rates for adverse events.[14] Meanwhile, however, the absence in international literature of reports on higher discontinuations with other InSTIs, raltegravir and elvitegravir boosted with cobicistat, after their introduction, is remarkable.

In this study, we evaluated the incidence and reasons for cART discontinuation after commencing either dolutegravir- or elvitegravir-based cART among HIV-1 positive adults using data from the AIDS Therapy Evaluation in the Netherlands (ATHENA) national observational HIV cohort.[25] Furthermore, we aim to identify patient specific risk factors

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for discontinuing dolutegravir and elvitegravir-based cART for any reason, for adverse events, and for NPAEs in particular.

## METHODS

### Design

The ATHENA national observational HIV cohort[25] monitors all HIV-positive patients from the moment of entry into HIV care in the Netherlands, except for the approximately 2% who opt out of having their data registered. Follow-up time in the ATHENA cohort equals follow-up time in HIV care in one or more of the 26 designated HIV treatment centres. We included all HIV-1 positive adults who first initiated either dolutegravir- or elvitegravir-based cART between December 2013 and February 2016, who had at least one visit after initiating such regimen, and had at least three months follow-up at time of database closure (Figure 1). Patients on raltegravir were not included for two reasons; (1) raltegravir was introduced many years earlier and prescription practices may have been different than for dolutegravir and elvitegravir, which were introduced later and more closely to each other, (2) use of raltegravir for first-line treatment in the Netherlands has been limited in view of the need for twice daily dosing of the original raltegravir formulation.[26] As of May 2016, reports from smaller studies suggested high rates of discontinuations with the use of dolutegravir and in order to minimize bias resulting from physicians being more inclined to discontinue dolutegravir after publication of these reports, database closure was set on May 1st 2016.[27] Patients were considered cART-naïve if they had never received cART before receiving dolutegravir or elvitegravir-based cART. We defined three outcomes: 1) any discontinuation; 2) discontinuation for adverse events, and 3) discontinuation because of NPAEs.

### Definitions

Baseline was defined as the date of first starting a dolutegravir- or elvitegravir-based cART. Time was censored at the time of last recorded clinical visit, loss-to-follow-up (no recorded evidence of being in care for >1 year), transfer out or time of database closure; whichever came first. Intermittent treatment interruptions of dolutegravir or elvitegravir, dolutegravir dose changes, or changes in backbone composition were not considered as discontinuations. Reasons for discontinuations were hierarchically classified, in case there was more than one reason, into virological failure (HIV-1 RNA ≥ 200 copies/mL), therapy or immunological failure[28], treatment-limiting toxicity, simplification, drug-drug interactions, patient decision, other and unknown. CD4 cell-count at baseline reflects the most recent available CD4 cell-count within 12 months before start, or within the first week after start of dolutegravir- or elvitegravir-based cART. Data on current and previous use of non-HIV co-medications was restricted to medications pre-specified in standardized

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patient data collection protocols for the ATHENA-cohort[29] and included cardiovascular drugs, drugs to prevent cardiovascular events, anti-diabetic drugs, and medications with a risk for drug-drug interactions. If a patient used no co-medication at all this was recorded in the database. Any time use of psychotropic drugs was defined as previous or current use of antidepressants (Anatomical Therapeutic Chemical Classification System (ATC)-code; N06A), psychostimulants (N06B), antipsychotics (N05A), anxiolytics (N05B), and/or hypnotics and sedatives (N05C), provided that these were collected according to the protocol. To investigate the relation between having a history of frequent switching for adverse events and discontinuation of elivitegravir or dolutegravir, a ratio – referred to as 'previous discontinuations for adverse events' – was calculated by dividing the total number of previous cART switches because of adverse events by the total number of previous cART switches for any reason summed up for each patient from the date at which cART was started for the first time. Adverse events associated with discontinuation of dolutegravir or elvitegravir-based cART were extracted from the database and were classified into categories by organ system according to the Division of AIDS (DAIDS) system for grading of adverse events.[30] Psychiatric adverse events were further analysed separately, and divided in the categories insomnia, depression and mood disorders, suicidal ideation or attempt, and other psychiatric adverse events. Neurologic adverse events were categorized into headache and other neurologic adverse events.

## Statistical analyses

Incidence rates for cART discontinuation were calculated by dividing the number of such discontinuations by the total time at risk after starting dolutegravir- or elvitegravir-based cART. We used Kaplan-Meier and Cox proportional hazards-models to determine the incidence and determinants of discontinuation of dolutegravir- and elvitegravir-based cART, separately for cART-naïve and -experienced patients. By stepwise forward selection the following variables were included in the multivariable model if $P$ was <·10 in the univariable analysis; cART  regimen, most likely route of HIV acquisition, region of origin, CD4 cell-count at baseline, ever use of psychotropic drugs, time since HIV-diagnosis, and HCV co-infection at baseline. Additionally, we assessed the following factors in the cART-experienced group; viral suppression at baseline (HIV-1 RNA <200/≥200 copies/ml), nadir CD4 cell-count (<200 or ≥200 cells/mm$^3$), previous use of efavirenz, and previous discontinuations due to adverse events. The cART composition (elvitegravir/cobicistat/tenofovir disoproxil fumarate (DF)/emtricitabine, dolutegravir/abacavir/lamivudine, dolutegravir/tenofovir  DF/emtricitabine or other dolutegravir-based cART) was always included in the model to evaluate the risk difference for discontinuation between dolutegravir- and elvitegravir-based cART; sex and age were also included in the multivariable model, regardless of significance, owing to biological plausibility and based on prior reports.[16] Cox-proportional hazards modelling enabled us to evaluate discontinuations for cART naïve- and experienced-patients in more detail by

zooming in, step-by-step, on discontinuations due to any reason, thereafter evaluating discontinuations due to adverse events and finally discontinuations due to NPAEs. Of note, cART-naïve patients were excluded from multivariable analysis for risk of treatment limiting NPAEs given the limited number of discontinuations for this reason in this group.

The proportional-hazard assumption was checked using log-log plots and testing the Schoenfeld residuals. Potential statistical interaction, i.e. effect modification, was evaluated. Group comparisons were performed using unpaired $t$-tests, Wilcoxon rank–sum, x2 or Fisher's exact tests and Kaplan-Meier estimates (log-rank test), as appropriate. All statistical inferential frameworks were based on the two-sided p-value, and statistical significance was based on the 5% error rate. Data were analysed using IBM SPSS version 22 and SAS version 9.4.

## RESULTS

Out of 18,866 HIV-1 positive adults, 3,416 patients initiating either dolutegravir- or elvitegravir-based cART were included (Figure 1); 1,051 (31%) were cART-naïve, and 2,365 (69%) were cART-experienced (Table 1). Median (IQR) follow-up was 8·4 (5·6-11·9) and 9·0 (6·0-12·4) months for cART-naïve and -experienced patients on dolutegravir, respectively, and 16·0 (11·5-20·1) and 11·7 (7·4-15·2) months for cART-naïve and –experienced patients on elvitegravir, respectively. During follow-up, a total of 14 (0·6%) patients in the dolutegravir group and 12 (0·1%) in the elvitegravir group died (all-cause mortality), and 133 patients (62 on dolutegravir; 71 on elvitegravir) were loss to follow-up. In our cohort 12·7% (434/3416) of patients discontinued their dolutegravir- or elvitegravir-based cART, because of a variety of reasons, but toxicity was mentioned most frequently (Table 2). Overall, in 254/3416 (7·4%) of patients toxicity was reported as a reason to change cART. Switching cART because of virological, therapy or immunological failure was rare, but highest for cART-naïve patients starting elvitegravir-based cART (Table 2). In general, cART-experienced patients, starting either dolutegravir or elvitegravir, were more likely to discontinue their cART for any reason and for adverse events than cART-naïve patients (log-rank; $P<·001$) (Figure 2).
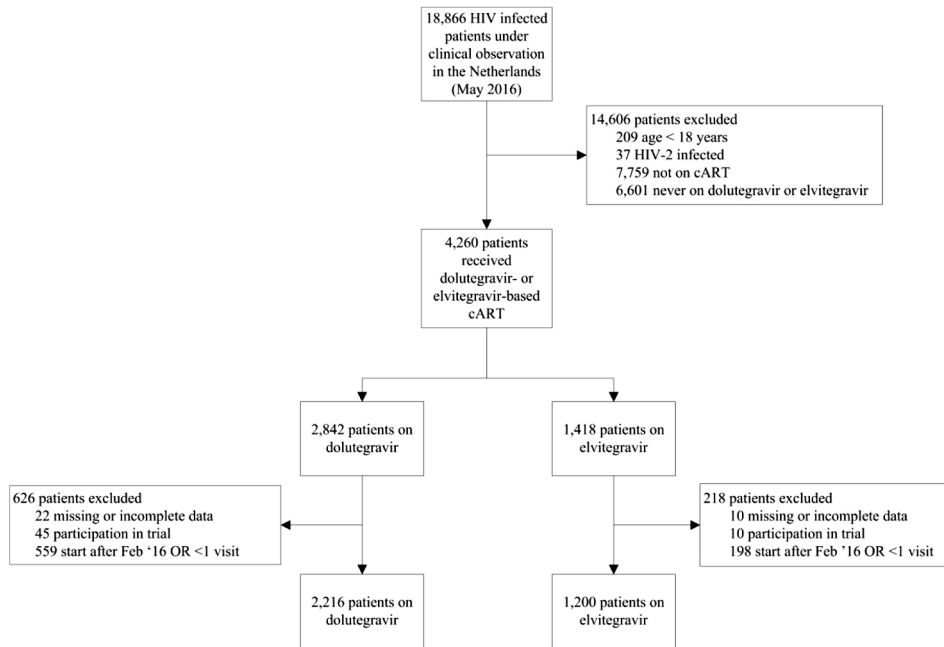
**FIGURE 1.** Inclusion flowchart
cART; combination antiretroviral therapy.

**TABLE 1.** Patient characteristics at start of treatment with dolutegravir- or elvitegravir-based cART.

| Characteristic | cART-naïve | | | cART-experienced | | |
|---|---|---|---|---|---|---|
| | dolutegravir (n=420) | elvitegravir (n=631) | *P* value | dolutegravir (n=1796) | elvitegravir (n=569) | *P* value |
| Age, years, mean (SD) | 40.7 (12.4) | 38.4 (10.9) | .009 | 47.1 (11.3) | 44.9 (10.5) | <.001 |
| Sex, male | 384 (91.4) | 592 (93.8) | .140 | 1466 (81.6) | 459 (80.7) | .609 |
| Region of origin | | | .313 | | | .567 |
| The Netherlands | 261 (62.1) | 429 (68.0) | | 1133 (63.1) | 356 (62.6) | |
| Europe | 49 (11.7) | 64 (10.1) | | 172 (9.6) | 44 (7.7) | |
| Africa and middle East | 35 (8.3) | 45 (7.1) | | 228 (12.7) | 82 (14.4) | |
| South America and Caribbean | 54 (12.9) | 70 (11.1) | | 168 (9.4) | 57 (10.0) | |
| Other | 21 (5.0) | 21 (3.3) | | 87 (4.8) | 30 (5.3) | |
| Unknown | - | 2 (0.3) | | 8 (0.4) | - | |
| Transmission route | | | .005 | | | .016 |
| MSM | 306 (72.9) | 516 (81.8) | .001 | 1126 (62.7) | 374 (65.7) | .190 |
| Heterosexual | 92 (21.9) | 90 (14.3) | | 498 (27.7) | 166 (29.2) | |
| Intravenous drug use | 1 (0.2) | 1 (0.2) | | 41 (2.3) | 4 (0.7) | |
| Other or unknown | 21 (5.0) | 24 (3.8) | | 131 (7.3) | 25 (4.4) | |
| HIV RNA level < 50 copies/ml, n=2218 | n/a | n/a | | 1436 (85.0) | 432 (81.9) | .083 |
| CD4-cell count, cells/mm³, n=3255 | 460 (280-630) | 460 (347-620) | .216 | 620 (449-780) | 630 (450-800) | .365 |
| CD4-cell count <200 cells/mm³, n=3255 | 74 (17.7) | 60 (9.6) | <.001 | 79 (4.7) | 29 (5.4) | .487 |
| Hepatitis co-infection | | | | | | |

4

**TABLE 1. Continued.**

| Characteristic | cART-naïve | | | cART-experienced | | |
|---|---|---|---|---|---|---|
| | dolutegravir (n=420) | elvitegravir (n=631) | P value | dolutegravir (n=1796) | elvitegravir (n=569) | P value |
| Hepatitis B | 6 (1.4) | 19 (3.0) | .099 | 81 (4.5) | 42 (7.4) | .007 |
| Hepatitis C | 11 (2.6) | 8 (1.3) | .107 | 124 (6.9) | 19 (3.3) | .002 |
| Regimen | | | | | | |
| Classic cART with ABC/3TC | 283 (67.4) | - | | 1033 (57.5) | - | |
| Classic cART with TDF/FTC | 102 (24.3) | 630 (99.8) | | 498 (27.7) | 557 (97.9) | |
| Classic cART with other NRTI combinations | - | - | | 16 (0.9) | - | |
| Non-classic cART | 35 (8.3) | 1 (0.2) | | 249 (13.9) | 12 (2.1) | |
| Pill burden[a] | | | <.001 | | | <.001 |
| 0 or unknown | 206 (49.0) | 383 (60.7) | | 629 (35.0) | 242 (42.5) | |
| > 1-5 | 189 (45.0) | 238 (37.7) | | 1063 (59.2) | 311 (54.7) | |
| >5 | 25 (6.0) | 10 (1.6) | | 104 (5.8) | 16 (2.8) | |
| Use of psychotropic drugs[b] | | | | | | |
| Current | 28 (6.7) | 27 (4.3) | .089 | 119 (6.6) | 27 (4.7) | .104 |
| Ever | 58 (13.8) | 92 (14.6) | .726 | 515 (28.7) | 127 (22.3) | .003 |
| Time on (c)ART before start, months | n/a | n/a | | 80 (38-149) | 63 (25-120) | <.001 |
| Previous ART[c] | | | | | | |
| Third agent - NNRTI | n/a | n/a | | 738 (41.1) | 254 (44.6) | |
| Third agent - PI | n/a | n/a | | 585 (35.6) | 181 (31.8) | |
| Third agent - raltegravir | n/a | n/a | | 149 (8.3) | 41 (7.2) | |
| Non-classic cART | n/a | n/a | | 218 (12.1) | 28 (4.9) | |
| No (c)ART | n/a | n/a | | 106 (5.9) | 65 (11.4) | |
| Ever use of efavirenz | n/a | n/a | | 931 (51.8) | 329 (57.8) | .013 |
| No. of previous DCs due to adverse events | n/a | n/a | | 2 (1-3) | 1 (1-2) | <.001 |

Values are expressed as n(%) or median (IQR) unless otherwise specified. cART; combination antiretroviral therapy, MSM; men who have sex with men, NNRTI; non-nucleoside reverse transcriptase inhibitor, PI; protease inhibitor, DCs; discontinuations, n/a; non applicable. [a]Number of currently used non-HIV co-medications, restricted to drugs as specified in data-collection guidelines for the ATHENA cohort. [b]Note; only selected co-medications are registered in the ATHENA database owing to restricted data collection for co-medications. [c] Last regimen prior to start of dolutegravir- or elvitegravir based cART.

**TABLE 2.** Reasons for discontinuation of dolutegravir- or elvitegravir-based cART.

| Reason for discontinuation | Total | cART-naïve | | cART-experienced | |
|---|---|---|---|---|---|
| | | dolutegravir | elvitegravir | dolutegravir | Elvitegravir |
| Virological, therapy or immunological failure | 15 (0.4) | 0 (0) | 9 (1.4) | 2 (0.1) | 4 (0.7) |
| Toxicity | 254 (7.3) | 19 (4.5) | 40 (6.3) | 142 (7.9) | 53 (9.3) |
| Simplification | 13 (0.4) | 3 (0.7) | 7 (1.1) | 3 (0.2) | 0 (0) |
| Interaction co-medication | 18 (0.5) | 0 (0) | 4 (0.6) | 5 (0.3) | 9 (1.6) |
| Patient decision | 36 (1.1) | 1 (0.2) | 5 (0.8) | 20 (1.1) | 10 (1.8) |
| Other | 86 (2.5) | 8 (1.9) | 13 (2.1) | 39 (2.2) | 26 (4.6) |
| Unknown | 12 (0.4) | 0 (0) | 3 (0.5) | 6 (0.3) | 3 (0.5) |
| Total | 434/3416 (12.7) | 31/420 (7.4) | 81/631 (12.8) | 217/1796 (12.1) | 105/569 (18.5) |

Values are expressed as number of discontinuations (%) per subgroup. cART; combination antiretroviral therapy.

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Discontinuation of Dolutegravir- and Elvitegravir-containing cART for HIV in the Netherlands; incidence rates and risk factors

cART-naïve
| | | | | | |
|---|---|---|---|---|---|
| Number at risk | 420 | 409 | 285 | 164 | 71 |

| | | | | | |
|---|---|---|---|---|---|
| | 631 | 582 | 506 | 404 | 297 |

Number of DCs
| | | | | | |
|---|---|---|---|---|---|
| Because of any reason | 0 | 12 | 23 | 30 | 31 |
| Because of AE | 0 | 7 | 18 | 19 | 19 |

| | | | | | |
|---|---|---|---|---|---|
| | 0 | 19 | 37 | 52 | 62 |
| | 0 | 12 | 23 | 28 | 34 |

cART-experienced
| | | | | | |
|---|---|---|---|---|---|
| Number at risk | 1796 | 1688 | 1105 | 704 | 346 |

| | | | | | |
|---|---|---|---|---|---|
| | 569 | 459 | 335 | 224 | 113 |

Number of DCs
| | | | | | |
|---|---|---|---|---|---|
| Because of any reason | 0 | 109 | 182 | 207 | 213 |
| Because of AE | 0 | 85 | 123 | 136 | 142 |

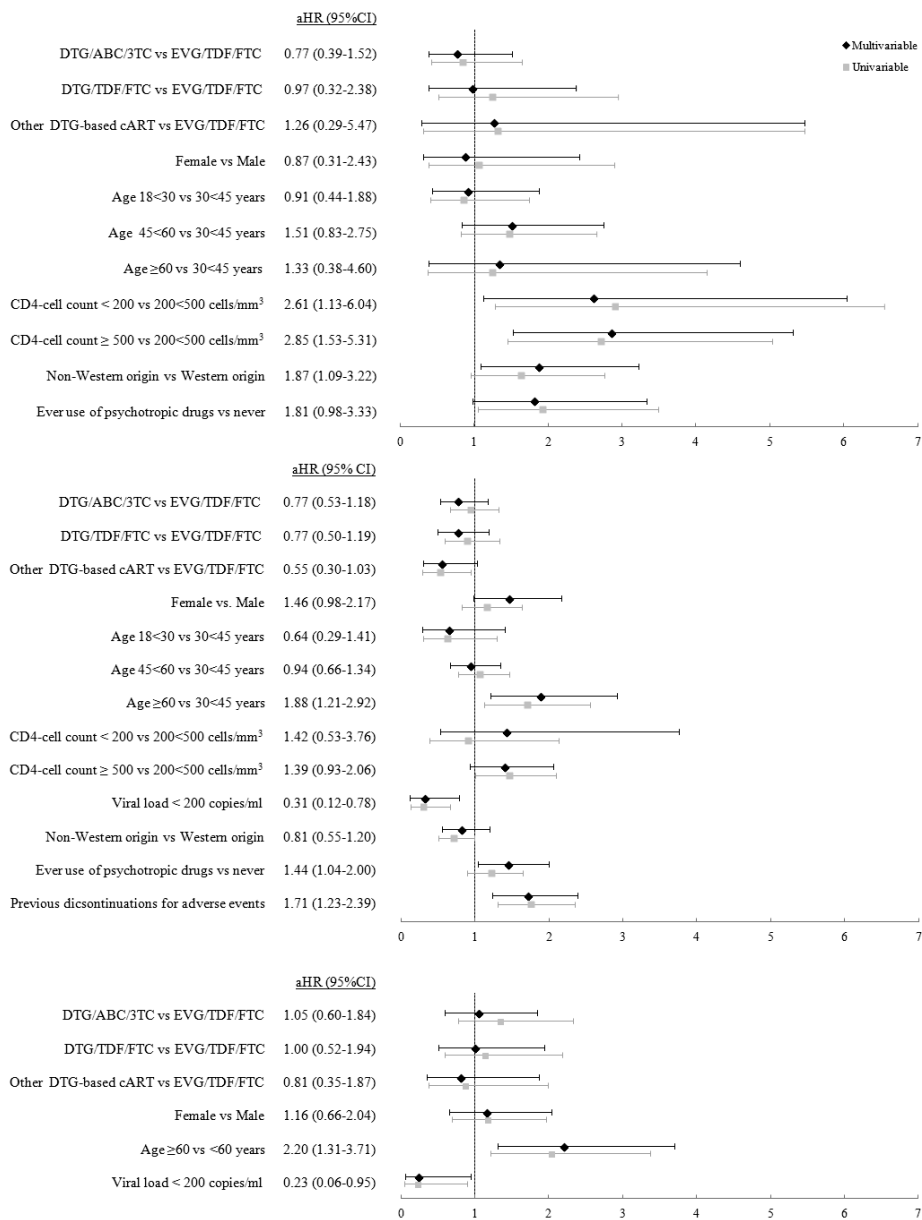| | | | | | |
|---|---|---|---|---|---|
| | 0 | 48 | 71 | 82 | 93 |
| | 0 | 31 | 40 | 44 | 49 |

**FIGURE 2. Discontinuations for any reason and associated with adverse events, and for cART-naï-ve and -experienced patients separately.**
AE; adverse events, DC; discontinuations, cART; combination antiretroviral therapy.

## cART-experienced patients

cART-experienced patients starting dolutegravir were older, had a longer history of prior treatment, used more co-medications and more often had HCV co-infection. Also, they had more frequently discontinued prior regimens for adverse events and were more likely to have ever used psychotropic medication compared to cART-experienced patients that received elvitegravir-based cART (Table 1). cART discontinuation associated with adverse events twelve months after treatment initiation was observed in 8·6% and 7·9% of cART–experienced patients initiating elvitegravir and dolutegravir, respectively. Uni- and multivariable analysis showed no difference in terms of risk for cART discontinuation for adverse events between dolutegravir/abacavir/lamivudine (unadjusted Hazard Rate (uHR) 0·94, 95% CI 0·67-1·32; adjusted HR (aHR) 0·77, 95% CI 0·53-1·12) or dolutegravir/tenofovir DF/emtricitabine (uHR 0·89, 95% CI 0·59-1·33; aHR 0·77, 95% CI 0·50-1·19; $P$=·239) versus elvitegravir/cobicistat/tenofovir DF/emtricitabine (Table 3, Figure 3 and Supplementary Material Table 1). Risk factors for discontinuation due to adverse events were age ≥60 years old (aHR 1·88, 95% CI 1·21-2·92), prior or current use of psychotropic medications (aHR 1·44 95% CI 1·04-2·00), and having a high ratio of previous cART discontinuations for adverse events (aHR 1·71, 95% CI 1·23-2·39) (Figure 3). In addition, HIV-1 RNA < 200 copies/mL at baseline reduced the risk for treatment discontinuation for adverse events (aHR 0·31, 95% CI 0·12-0·78). A trend towards a higher risk of discontinuation for adverse events was observed for female patients (aHR 1·46, 95% CI 0·98-2·17).

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**FIGURE 3. Risk factors for discontinuation of dolutegravir- or elvitegravir-based cART associated with adverse events in cART-naïve (A) and –experienced patients (B), and risk factors for discontinuation associated with neuropsychiatric adverse events in cART-experienced patients (C).**
aHR; adjusted hazard rate, cART; combination antiretroviral therapy, CI; confidence interval, DCs; discontinuations, DTG/ABC/3TC; dolutegravir/abacavir/lamivudine,  DTG/TDF/FTC; dolutegravir/ tenofovir disoproxil fumarate/emtricitabine, EVG/TDF/FTC; elvitegravir/tenofovir disoproxil fumarate/emtricitabine.

Discontinuation associated with NPAEs within twelve months after treatment initiation was observed in 3·7% (87/2365) of cART-experienced patients; 3·8% (69/1796) of patients on dolutegravir and 3·2% (18/569) of patients on elvitegravir. Multivariable analysis in cART-experienced patients revealed no differences in discontinuation due to NPAEs after start of dolutegravir- versus elvitegravir- based cART (Figure 3). Age ≥60 years old (aHR 2·20, 95% CI 1·31-3·71) and HIV-1 RNA <200 copies/mL at baseline (aHR 0·23, 95% CI 0·06-2·04) were the only factors that were associated with discontinuation for NPAEs. In the univariable analysis, use of psychotropic drugs and ever use of efavirenz were not significantly associated with discontinuation due to NPAEs and were therefore not included in the multivariable model. In addition, although also not statistically significant, we observed a trend towards a higher discontinuation rate due to NPAEs in patients with a higher ratio of previous discontinuations due to adverse events relative to all their previous cART discontinuations (uHR 1·56, 95% CI 0·98-2·47; Supplementary Material, Table 2).

**TABLE 3.** Incidence rates of cART discontinuation associated with any adverse event

| Risk factor | cART-naïve | | | | cART- experienced | | | |
|---|---|---|---|---|---|---|---|---|
| | No. of DCs | Person time, person-years | Rate per 1000 person-years | Unadjusted hazard ratio (95% CI) | No. of DCs | Person time, person-years | Rate per 1000 person-years | Unadjusted hazard ratio (95% CI) |
| cART regimen | | | | | | | | |
| EVG/TDF/FTC | 40 | 763 | 52 | 1 | 53 | 489 | 108 | 1 |
| DTG/ABC/3TC | 11 | 202 | 54 | 0.83 (0.42-1.65) | 87 | 704 | 124 | 0.94 (0.67-1.32) |
| DTG/TDF/FTC | 6 | 75 | 80 | 1.24 (0.52-2.95) | 41 | 372 | 110 | 0.89 (0.59-1.33) |
| Other | 2 | 22 | 90 | 1.31 (0.32-5.47) | 14 | 221 | 63 | 0.52 (0.29-0.94) |
| Sex | | | | | | | | |
| Male | 55 | 996 | 55 | 1 | 155 | 1476 | 105 | 1 |
| Female | 4 | 67 | 60 | 1.05 (0.38-2.90) | 40 | 324 | 124 | 1.16 (0.82-1.64) |
| Age, years | | | | | | | | |
| 18-<30 | 11 | 253 | 44 | 0.85 (0.41-1.74) | 8 | 128 | 62 | 0.62 (0.30-1.30) |
| 30-<45 | 23 | 458 | 50 | 1 | 63 | 634 | 99 | 1 |
| 45-<60 | 22 | 306 | 72 | 1.47 (0.82-2.65) | 87 | 822 | 106 | 1.06 (0.77-1.47) |
| ≥60 | 3 | 46 | 65 | 1.24 (0.37-4.14) | 37 | 216 | 172 | 1.70 (1.13-2.56) |
| Region of origin | | | | | | | | |
| Western | 37 | 782 | 47 | 1 | 149 | 1263 | 118 | 1 |
| Non-Western | 22 | 282 | 78 | 1.63 (0.96-2.76) | 46 | 537 | 86 | 0.71 (0.51-0.99) |
| CD4-cell count, cells/mm$^3$ | | | | | | | | |
| <200 | 10 | 116 | 86 | 2.90 (1.28-6.55) | 6 | 80 | 75 | 0.90 (0.38-2.13) |
| 200-499 | 14 | 491 | 29 | 1 | 36 | 445 | 81 | 1 |
| ≥500 | 35 | 449 | 78 | 2.70 (1.45-5.03) | 139 | 1159 | 120 | 1.46 (1.01-2.10) |
| Viral load | | | | | | | | |
| < 200 copies/mL | n/a | n/a | n/a | - | 174 | 1502 | 115 | 0.30 (0.13-0.67) |
| ≥ 200 copies/mL | n/a | n/a | n/a | - | 6 | 185 | 32 | 1 |
| Use of psychotropic drugs | | | | | | | | |
| Never | 45 | 916 | 49 | 1 | 134 | 1229 | 103 | 1 |
| Ever | 14 | 147 | 95 | 1.91 (1.05-3.49) | 61 | 500 | 122 | 1.22 (0.90-1.65) |
| Total | 59 | 1063 | 55 | - | 195 | 1800 | 108 | - |
| Total | 59 | 1063 | 55 | - | 195 | 1800 | 108 | - |

cART; combination antiretroviral therapy, CI; confidence interval, DCs; discontinuations, DTG/ABC/3TC; dolutegra-vir/abacavir/lamivudine, DTG/TDF/FTC; dolutegravir/tenofovir disoproxil fumarate/emtricitabine EVG/TDF/FTC; elvitegravir/tenofovir disoproxil fumarate/emtricitabine.

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### cART-naïve patients

Among previously cART-naïve patients, those who started dolutegravir-based cART were older, received more co-medications, and more often had advanced HIV-infection, than those starting elvitegravir-based cART. cART-naïve patients starting elvitegravir-based cART were more often MSM (Table 1). cART discontinuation associated with adverse events twelve months after treatment initiation was observed in 5·4% and in 4·5% of cART-naïve patients initiating elvitegravir and dolutegravir, respectively. Again, uni- and multivariable analysis showed no difference in cART discontinuation for adverse events between dolutegravir/abacavir/lamivudine or dolutegravir/tenofovir DF/emtricitabine versus elvitegravir/cobicistat/tenofovir DF/emtricitabine (Table 3, Figure 3 and Supplementary Material Table 1). Factors that were independently associated with discontinuation associated with adverse events were being of non-Western origin (aHR 1·87, 95% CI 1·09-3·22), and having a high (>500 cells/mm$^3$) (aHR 2·85, 95% CI 1·53-5·31) or low (<200 cells/mm$^3$) (aHR 2·61, 95% CI 1·13-6·04) CD4 cell-count at start of dolutegravir- or elvitegravir-based cART. A trend towards a higher risk of discontinuation for adverse events was observed for patients who concomitantly use psychotropic medications, or used these medications in the past (aHR 1·81, 95% CI 0·98-3·33) (Figure 3). Discontinuations associated with NPAEs within 12 months after treatment initiation were observed in 2·1% (22/1051) of cART-naïve patients.

### Adverse events

In cART-naïve and -experienced patients combined, 363 treatment limiting adverse events were recorded for a total of 254 patients. Adverse events were mainly of psychiatric nature (27·4%), followed by gastrointestinal (19·6%) and neurologic (11·6%) (Table 4). Insomnia was especially reported by patients that discontinued dolutegravir-based cART; 15-18% versus 7-9% for elvitegravir-based cART. Headache was reported more by cART-experienced patients (8.0% and 8.7% for dolutegravir and elvitegravir, respectively), than by cART-naïve patients (0% and 1.8% for dolutegravir and elvitegravir, respectively) (Table 4).

**TABLE 4.** Reported adverse events associated with discontinuation of dolutegravir- or elvitegravir-based cART.

| Adverse event category | Time to DC, median (IQR), days | Total | Adverse events, times reported* (%) | | | |
|---|---|---|---|---|---|---|
| | | | cART-naïve | | cART-experienced | |
| | | | dolutegravir | elvitegravir | dolutegravir | elvitegravir |
| Psychiatric | 102 (38-184) | 98 (27.4%) | 14 (42.4%) | 13 (23.2%) | 60 (30.0%) | 11 (15.9%) |
| Insomnia | 90 (31-174) | 45 (12.6%) | 6 (18.2%) | 5 (8.9%) | 29 (14.5%) | 5 (7.2%) |
| Depression and mood disorders | 114 (40-214) | 19 (5.3%) | 3 (9.1%) | 3 (5.4%) | 13 (6.5%) | 0 (0.0%) |
| Suicidal ideation or attempt | 66 (22-128) | 4 (1.1%) | 1 (3.0%) | 0 (0.0%) | 3 (1.5%) | 0 (0.0%) |

**TABLE 4. Continued.**

| Adverse event category | Time to DC, median (IQR), days | Adverse events, times reported* (%) | | | | |
| --- | --- | --- | --- | --- | --- | --- |
| | | | cART-naïve | | cART-experienced | |
| | | Total | dolutegravir | elvitegravir | dolutegravir | elvitegravir |
| Other | 81 (36-150) | 30 (8.4%) | 4 (12.1%) | 5 (8.9%) | 15 (7.5%) | 6 (8.7%) |
| Gastrointestinal | 100 (21-160) | 71 (19.8%) | 6 (18.2%) | 12 (21.4%) | 40 (20.0%) | 13 (18.8%) |
| Systemic | 70 (21-110) | 44 (12.3%) | 5 (15.2%) | 8 (14.3%) | 21 (10.5%) | 10 (14.5%) |
| Rash | 58 (26-111) | 12 (3.4%) | 1 (3.0%) | 3 (5.4%) | 4 (2.0%) | 4 (5.8%) |
| Other | 74 (18-133) | 32 (8.9%) | 4 (12.1%) | 5 (8.9%) | 17 (8.5%) | 6 (8.7%) |
| Neurologic | 66 (22-104) | 42 (11.7%) | 2 (6.1%) | 2 (3.6%) | 30 (15.0%) | 8 (11.6%) |
| Headache | 47 (20-79) | 23 (6.4%) | 0 (0.0%) | 1 (1.8%) | 16 (8.0%) | 6 (8.7%) |
| Other | 57 (29-114) | 19 (5.3%) | 2 (6.1%) | 1 (1.8%) | 14 (7.0%) | 2 (2.9%) |
| Renal dysfunction | 129 (47-308) | 23 (6.4%) | 0 (0.0%) | 8 (14.3%) | 5 (2.5%) | 10 (14.5%) |
| Dermatologic | 85 (28-164) | 20 (5.6%) | 1 (3.0%) | 2 (3.6%) | 11 (5.5%) | 6 (8.7%) |
| Musculoskeletal | 103 (48-199) | 20 (5.6%) | 1 (3.0%) | 2 (3.6%) | 12 (6.0%) | 5 (7.2%) |
| Liver dysfunction | 86 (55-146) | 10 (2.8%) | 0 (0.0%) | 4 (7.1%) | 4 (2.0%) | 2 (2.9%) |
| Sensory | 59 (15-111) | 6 (1.7%) | 2 (6.1%) | 0 (0.0%) | 4 (2.0%) | 0 (0.0%) |
| Other | 114 (52-232) | 29 (7.0%) | 2 (6.1%) | 5 (8.9%) | 16 (8.0%) | 6 (8.5%) |
| **Total** | | 363 | 33 | 56 | 203 | 71 |

AE, adverse event; cART, combination antiretroviral therapy; DC, discontinuation; IQR, Inter Quartile Range. *More than one AE per patient possible.

# DISCUSSION

Based on the data from the nationwide HIV cohort study in the Netherlands, we provide evidence that both the InSTIs dolutegravir and elvitegravir are well tolerated when used in HIV-positive adults. Interestingly, cART-experienced patients were more prone to discontinue integrase-based cART than cART-naïve patients and treatment limiting adverse events within the first year after start of dolutegravir- or elvitegravir-based cART occurred in ~5% and ~8-9% of cART-naïve and –experienced patients, respectively. In general, discontinuations because of NPAEs in our cohort were low (<4%) and we observed no difference in risk for discontinuation for (NP)AE between patients on dolutegravir- versus elvitegravir-based cART.

Rates of discontinuation because of adverse events exceeded those reported in large phase III/IV clinical trials after 48 weeks of treatment for both these InSTIs.[2,3,31-37] Our results seem comparable to the results from some other observational cohort studies, in which ~3·6-8% of patients discontinued dolutegravir for adverse events within one year after start.[14,16,38] However, these particular studies did not distinguish between cART-naïve and –experienced patients when calculating discontinuation rates. In the Swiss HIV Cohort Study no association was found between being cART-naïve and discontinuation for adverse events for either dolutegravir or raltegravir. In general, especially smaller cohort

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studies showed higher rates of discontinuations due to adverse events for dolutegravir compared to our results.[19,27] Although, our national dataset included the patients presented previously by v/d Berk *et al.*, there were some differences in demographics. For instance, in our dataset ~60% of included patients were of Dutch origin versus ~40% in the study by v/d Berk *et al.* Because being of non-Western origin was the only risk factor significantly associated with discontinuation of dolutegravir- or elvitegravir-based cART for adverse events in cART-naïve patients, this may have contributed to the difference in reported unadjusted discontinuation rates between both studies.

This current study is distinct given that the ATHENA cohort database contains extensive data on patient characteristics and (non-)HIV-treatment history from 98% of all HIV-positive adults in care in The Netherlands since the '90s.[25] Stratified analysis for cART-naïve and –experienced patients enabled us to look into these patient specific risk factors for discontinuation of dolutegravir- or elvitegravir based cART in more detail. Although no significant difference in discontinuation profile was observed between patients starting dolutegravir- or elvitegravir-based cART, risk factors for discontinuation between cART-experienced and -naïve patients were different.

In cART-experienced patients, age above 60 years old was associated with a twofold higher risk for all types of discontinuations (because of any reason, adverse events and NPAEs). Older age was also identified as a risk factor in German, Spanish and French HIV cohort studies[15,16,22], but not in the Swiss HIV-cohort.[14] A history of psychotropic medication use and prior discontinuation of cART due to adverse events in our analysis were both found to be associated with discontinuation because of adverse events in cART-experienced patients, independent of which InSTI was used. These important risk factors have not been analyzed by other cohorts that evaluated patients who discontinued InSTIs. As more patients with these risk factors were selected for use of dolutegravir, it may partly explain the seemingly higher rates of discontinuation because of adverse events with dolutegravir observed in outpatient care. In contrast to some other studies, we did not see higher discontinuations rates if cART included abacavir[16,17,19], but we did see a trend towards higher discontinuation for adverse events in cART-experienced women.[14]

Specifically in cART-naïve patients, both a high and a low CD4 cell-count at baseline resulted in an almost threefold increase in risk of discontinuation because of adverse events. The reason for this rather contradictory result is not completely clear. A possible explanation is that an advanced state of immunodeficiency (low CD4-cell count) at start of cART increases the risk for immune reconstitution inflammatory syndrome (IRIS) leading to early discontinuation of cART.[39] As for the high CD4-cell count, it might be possible that patients, who feel well and for whom multiple treatment options are available, are more inclined to switch cART because of less serious side-effects.

Considering the results of our study and the risk profiles that we identified, there are several possible explanations for the higher discontinuations rates in observational data compared to RCTs. First of all, in general, registration studies include highly selected HIV-infected patients of younger age (approx. 35 years old). In real-life, the average age of HIV-infected patients has increased over the years and was ~40-45 years in this cohort. Our data show that increasing age is a significant risk factor for treatment-limiting adverse events in cART-experienced patients, and hence it is logical to find higher discontinuation rates in clinical practice than in clinical trials. Second, the fact that many patients started dolutegravir in a relatively short period of time has led to early identification of specific toxicities. Dolutegravir was started in a large subset of patients with risk factors for toxicity, besides age, these were the use of more psychotropic co-medications and prior cART discontinuations for adverse events, that could have attributed to some substantial channeling bias. Another explanation for this discrepancy could be the specific NPAEs profile (e.g. insomnia and mood disorders) seen with the use of dolutegravir. It is known that insomnia can lead to development of other psychological distress, ultimately leading to mood disorders or depression[40], and thus one could speculate that psychiatric symptoms may develop more easily with the use dolutegravir as a result of insomnia. Notably, insomnia was the only NPAE that was reported more frequent by subjects on dolutegravir-based cART than by those on efavirenz-based cART in the SINGLE study[2], and a significantly higher risk of insomnia was confirmed in a recent meta-analysis by Hill *et al.*[41] In our opinion, this observation was neglected at the time of introduction of dolutegravir, when its favourable safety profile was stressed as an important benefit.

A limitation of our study is that, although we aimed to explore the reasons for discontinuation of solely dolutegravir or elvitegravir, we cannot rule out that we included discontinuations that were driven by backbone components. This is especially a risk for elvitegravir-based cART as this InSTI is only available as a single tablet regimen. Furthermore, we were only able to report on adverse events that led to the discontinuation of cART and not on adverse events that were not treatment limiting.

Unfortunately, information on the use of psychotropic drugs in the database was not complete, due to restricted collection guidelines for co-medications. The effect of prior or current psychotropic drug use on discontinuations for (NP)AEs could therefore have been underestimated and may be even stronger in reality. Despite its limitations, this study provides more detailed information on a large real-life cohort of patients on dolutegravir- and elvitegravir-based cART in a Western setting than most other studies have reported so far. Future studies should focus on (NP)AEs in specific patient populations that are underrepresented in phase III clinical trials, e.g. the elderly and patients of non-Western origin.

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In conclusion, this study demonstrated that patient characteristics, i.e. including socio-demographic and clinical characteristics, are the main predictor for discontinuation of dolutegravir and elvitegravir-based cART because of adverse events in cART-naïve and -experienced patients. These patient characteristics contribute more to the risk for cART discontinuation than the use of dolutegravir or elvitegravir itself. Both InSTIs can be considered as ARVs that cause limited discontinuations due to adverse events, however caution is warranted for populations susceptible for cART discontinuation, especially ageing populations. Our findings suggest that patient characteristics should be considered more when selecting an optimal regimen, and also reinforce the need for independently conducted phase IV pharmacovigilance studies to help position new antiretrovirals in our treatment armamentarium.

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Discontinuation of Dolutegravir- and Elvitegravir-containing cART
for HIV in the Netherlands; incidence rates and risk factors

# PART 2

occurrence and detection
of neurocognitive decline
in HIV patients

# CHAPTER 5

Cognitive impairment in clinical practice: the evaluation of a stepwise screening protocol in relation to clinical outcomes and management

## ABSTRACT

Neurocognitive impairment (NCI) is an increasingly important comorbidity in an ageing HIV+ population. Despite the lack of available treatment modalities, screening for NCI is recommended. In the UMC Utrecht, yearly NCI-screening is done using the Montreal Cognitive Assessment (MoCa) tool and the HIV Dementia Scale (HDS). The aim of this study is to evaluate this screening protocol in relation to clinical outcomes and management. A retrospective cohort study was performed in suppressed adult HIV+ patients. Next to the MoCa and HDS, the Utrecht scale for Evaluation of Rehabilitation-Participation (USER-P) and the Hospital Anxiety and Depression Scale (HADS) were used. Patients scoring below average on cognitive screening tests or with subjective cognitive complaints were further evaluated using a standardized protocol including optimizing cART and checking for somatic disorders. In patients with subjective complaints and participation restrictions, cognitive rehabilitation was proposed. Two hundred eighty-six patients were screened. The vast majority were MSM with an average age of 49 years. One hundred forty-four out of 286 patients (50%) had an abnormal test score and/or had subjective cognitive complaints. Restrictions in participation were present in 23% of patients. Six patients on Efavirenz switched their regimes, as this drug is known for its potential central nervous system (CNS) side effects. A depressive component was present in 58 patients (40%). Five patients had a clinical relevant laboratory abnormality. Moreover, six patients were referred for cognitive rehabilitation, which resulted in a 100% success rate in set goals in the five evaluable patients. Although the protocol was not fully adhered to in all patients, it did result in detectable underlying causes of NCI in 59% of patients, and 21% was referred for further treatment. Moreover, cognitive rehabilitation appears to be a successful intervention for patients with NCI who experience subjective complaints and participation restrictions.

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# INTRODUCTION

Neurocognitive impairment (NCI) is an important comorbidity in Human Immunodeficiency Virus (HIV) infected patients, and thorough investigation of NCI can lead to the diagnosis HIV Associated Neurocognitive Disorder (HAND).[1] The introduction of potent combination antiretroviral therapy (cART) led to a significant decrease of HANDs most severe form, HIV associated dementia (HAD). Still, HIV positive (HIV+) patients have a 15-50% change to develop any form of HAND even when properly treated.[2,3] This will likely increase due to ageing of the HIV+ population.[4,5] In mild forms of HAND, patients can already experience difficulties in, for example, medication compliance, employment, and quality of life, which challenges the care for HIV+ patients.

Screening is an important step in addressing NCI and doing so is recommended by international guidelines.[6,7] Several screening tests for NCI have been developed but there is no international consensus on how to screen. Moreover, no recommendations are in place regarding therapeutic strategies after HAND diagnosis, as no evidence-based treatment modalities for HAND are yet available.[8–11] As the cause of HAND appears to be multifactorial, several therapeutic strategies can be attempted. At first, cART may need to be switched if it contains agents known for their cognitive side effects. In addition, somatic factors such as hypothyroidism, neuro-syphilis, vitamin deficiencies and HIV replication in the cerebrospinal fluid (CSF) should be considered when exploring causes for cognitive impairment.[7] Finally, depression may play a role in the development of cognitive impairment and should be addressed.[10,12] In cases were recovery of cognitive impairment is not possible, a patient has to find a way to cope with emerging limitations in everyday life. This can be obtained with cognitive rehabilitation programs.

Based on these considerations, in our treatment center, a standardized protocol was developed, in which HIV+ patients were offered screening for NCI with subsequent structured analysis of possible underlying somatic and psychological causes in case of cognitive abnormalities. The aim of the study is to evaluate the adherence and outcomes of this protocol in order to be able to determine therapeutic strategies that are effective in routine clinical practice.

# METHODS

### Study design
For this retrospective cohort study, neurocognitive screening data of adult HIV+ patients on cART from the outpatient department of the UMCU in the period May 2012 till March 2016 were used. NCI screening was offered annually in clinical practice to patients

with proper command and understanding of the Dutch language at their routine outpatient visit. Informed consent was obtained through an opting-out procedure for all HIV-infected patients in care, and data were coded and collected in a database by the Stichting HIV Monitoring (SHM).

### Screening protocol

During the annual screening visit, patients were subjected to two neurocognitive screening tests and three questionnaires. Screening for NCI was performed by using the Montreal Cognitive Assessment (MoCA)[13] and the HIV Dementia Scale (HDS)[14]. Both tests are designed to assess several cognitive domains. The maximum score of the MoCA is 30 with a cut-off score of 25. The total score is corrected for education; patients below 12 years of experienced education from elementary school gained one additional point. Cut-off score for the HDS is 9 out of a maximum of 16.

In addition, three questionnaires were used. First, in order to determine the effect on daily activities, the Utrecht Scale of Evaluation Rehabilitation-Participation (USER-P)[15] was used which comprises of 10 items on experienced participation restrictions in several areas in relation to a person's health or disability. Each item score ranges from 0 (not possible at all) to 3 (independent without difficulty); scoring below the maximum score on two or more items of experienced participation (a total score of less than 80%) was defined as abnormal. Secondly, three European AIDS Clinical Society (EACS) questions for subjective cognitive complaints, ranging from none to severe, were used; if one of the questions was answered with severe it was accounted as abnormal. Finally, the Hospital Anxiety and Depression Scale (HADS)[16] was performed to screen for depressive or anxiety symptoms. Scores above 12 points were indicative for considerable anxiety or depression complaints and symptoms.

### Screening flow chart

Patients scoring below average on at least one of the cognitive screening tests or mentioning subjective cognitive complaints in the 3 EACS questions were further evaluated. The first step was to optimize antiretroviral treatment through ruling out virological failure and switch Efavirenz. An undetectable viral load was defined as <50 copies/ml, low-level viremia as >50 but <200 copies/ml and if a detectable viral load was undetectable on the following visit, it was defined as a blip. The second step was assessment of Thyroid-stimulating hormone (TSH), Folic acid, Vitamin B12 and TPHA/ VDRL, and the treatment of any abnormalities arising from these assessments. In order to guarantee lab results were representative for the time of screening, a maximum interval of 3 months between assessment of the neurocognitive tests and measurement of the viral load was accepted. For the other laboratory testing a timeframe of 12 months before and 6 months after neurocognitive testing was accepted with an

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exception for TPHA/VDRL testing (6 months before). Alongside laboratory testing, HADS scores were evaluated and referral for psychological treatment was initiated in patients with significant depressive or anxiety symptoms. In those patients without any of the aforementioned abnormalities, referral for lumbar puncture (LP) and Magnetic resonance imaging (MRI) was proposed to respectively detect viral replication in the cerebrospinal fluid or profound abnormalities in the brain. Finally, patients could be referred for cognitive revalidation adapted for HAND. This neurocognitive revalidation was performed by occupational therapists in a 12-week program. It started with giving the patient general information about cognition, and an analysis of the cognitive difficulties patients experienced in everyday functioning. Patients graded relevant domains from 0 to 10 and created personal goals for improvement which were mainly focused on planning and memory. They had multiple visits with their therapist and received homework assignments. The patients evaluated the program and set goals at the end of the program. They had to grade their performance on these domains before, and after the program (maximum 12 weeks). This measuring of goal attainment in a standardized way is used as an individualized outcome measure.

### Statistical analysis

Each step of the flowchart performed and subsequent results of the tests were captured for each patient with the use of the hospitals' electronic health care system. Baseline characteristics were outlined and stratified for normal and abnormal screening outcome. Values in all parameters were expressed as means (±SD) when normally distributed and as medians (interquartile range) in case of skewed distribution. Baseline differences were calculated with SPSS (version 21 Inc., Chicago, IL) as univariate analyses with the use of an unpaired t-test for continuous variables when normally distributed and the Mann-Whitney test for a skewed distribution. For categorical variables, the Fishers exact test was used. P-values (2-sided) of <0.05 were considered statistically significant.

In evaluating the adherence and outcomes of the flowchart, we examined if patients were on an Efavirenz-containing regime, if the various laboratory values were tested and if patients were referred for psychological treatment and/or further neurological examination. Furthermore, outcomes of the performed steps were determined as deficiency rates for the laboratory and neurological assessments. At last, neurocognitive revalidation results were examined by evaluating patient's initial scores compared to the scores at the end of each therapy program.

## RESULTS

A total of 286 patients were screened for NCI (figure 1). Subjective cognitive complaints

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were reported by 122/286 patients (43%) on the EAC's questions whereas 45 of those 122 (37%) scored abnormal on at least one of the two screening test (MoCA or HDS). Additionally, there were 16 patients without subjective complaints on the EAC's questions, and 10 patients who weren't tested with the EAC's questions, who scored abnormal on the MoCA or the HDS. Four patients were excluded and therefore, a final 144/286 (50%) patients were further analysed of which patients characteristics are depicted in table 1. The majority was male and of Dutch origin. There was no significant difference in the mean age between the group with and without indications for cognitive impairment (47.7 versus 49.9 years; p=0.08). The group with indications for cognitive impairment had a longer duration of HIV-infection (94.6 months versus 75.5 months, p=0.014) and a longer duration of cART use (77.1 months vs 55.6 months p=0.01). For the subgroup of patients experiencing subjective complaints as measured by the EAC's questions there were no differences between both groups.
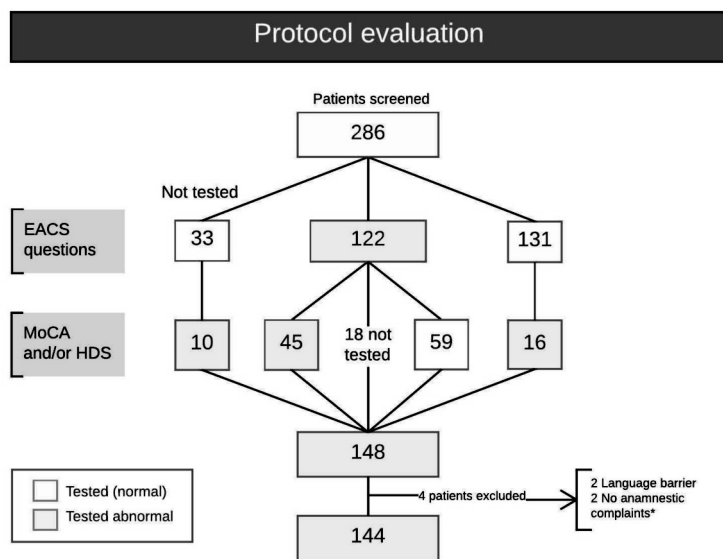


**FIGURE 1. Screening protocol**
EAC = European AIDS Clinical Society; MoCA = Montreal Cognitive Assessment; HDS = HIV Dementia Scale. *After consultation with MD, no subjective complaints were indicated

A substantial part of the patients with an abnormal result on one of the screening tests also had signs of a mood disorder. Formal screening with HADS resulted in abnormal scores in 58 out of 133 patients tested (40%). Furthermore, 18/144 (13%) patients were referred to psychological treatment for mood complaints.

As around 1200 HIV-infected patients are being followed at the UMCU, the study cohort is only a subgroup of all patients in care. However, the study group is rather comparable to the whole patient population, apart from nationality and duration of HIV infection and ART use. The percentage of non-native Dutch speakers was lower in the study and the duration of HIV and ART use was somewhat shorter in the study population, as compared with the entire population (data not shown).

**TABLE 1.** Baseline characteristics

| Characteristics | Neurocognitive screening (n=286) | |
| --- | --- | --- |
| | Screening tests and subjective (EACS) normal (n=142) | Screening tests and/ or subjective (EACS) abnormal (n=144) |
| Demographic | | |
| Age, years (mean (SD)) | 47.7 (10.6) | 49.9 (11.2) |
| Sex, men | 134 (94.4%) | 128 (88.9%) |
| Nationality, NL | 128 (90.1%) | 130 (90.3%) |
| Experienced education, years | 14 (11-15) | 14 (11-15) |
| HIV-related factors | | |
| **Diagnosis, months** | **75.5 (40.4-123.6)** | **94.6 (51.6-167.3)** |
| **Transmission, MSM** | **115 (81.0%)** | **98 (68.1%)** |
| Nadir CD4 count, cells/mm3 | 243 (106-326) | 225 (95-320) |
| cART regime, NRTI backbone + | | |
|   - PI | 66 (46.5%) | 69 (47.9%) |
|   - NNRTI | 64 (45.1%) | 52 (36.1%) |
|   - Other | 12 (8.5%) | 23 (16.0) |
| **cART, EFV containing regime** | **53 (37.3%)** | **33 (22.9%)** |
| **cART use, months** | **55.6 (22.5-110.8)** | **77.1 (38.6-152.0)** |
| CD4 count, cells/mm3 | 574 (439-713) | 555 (409-725) |
| HIV-RNA <50 copies/ml | 134 (94.4%) | 135 (93.8%) |
| HBV, chronic | 5 (3.5%) | 7 (4.9%) |
| HCV, chronic | 2 (1.4%) | 7 (4.9%) |
| Co-medication | | |
| Anti-depressive drugs | 3 (2.1%) | 8 (5.6%) |
| Cognitive inhibitive medication | 2 (1.4%) | 9 (6.3%) |

Results presented in total (%) or median (IQR). Cognitive-inhibitive drugs: antipsychotics, benzodiazepines, opiates, Alzheimer's medication and Antiparkinson medication. The characteristics presented in bold differ statistically significantly between groups (p<0.05). EACS, European AIDS Clinical Society; NL, Netherlands; MSM, men who have sex with men; NRTI, nucleoside reverse transcriptase inhibitor; cART, combination antiretroviral tehrapy; PI, protease inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; EFV, efavirenz; HBV, hepatitis B virus; HCV, hepatitis C virus.

**Adherence to and outcomes of the flowchart**

First, adherence to the flowchart with its four steps was evaluated (figure 2). The first step, analysing the viral load within a 3 months interval, was completed in 141 out of 144 (97%). In the second step, 15/144 patients (10%) had all laboratory values tested, 101/144 patients (70%) at least one, and there were only 28/144 patients (20%) who did not have any laboratory value tested. Subsequent analysis of the 3rd steps showed

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that, in total, 13/144 patients (9%) were referred for further neurological examination and only six patients were included in the final step and participated in a cognitive rehabilitation program.
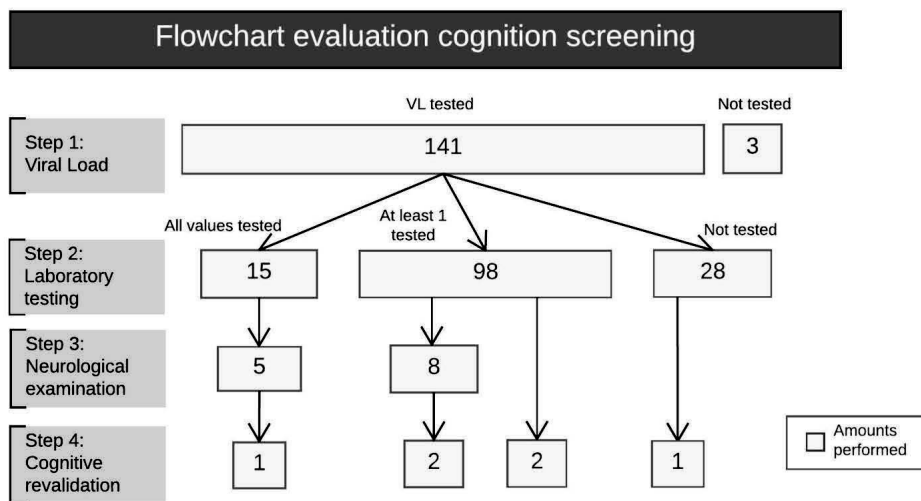


**FIGURE 2.** Flowchart evaluation

When analysing the outcomes of the various tests in the subsequent steps of the flowchart, 10/144 patients (9%) had a detectable viral load (table 2) of whom six were labelled as low level viremia and four as blips. A total of 33/144 (23%) patients were on an Efavirenz-containing regime, of which only six patients (18%) subsequently switched their regimes. Amongst 115 patients (18.3%) tested for laboratory abnormalities, 21 showed abnormal findings, of which folic acid deficiencies were predominant with 10/31 patients (32%). However, none of these patients had an anaemia as a result of this deficiency. Two patients had a positive syphilis test and three patients had TSH abnormalities. When evaluating step 3, an LP (for intrathecal viral replication) was performed in six patients, a MRI in one patient and both MRI and LP testing was performed in four patients. Two patients had abnormal findings on their MRI, which were suggestive for HAND (e.g. generalized atrophy, white matter abnormalities). These two patients had a folic acid deficiency, but no further laboratory abnormalities. One patient had an abnormal lymphocyte count in his cerebral spinal fluid; however he was lost to follow up before further analysis could take place.
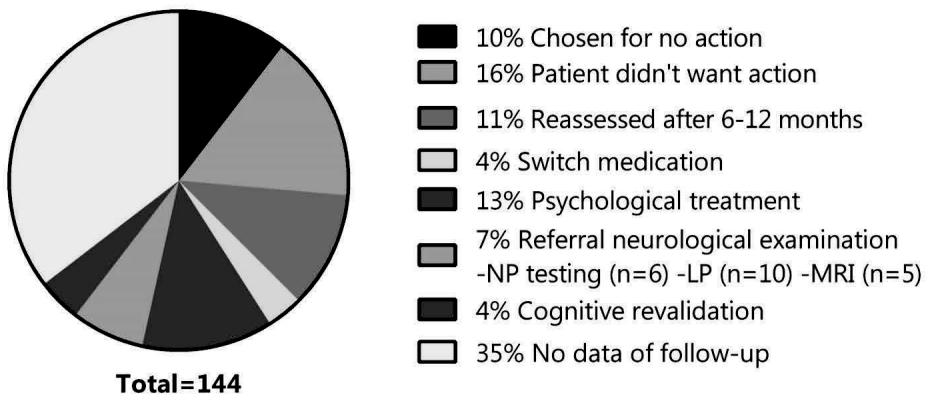
In a total of 30 patients (21%) further action was taken upon deviating screening test results (fig3). In 13%, patients were referred for psychological treatment, in 4% cART

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was adjusted, and in 4%, patients were referred for cognitive rehabilitation. In about a quarter of cases, either patient or physician chose to undertake no further action for unknown reasons. In 11%, cognitive screening was repeated after 6 or 12 months.

**TABLE 2.** Results of flowchart testing

| Steps | Performed | Abnormal outcomes | |
|---|---|---|---|
| | N | N | % |
| **Step 1 – Virological treatment** | | | |
| EFV switched | 33 | 6 switched | 18.2% |
| VL within 3 months | 141 | 10 | 7.1% |
| **Step 2 – Laboratory testing** | | | |
| Lues TPHA/VDRL | 62 | 4 | 6.5% |
| TSH | 77 | 6 | 7.8% |
| Folic acid | 32 | 10 | 31.2% |
| Vit B12 | 30 | 1 | 3.3% |
| **Step 3 – Neurological examination** | | | |
| LP | 10 | 1 | 10% |
| MRI | 5 | 2 | 40% |
| NP testing | 5 | 3 | 60% |

EFV, efavirenz. Viral load (VL) testing within 3 months; laboratory testing: Thyroid-stimulating hormone (TSH), Folic acid, Vitamin B12 and TPHA/VDRL; neurological examination: Lumbar puncture (LP), Magnetic resonance imaging (MRI) and Neuropsychological (NP) testing.



10% Chosen for no action
16% Patient didn't want action
11% Reassessed after 6-12 months
4% Switch medication
13% Psychological treatment
7% Referral neurological examination -NP testing (n=6) -LP (n=10) -MRI (n=5)
4% Cognitive revalidation
35% No data of follow-up

Total=144

**FIGURE 3.  Actions taken after NCI screening**

### Outcomes after cognitive rehabilitation

A total of 132 tested were subjected to the USER-P questionnaire where 31 (23%) addressed substantial problems with participation in every day functioning. Six patients from these 31 were referred for cognitive rehabilitation, and results were available

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for 5 of them. Average scores on pre-defined domains were calculated per patient. All patients improved in the 12-week period with an average improvement of 3.2 points on a 10-point scale (p<0.01) (figure 4). Patients could better handle the symptoms of their cognitive impairment and were more satisfied in everyday functioning.
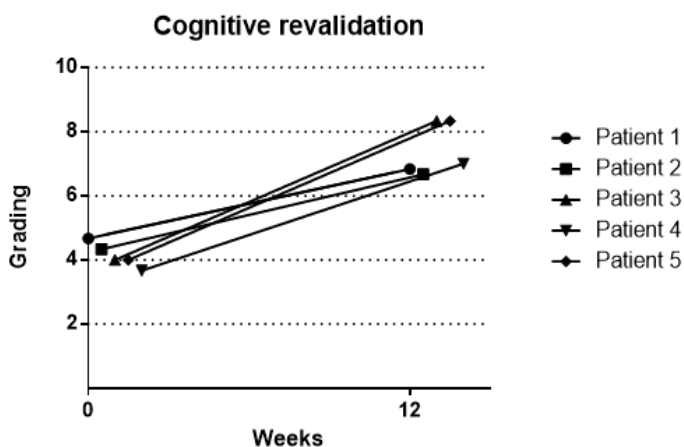


**FIGURE 4. Improvement after cognitive rehabilitation**

## DISCUSSION

This study demonstrated that signs of neurocognitive impairment were present in 50% of our patients, probably in part caused by depression. Despite using a concise flowchart for detection of underlying causes of NCI, it was only completely followed in a small group (10%) of patients. However, when further investigations were performed, clinically relevant outcomes were found in 59% of patients. Moreover, in 21% of the patients, further action was taken to optimize patient outcomes. Finally, the cognitive rehabilitation program was highly successful in all 5 evaluable referred patients.

It is well known in literature that mood disorders can cause problems with cognition.[17–19] Indeed, 40% of the patients who scored abnormal on the NCI screening reported mood disorders, and 33% of them was subsequently referred for psychological treatment of their depression- or anxiety-complaints. Therefore, it is an important confounding factor that should be taken into account when screening HIV-positive patients for NCI, or when they are reporting cognitive problems. This means that healthcare professionals should actively ask for mood complaints in this population. A contra-intuitive finding was that the use of Efavirenz was higher in the group who scored normal on the screening tests. This could be due to selection bias occurring when physicians only select patients eligible for Efavirenz in whom no suspicion of mood disorders is raised.

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In addition, increasingly more data are starting to show the negative effects of Efavirenz on the brain and on neurocognition.[20] For instance, stopping Efavirenz resulted in an increase of 0.96 on a total NPA Z-score after 96 weeks and in vitro studies have shown Efavirenz has neurotoxic effects.[21,22] Furthermore, Efavirenz also had an adverse effect on mood, with patients reporting depressive symptoms linking them to Efavirenz use.[23–25] With this in mind, it is remarkable that in this study, only 18% of patients on Efavirenz with cognitive complaints detected trough screening were switched to another cART regime. It is not clear whether this was because of the patients' reluctance to switch, or because the patients' physician did not offer the switch. It is important to be wary for cognition-impairing antiretrovirals, especially since recent studies have lined the new integrase-inhibitors, which are currently the first-line treatment option recommended in international guidelines, to occurrence of neurocognitive complaints.[7,26–29] Based on the findings in this study, a medication switch should be considered in every patient with NCI who is currently on a regime containing a cognition-impairing antiretroviral. After all, screening is only useful in circumstances where abnormal result can be dealt with by appropriate interventions.

Moreover, screening every HIV-patient with cognitive complaints for other, non-HIV-relatable causes for cognitive impairment, such as hypothyroidism or neurosyphilis, seems important. In this study 86 patients (59%) had a possible underlying cause for NCI such as mood disorders (58 patients), cognition impairing cART (23 patients), or laboratory abnormalities such as positive syfillis tests (2 patients) or thyroïd abnormalities (3 patients). Even though it is not clear whether these outcomes have contributed to the neurocognitive decline, it seems reasonable to correct abnormalities before referring patients for a full neuropsychological assessment or neurocognitive revalidation.

Therefore, implementing a protocol for actions to be taken in those patients with positive scores after NCI-screening is important. When a protocol is standardized, it is easier to implement in routine clinical practice and it gives physicians practical guidance to deal with this specific patient population. Poor adherence to the aforementioned protocol has led to a more concise and manageable new protocol containing a three-step plan. Step one involves optimizing cART and diagnosing and treating possible mood disorders. Step two involves excluding somatic factors for cognitive impairment, and step three is a referral to cognitive rehabilitation. The latter is important since it should only be offered to patients with cognitive complaints, who also experience a negative effect of their cognitive complaints on their participation in daily activities. In this study, such rehabilitation was shown to be very beneficial.

Several discrepancies were found between the subjective complaints experienced by patients in everyday life, and the objective results from screening tests. There were

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patients experiencing problems who did not score abnormal on the screening tests, and vice versa. This could be due to the fact that these pen and paper screening tests lack sensitivity as they do not capture complex daily life situations. However, it has also been stated before that patient-provided information or self-reports can be poorly associated with objective results.[30,31] This is also evident from the large portion of patients diagnosed with the asymptomatic form of HAND in HIV-positive cohorts.[2] Not only does this complicate diagnosing HAND, it also restricts options for treatment. A solid and reliable method for assessing subjective complaints is advised in diagnosing as well as treating HAND.

There are several limitations to this study. First, not all patients seen at the outpatient department were screened with the NCI screening protocol. This could have imposed inclusion bias. As main reasons for not screening were language barriers, refusal of patients, of reluctance of care givers, we believe that the screened patients were a rather good representation of the full HIV-infected patient population at our hospital. However, we cannot rule out the possibility that the percentage of patients with signs of NCI in the total population is lower or somewhat higher still. Furthermore, this study has a retrospective design, with its known limitations. Subsequently, there is no information on the reason why some patients went through the entire flowchart, and some only completed a few steps. Whether this was due to a decision of the patient or the physician makes a profound difference. Other reasons behind not completing the flowchart, like comorbidities or adherence problems, could mean the most impaired patients were not further analysed. This can result in an underestimation of the problem. Finally, there is no information on the selection of the six patients undergoing cognitive rehabilitation, which also could have imposed selection bias. On the other hand, the strength of this study is that it shows how physicians handle this complex problem in daily practice. It illustrates that the guidelines concerning HAND screening are interpreted differently and are not always easy to comply.

In conclusion, NCI is a frequently occurring problem in HIV-infected patients, for which screening is advised but limited solutions are available. Although the comprehensive NCI protocol described in this study was only moderately adhered to, it did result in detectable underlying causes of NCI in 59% of patients, and referral for further treatment in 21% of patients. Implementation of a concise screening flow chart is therefore recommended in the out-patient departments treating HIV-infected patients.

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Cognitive impairment in clinical practice: the evaluation of a stepwise
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# CHAPTER **6**

The MoCA basic is not
a reliable screening tool for cognitive decline in HIV
patients in sub-Saharan settings

## ABSTRACT

**Background**

HIV Associated Neurocognitive Disorders (HAND) is a frequently occurring comorbidity in HIV-positive patients, diagnosed by means of a neuropsychological Assessment (NPA). Due to the magnitude of the HIV-positive population in sub-Saharan Africa, easy-to-use cognitive screening tools are essential.

**Methods**

This was a cross-sectional clinical trial involving 44 HIV-positive patients (on stable cART) and 73 HIV-negative controls completing an NPA, the International HIV Dementia Scale (IHDS), and a culturally appropriate cognitive screening tool; the Montreal Cognitive Assessment-Basic (MoCa-B). HAND was diagnosed by calculating Z-scores using internationally published normative data on NPA, as well as by using data from the HIV-negative group to validate the MoCA-B.

**Results**

117 patients (25% male, median age 35 years, median years of education 11) were included. A moderate correlation was found between the MoCA-B and NPA total Z-score (Pearson's r: 0.36, p=0.02). Area under the curve (AUC) values for MoCa-B and IHDS were 0.59 and 0.70 respectively. The prevalence of HAND in HIV-positive patients was 66% when calculating Z-scores using published normative data versus 48% when using the data from our own HIV- cohort.

**Conclusion**

The MoCA-B appeared not to be a valid HAND screening tool in this setting. The prevalence of HAND in this setting is high, but appeared overestimated when using published norms.

# INTRODUCTION

One of the most frequent comorbidities of HIV-infection is neurocognitive impairment in the form of HIV Associated Neurocognitive Disorder (HAND).[1–4] Timely recognition of HAND is important, as HAND can lead to loss of quality of life and everyday functioning, and virological failure due to diminished compliance with combination antiretroviral therapy (cART).[5,6] A recent study showed a prevalence of 50% of any form of HAND in cART-treated HIV-patients in the United States.[3] However, depending on the used definitions and nomenclatures, HAND prevalence in the Western world differs widely, ranging from 17% to 70%.[7,8] This variability is even larger - between 17% and 88%- and less studied in sub-Saharan Africa, were the majority of people living with HIV resides.[9–12] By definition, HAND is diagnosed by applying diagnostic criteria – e.g. Frascati's or Gisslèn's - to a neuropsychological assessment (NPA) examining at least five cognitive domains.[7] Since this is time-consuming and requires specifically trained personnel, easy-to-use screening tests have been developed to detect neurocognitive decline. These tests are especially needed for the mild forms of HAND, because severe forms are easily recognized in clinical practice. To date, the only cognitive screening test available intended for international settings is the International HIV Dementia Scale (IHDS).[13] The IHDS, although widely used and claimed to be language- and culturally neutral, has shown poor test statistics, especially in screening for milder, and far more prevalent forms of HAND.[12–15] This might be explained by the fact that the IHDS is not based on nor intended for testing and interpreting in terms of cognitive domains.

An alternative for the IHDS is the Montreal Cognitive Assessment (MoCA) that has shown to adequately measure cognitive functioning with respect to milder forms of HAND in HIV-positive individuals in populations of developed countries.[16] A more language- and culturally neutral version for administration in resource-limited settings was recently developed: the MoCA basic (MoCA-B).[17] Thus far, the MoCA-B has been compared with an NPA in two clinical trials in Asia, where it appeared to have outstanding validity.[18,19] However, no studies have been conducted in sub-Saharan countries. We therefore conducted a pilot study analyzing the feasibility and validity of the MoCA-B compared to an NPA and the IHDS in diagnosing HAND in stable, cART-treated HIV-patients in sub-Saharan Africa.

# METHODS

This study took place at the research facility in Elandsdoorn, a rural township in Limpopo, South Africa, from December 2015 till March 2016. The facility is part of the Ndlovu Medical Care Group, a non-governmental organization that provides, among other

things, free HIV/AIDS-programs.[20] This study was reviewed and approved by the local ethics committee of the University of Pretoria, South Africa.

### Participants

Patients were recruited from the Ndlovu cohort study, the methods of which are published elsewhere.[21] For this sub-study, a random sample of HIV-positive and HIV-negative study participants who met the eligibility criteria were contacted by telephone and asked to participate. HIV-positive patients were included when they had a CD4-count of at least $100/mm^3$, a viral load of <50 copies/ml (RNA-PCR assay) and were on stable cART for at least 6 months. HIV-negative participants needed to have been tested negative for HIV maximum 6 months prior to inclusion. All patients needed to be aged 18 years or older, and be able to provide written informed consent.

Exclusion criteria for both groups were the following: a previously diagnosed and documented neurological disease or neurological opportunistic infection, a documented depression according to the DSM-IV criteria, or use of anti-depressants. As a result of our recruitment process, our population consisted of patients who already proved compliant to attending study visits at least once, and who mostly lived nearby the research facility.

### Materials

The MoCA-B is a pen-and-paper cognitive screening tool examining multiple cognitive domains (e.g. executive functioning, memory, fluency, and attention). The sub-tests were chosen specifically to optimize testing in individuals with a limited level of education. For instance, it does not contain literacy-dependent tasks, and complex problem-solving tasks are designed to describe scenarios that pertain to everyday life.[19] A subject can earn a maximum of 30 points from 10 subtests. A cut-off score of below 25/30 was used. The concise NPA consisted of the WHO UCLA Auditory Verbal Learning Task (WHO UCLA AVLT) learning and recall[22], the Timed Gait Test[23], the Grooved Pegboard Test[24], the Symbol Digit Modalities Test (SDMT)[24], The Color Trail Test (CTT)[25], and the Digit Span test (see table 1).[24] Although the original forms were used, the patient's own language was used for instructions and, if applicable, content of the neuropsychological tests. For instance, the words used in the fluency tasks were given in the participants' native language. A standardized administration manual in English was translated (forward and backward) into isiZulu and Northern Soto by a certified translation company. At the start of the assessment, the participant chose his or her most fluent language as the preferred administration language: English, isiZulu or Northern Soto.

### Data collection

Detailed information on sex, age, paid work, mental health, smoking and alcohol habits, cART, concomitant medication, and laboratory values (CD4, viral load) were collected

from the Ndlovu cohort study. For the cognition sub-study we added the IHDS, the MoCA-B, and a concise NPA. All tests and questionnaires were administered by a trained local counselor using standard procedures described in the accompanying manuals and/ or from published instructions.[13,23,24] The MoCA-B was conducted using the standard instructions on the MoCA website (www.mocatest.org).

**TABLE 1.** Tested NPA subtests and explanation

| Test | Domain | Explanation: patient is asked to... |
| --- | --- | --- |
| **Timed gait** | (gross) motor function | Walk a predetermined distance as fast as possible |
| **Grooved Pegboard** | (fine) motor function | Place pegs in a pegboard as fast as possible |
| **Digit symbol modalities** | Information processing | Match digits with their corresponding symbols as fast as possible |
| **Color trails 1** | Information processing | Connect numbers in ascending order as fast as possible |
| **Color trails 2** | Information processing/ Executive functioning | Connect numbers in ascending order when alternating between two colors. |
| **Color trails ratio** | Color trails 2/color trails 1 | Ratio between the two color trails |
| **Digit span – forwards** | Attention | Repeat an increasing span of numbers forwards |
| **Digit span - backwards** | Executive functioning | Repeat an increasing span of numbers backwards |
| **WHO UCLA AVLT** | Verbal Memory | Remember a list of 15 words after 5 repetitions. A1-A5 is the sum of all words remembered after 5 repetitions; A7 is the amount of words remembered after 30 minutes. |

ALVT=Auditory Verbal Learning Test, UCLA= University of California Los Angeles, WHO = World Health Organization

### Statistical analysis

Shapiro-Wilk tests were used to test for normality. Mann-Whitney U tests or Chi Square tests were performed to analyze group differences on demographics. A Pearson's correlation coefficient was used to assess correlation.

Raw NPA test scores were transformed into age/education-adjusted Z-scores using: 1) published normative data from comparable sub-Saharan populations[23,26,27], and 2) data from our HIV-negative population. Z-scores were calculated by subtracting the mean of either norm group from an individual's raw score, and dividing the result by the standard deviation of the normative data. Because of a significant difference in age between the HIV-positive and the HIV-negative subjects, we adjusted the mean and standard deviation of the HIV-negative group using a regression coefficient for age. These Z-scores were summed and averaged to create a composite Z-score. We multiplied the timed gait, CTT, and grooved pegboard score with -1 because for these tests a higher score means a worse performance.

To diagnose HAND, Frascati and Gisslèn criteria were used.[7,28] According to the Frascati criteria, a person has severe HAND (HIV Dementia) when he or she scores at least two

SD below the mean on at least one subtest in at least two domains. The qualification for mild HAND is met when at least one SD below the mean is scored on at least one test in at least two domains. Information on interference with daily functioning is needed to differentiate between asymptomatic neurocognitive impairment and mild neurocognitive disorder. For the Gisslèn criteria, an average domain score of 1.5 SD below the mean on at least two domains is used to define mild HAND, and two SD below the mean for severe HAND.

As NPA is the gold standard for diagnosing HAND, the feasibility and validity of the MoCA-B was measured by performing a ROC-analysis and calculating Area Under the Curve (AUC) values. In this ROC analysis, the test variable, i.e. the MoCA-B with a cut-off score of below 25, is compared to a state variable, i.e. HAND or no HAND.

Furthermore, we compared the total and domain-scores to the results of the NPA. The MoCA-B evaluates three cognitive domains that were also evaluated in the NPA, namely executive functioning, attention, and memory. All statistical tests and procedures were conducted using IBM SPSS statistics (version 21, New York, USA).

## RESULTS

**Participants**

At the start of this study, 1173 participants (ratio of HIV-positive to HIV-negative 1:2) had been enrolled in the Ndlovu Cohort study since November 2014. 117 participants (10%) were included in this sub-study of whom 44 were HIV-positive and 73 HIV-negative. All participants had visited the research facility for the Ndlovu cohort study at least once already, and 10 in the HIV-positive group (23%) and 20 in the HIV-negative group (26%) had visited the site twice before. The main reason for eligible participants not to participate was logistical; they lived too far away from the research site.

Participants were predominantly female (76%) and there was a high rate of unemployment (84%) (table 2). HIV-positive patients were older than HIV-negative controls (40.5 years versus 32.4 years; p<0.05) with no difference in years of education, which averaged 11 years. There was a significant difference in native language with more IsiZulu speakers in the HIV-positive group and more Sepedi speakers in the HIV-negative group (p=0.04). HIV-positive participants had a mean CD4 count of 530/mm$^3$.

**TABLE 2.** Baseline patient characteristics

|  | total | HIV+ | HIV- | p-value |
|---|---|---|---|---|
| N | 117 | 44 | 73 |  |
| Male (%) | 24.6 | 18.6 | 28.2 | .25 |
| Age, median years (IQR) | 35(15) | 41(13) | 29(15) | <0.01 |
| Education, median years (IQR) | 11(3) | 11(3) | 11(3) | 0.95 |
| Illiteracy (%) | 4.4 | 7.0 | 2.8 | 0.35 |
| % Unemployment | 83.8 | 88.6 | 80.8 | 0.43 |
| % Smoking | 19 | 20 | 18 | 0.91 |
| % Alcohol | 32 | 25 | 37 | 0.26 |
| Language – % Isizulu/Sepedi | 50.4/44.4 | 63.6/29.5 | 42.5/53.4 | 0.04 |
| CD4 /mm³ (SD) |  | 530 (232.1) |  | NA |
| % Suppressed viral load |  | 100 |  | NA |
| Months since diagnosis |  | 85.12(55.9) |  | NA |
| Months on cART |  | 70.33(53.9) |  | NA |
| % on EFV |  | 93 |  |  |
| Depressive symptoms (PHQ-9>10) |  | 11% | 6% | 0.29 |

cART: combination AntiRetroviral Therapy, EFV: Efavirenz, PHQ-9: Patient Health Questionnaire, SD: standard deviation

## Feasibility of the MoCA-B

Administration of the MoCA-B was feasible in all participants with a mean administration time of 13.6 (SD 3.3) minutes. The mean score on the MoCA-B was 22.1/30 for the HIV-positive group and 24.2/30 for the HIV-negative group (p<0.05). We compared the MoCA-B total score with NPA composite Z-scores. Based on published normative data for the NPA, a moderate correlation (Pearson's r: 0.36; p=0.02) was found. This is comparable to the correlation between the IHDS and NPA total Z-score (Pearson's r: 0.44; p<0.01). Moreover, when evaluating the three specific cognitive domains, there was only a moderate correlation for the memory subtests (r=0.44, p=0.03) and no significant correlation between attention (r=-0.09, p=0.58) or executive functioning (r=0.25, p=0.10) subtests of the MoCA-B and the NPA. The ROC curves for the MoCA-B and the IHDS screening for HAND diagnosed with Frascati criteria are depicted in figure 1. The Area Under the Curve (AUC) was 0.59 for the MoCA-B and 0.70 for the IHDS. When norms from our HIV-negative group were used for the NPA composite Z-scores, these results also showed only a moderate correlation with the MoCA-B scores (Pearson's r: 0.58; p<0.01). Test characteristics of the MoCA-B divided by different forms of HAND (mild/severe) and results of the NPA subtests are provided as supplemental data.

## HAND prevalence according to used diagnostic criteria

Using the Frascati criteria, the overall prevalence of HAND was 66%, and 54% for the mild forms of HAND based on the published norms. However, when using the HIV-negative group as reference, overall HAND prevalence declined to 48% and 43% respectively for HAND and the mild forms of HAND. A comparable pattern was seen for the Gisslèn criteria, namely 14% versus 9% for published norms and 7% versus 5% for comparable HIV- norms.
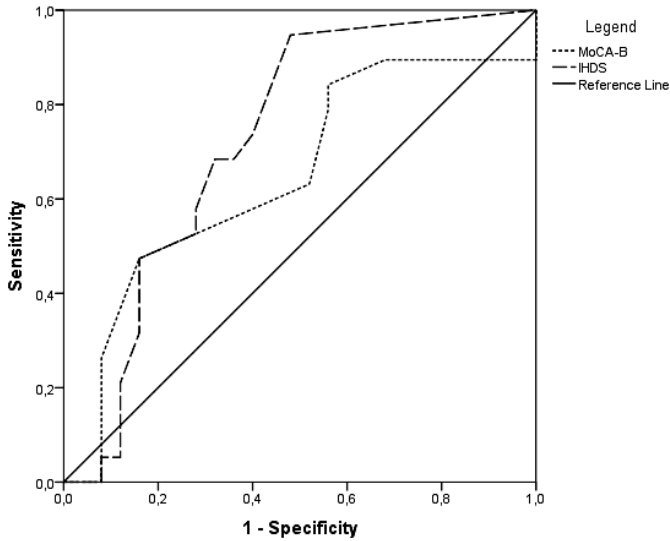
The MoCA basic is not a reliable screening tool for cognitive decline in HIV patients in sub-Saharan settings

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**FIGURE 1.** ROC-curve MoCA-B and IHDS for diagnosing mild forms of HAND

**TABLE 3.** Higher NPA scores in HIV+ patients compared to HIV- patients

| test | Raw score HIV- | Raw score HIV+ | p-value | Mean Z-score HIV+ *published norms* | Mean Z-score HIV+ *own norms* |
|---|---|---|---|---|---|
| **Timed gait (seconds)** | 11.5 | 11.3 | 0.529 | -1.08 | 0.22 |
| **Digit span forwards (# digits)** | 7.6 | 7.2 | 0.304 | -0.54 | -0.23 |
| **Digit span backwards (# digits)** | 3.9 | 4.1 | 0.633 | -0.22 | 0.19 |
| **Digit symbol modalities (#correct)** | 36.0 | 30.6 | 0.008 | -0.32 | -0.29 |
| **AVLT A1-5 (# words)** | 51.0 | 50.1 | 0.576 | 1.18 | -0.19 |
| **AVLT A7 (# words)** | 10.8 | 10.2 | 0.291 | 0.23 | -0.22 |
| **Grooved pegboard (seconds)** | 73.4 | 76.8 | 0.347 | 0.35 | -0.14 |
| **Color Trail test 1 (seconds)** | 51.9 | 54.5 | 0.459 | -0.94 | -0.23 |
| **Color trail test 2 (seconds)** | 112.1 | 126.6 | 0.036 | -0.90 | -0.49 |
| **CTTratio** | 2.3 | 2.4 | 0.50 | n.a. | -0.12 |
| **Total** | | | | -0.17 | -0.14 |

AVLT; Auditory Verbal Learning Test. A1-5: number of words cumulatively remembered after 5 trials, A7: number of words remembered after delay of 30 minutes. CTT: Color Trail Test, DSM: Digit symbol modalities

**TABLE 4.** Diagnosis of HAND according to the Frascati and the Gisslèn norms, using published as well as own normative data

| HAND diagnosis | Prevalence Mild | Prevalence Severe | Prevalence Either | PPV (95%CI) NPV (95%CI) mild | PPV (95%CI) NPV (95%CI) severe | PPV (95%CI) NPV (95%CI) either |
|---|---|---|---|---|---|---|
| Frascati published norms | 54% | 11% | 66% | 0.50 (0.23-0.77) 0.57 (0.37-0.75) | 0.93 (0.66-1.00) 0.13 (0.04-0.31) | 0.43 (0.18-0.71) 0.70 (0.51-0.85) |
| Frascati own norms | 43% | 5% | 48% | 0.79 (0.49-0.95) 0.53 (0.34-0.72) | 1.00 (0.68-1.00) 0.07 (0.01-0.22) | 0.79 (0.49-0.95) 0.60 (0.41-0.77) |
| Gisslèn published norms | 9% | 5% | 14% | | | |
| Gisslèn own norms | 5% | 2% | 7% | | | |

# DISCUSSION

We investigated the validity of cognitive screening tools to detect HAND and mild forms of HAND in a sub-Saharan African population. The MoCA-B appeared not to be a valid screening tool for HAND in this cohort in South-Africa. Additionally, the prevalence of HAND in this setting was, as expected, high, but could have been overestimated using currently available published norms.

Because of the difficulties associated with conducting a full NPA, valid and easy-to-use cognitive screening tests are much needed in resource-limited settings. However, insufficiently tested screening tools with poor validity should be avoided to prevent over- or under-diagnosing of the problem. The supply of language- and culturally appropriate cognitive screening tests is limited; only the IHDS is currently available. This study evaluated the validity of the MoCA-B. The finding that the MoCA-B has a poor validity is not consistent with what has previously been published about this test.[18,19] However, as mentioned before, the only two studies previously performed with the MoCA-B were carried out in Asia and not with HIV-patients.

The MoCA-B demonstrated the best test characteristics when screening for severe forms of HAND. However, easy and short diagnostics are more urgently needed for the mild forms of HAND which are not obviously detected in regular clinical consultation. The correlation between MoCA-B and the NPA total Z-score was only moderate. This might be explained by the fact that the MoCA-B does not include speed of information processing, which was found to be the most affected domain on the NPA in this study population. Therefore, a future screening tool incorporating speed of processing might be more appropriate.

Furthermore, we examined both HIV-positive and HIV-negative participants from the same cohort in order to have a highly comparable control group. The prevalence of HAND was at least 25% lower when using these normative data, as opposed to previously published normative data obtained from other sub-Saharan countries. This stresses the importance of collecting suitable normative data for different cultural groups in sub-Saharan settings because overestimation otherwise occurs. Therefore, caution is required when interpreting results obtained with ill-fitting normative data.

This study had a few limitations. Due to the nature of this pilot study, the sample size was relatively limited. The inclusion of mostly unemployed patients may have caused bias on the reported prevalence of HAND. On the other hand, this probably represents the subgroup of patients most affected by HAND in everyday life, and the target population for investigating diagnostic options. Moreover, as a result of our recruitment process, our sample is comprised of patients that had already shown compliance to study visits. This may have caused our study population to represent a more compliant, better-functioning group. However, if affected the HAND incidence rates, they would most likely be lower than the actual rates, meaning the incidence of HAND in the overall population would be even higher.

In conclusion, by using an appropriate NPA, and more importantly, fitting normative data in the form of HIV-negative individuals in the same socio-economic region, this study clearly showed that the easy-to-use MoCA-B was not reliable in screening for HAND. In addition, using a control group of HIV-negative individuals is of great importance when studying HAND in resource-limited settings.

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28    Gisslén M, Price RW, Nilsson S. The definition of HIV-associated neurocognitive disorders: are we overestimating the real prevalence? *BMC Infect Dis* 2011; **11**: 356.

The MoCA basic is not a reliable screening tool for cognitive decline in HIV patients in sub-Saharan settings

6

# PART 3

## value of functional MRI in diagnosing HAND

# CHAPTER **7**

Review of functional MRI in HIV: effects of aging and
medication

# ABSTRACT

**Background**

HIV Associated Neurocognitive Disorder (HAND) is a frequently occurring comorbidity of HIV-infection. Evidence suggests this condition starts subclinical before progression to a symptomatic stage. Blood Oxygenated Level Dependent (BOLD) fMRI has shown to be a sensitive tool to detect abnormal brain function in an early stage, and might therefore be useful to evaluate the effect of HIV-infection on brain function. An extensive literature search was performed in June 2015. Eligibility criteria for included studies were as follows; 1) conducted with HIV-positive patients, 2) using BOLD fMRI, 3) including a HIV-negative control group. A total of 19 studies were included in the review including 931 participants. Differences in activation between HIV-positive and –negative participants were found when testing multiple domains, ie attention, (working) memory, and especially executive functioning. Overall, HIV-positive patients showed hyperactivation in task-related brain regions despite equal performances as controls. Task performance was degraded only for the most complex tasks. A few studies investigated the effect of aging on fMRI, and most of them found no interaction with HIV-infection. Only three studies evaluated the effect of cART on functional data suggesting an increase in activation with the use of cART. fMRI is a sensitive instrument to detect subtle cognitive changes in HIV-patients. Open questions remain regarding the effects of cART on fMRI and the effects of aging on fMRI.

# INTRODUCTION

In the recent era of combination antiretroviral therapy (cART), infection with the human immunodeficiency virus (HIV) has changed from a rapidly fatal disease into a chronic condition with subsequent comorbidities.[1,2] One of the most important comorbidities in HIV infected patients is cognitive decline, resulting in HIV-associated Neurocognitive Disorders (HAND). It is estimated that around 50% of all HIV-infected patients has a form of HAND.[3] Moreover, in this aging population cognitive disorders are the most worrying aspect of the disease for the patients themselves. The advances in cART, over the past decades, have led to a shift in prevalence from the most severe form of HAND, HIV-associated dementia (HAD), towards milder forms of neurocognitive disorders like Asymptomatic Neurocognitive Impairment (ANI) and Mild Neurocognitive Disorder (MND).[3–6] The large proportion of HIV-infected patients suffering from ANI poses particular challenges for diagnosis, because by definition these patients do not experience nor report symptoms. Diagnosing ANI and other forms of HAND is important, as a recent study showed that patients with ANI have a two- to six fold increased risk of developing symptomatic cognitive problems as opposed to neurocognitive normal patients.[7] However, there is some debate on the diagnosis ANI, and whether the neurocognitive decline is not due to other comorbidities.[8] Sensitive screening instruments would therefore be a welcome addition to the diagnostic armamentarium.

Neuropsychological (NP) testing is the primary method for diagnosing HAND. However, this is time-consuming and may not be sensitive enough to detect subtle neurocognitive changes, which may underlie the milder forms of HAND such as ANI.[9] Several studies have shown that Blood Oxygenated Level Dependent (BOLD) functional Magnetic Resonance Imaging (fMRI) is more sensitive in detecting abnormal brain function compared to NP testing.[10–13] From 2001 onwards, there have been several studies evaluating the role of fMRI in the detection of neuronal dysfunction in HIV-infected patients; first focusing on attention and motor functions while later studies investigated executive functions and fronto-striatal networks.[14–17] In order to determine whether fMRI can be used as a diagnostic tool aiding in HAND diagnosis, it is important to summarize these studies and evaluate their usefulness in terms of applicability, risk of bias, and scientific limitations. A meta-analysis and concise systematic review was published in 2014, mostly focusing on the fronto-striatal system, and including different forms of fMRI than BOLD fMRI, the most frequently used form of fMRI.[18] To date, however, no extensive systematic review on solely BOLD fMRI, investigating all brain networks, and using only studies with a HIV-negative control group has been published. This can be explained by the fact that BOLD fMRI is a relatively new research tool and, as mentioned before, HIV-infection only recently became a chronic infection. In order to properly appraise the utility of fMRI in chronic HIV-infection, it is important to extensively outline the available data on this

subject. This can serve as a solid fundament for future research on this promising novelty in the field. Therefore, the objective of this review is to systematically analyze studies investigating BOLD fMRI in HIV-positive and –negative subjects in terms of differences in activation patterns, in order to evaluate the effect of HIV-infection on brain function, and the impact of age and medication.

## METHODS

### Search and selection

This systematic review was conducted according to the Preferred Reporting Items for Systemic review and Meta-Analysis (PRISMA) framework. The protocol for this study is included in the international prospective register of systematic reviews PROSPERO under registration number CRD42015015698. Eligibility criteria for included studies were as follows: one) conducted with HIV-positive patients, two) using BOLD fMRI, three) including a HIV negative control group.

A literature search was performed in June 2015 using three online databases: Embase, Pubmed, and the Cochrane database. The search terms are presented in supplementary document 1. Mesh terms were used if available.  All time frames were included because of the novelty of fMRI. We included only original research papers in English or in Dutch.

### Study selection

The first screening of papers for eligibility was done by one author (CH). Duplicates were identified and removed. A total of 538 papers were identified. Full text evaluation of the remaining studies for eligibility was performed independently by two authors (CH and JEA). In addition, references of the identified studies were cross-checked for any additional relevant studies. The process for selecting studies is summarized in figure 1. One reference from cross-checking studies was excluded because it was a conference report not published in a core medical journal.[19]

### Data extraction and validity

Data extraction was performed by two independent authors (CH and JEA) using a standardized data extraction form. Inconsistencies between study forms were discussed and when appropriate reviewed by a third author (MV) for majority decision. Where doubts remained, authors of the original paper in question were contacted. Variables included in the form were study setting, number of patients, patient characteristics including HIV specific variables, cART use, co-medication, substance abuse, and cognitive status, fMRI task used, form of analysis of fMRI data, and behavioral and fMRI results. Results were expressed as statistical significant differences in activation measured by

BOLD signal between HIV-positive and negative individuals. The statistical inferences used on fMRI data were summarized or simplified; if a multiple comparison correction was used, either by family wise error or false discovery rate, this was reported, together with the level of correction (voxel or cluster-level), and the *p* value used. A risk of bias assessment was performed for each individual study using a standardized risk of bias assessment form (QUADAS-2). In this assessment, we focused on the risk of bias in inclusion and possible confounders and not specifically on risks involved in the statistical inference of fMRI data since the latter information is presented in the results tables.
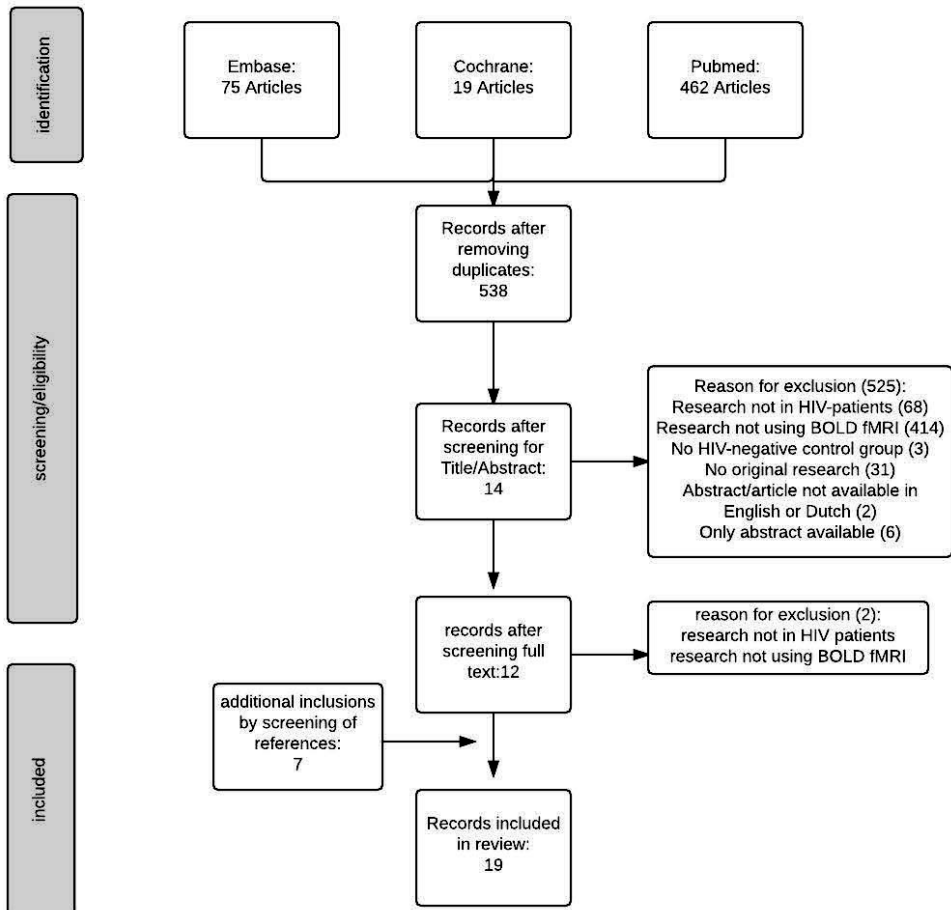


**FIGURE 1. process of study selection**
BOLD: blood oxygenated level dependent, fMRI: functional MRI

### Analysis

Medians and standard deviations for baseline characteristics were calculated when needed and when data was available. The results were grouped per form of analysis (whole brain or regions of interest), and furthermore by pairing studies that investigated the effect of HIV-infection on the characteristics of the BOLD signal, and those who specifically studied the interaction of HIV and aging.

### Role of the funding source

There was no role of the funding source in study design, in collection, analysis and interpretation of data, in the writing of the report or in the decision to submit the paper for publication.

## RESULTS

A total of 538 studies were identified after searching Embase, Pubmed, and Cochrane databases, of which 12 were eligible for inclusion after screening title and abstract. Reasons for exclusion and further process of study selection are depicted in figure 1. Finally, after cross-checking references of the included studies, another seven publications were included leading to 19 manuscripts in the final selection.

### Study Characteristics

A summary of study characteristics is given in table 1. All studies took place in the USA[14–16,20–34], except for one, which was situated in South-Africa.[17] With only two (12%) longitudinal studies[22,23], the majority (78%) was cross-sectional in design. In total, 19 studies included a total of 573 HIV-positive and 408 HIV-negative patients. Most of the patients were male, with six studies having solely male participants.[14,15,24,25,27,31] The mean age of all participants was 41.4 years (95% CI 41.06-41.64). The majority of HIV-patients were on cART with only the South-African study having no patients on cART.[17] Two studies did not report cART use, and average cART use was 56% (95% CI 53-58) in the other studies. Four studies specified the type of cART used and/or gave information on its CNS penetration effectiveness score.[14,15,26,27] Seven studies included patients with cognitive deficits ranging from mild impairment to HAD, either according to the former criteria or the new Frascati criteria.[15,21,24–26,28,30]

### Critical appraisal

All studies were appraised for risk of bias on four items (patient selection, index test, reference standard, and flow and timing), and for applicability on three items (patient selection, index test and reference standard) (supplementary document 2). There were four studies that did not use a reference standard (NeuroPsychological Assessment

(NPA)), and therefore could not be completely assessed.[15,16,28,31,32] The lack of a reference standard would normally be an issue; however it seems surmountable in this setting where the index test, i.e. fMRI, might be more sensitive than the reference standard. There were only four studies (24%) that scored inadequate on more than one item, indicating that the risk of bias on the aforementioned items seems low across studies.[16,20,25,27]

### Impact of HIV on BOLD characteristics

First, studies investigating the influence of HIV infection on the shape or the response of the BOLD signal were analyzed, since HIV replication in the brain has been shown to cause alterations in brain metabolism which might influence signal intensity.[35] The BOLD signal depends on the hemodynamic response of the brain, which causes a greater delivery of oxygen-rich blood to active neurons as opposed to inactive neurons. Four studies investigated the effect of HIV on the characteristics of the BOLD signal.[22,28,32,36] Two of them found no significant difference in mean peak values of the Haemodynamic Response Function (an indication of the shape and amplitude of the BOLD signal) when using a motor task in HIV-positive and HIV-negative subjects (table 2).[26,28] The third study, by Ances et al. (2010), using a visual task, found reduced functional changes in BOLD signal in the visual cortex in HIV-positive subjects as opposed to HIV-negative subjects.[32] Finally, using the same task, Ances et al. (2011) found in a subsequent study a statistically significant decrease in BOLD signal after one year in the HIV-positive group.[22] It must be noted, however, that the groups used for this latter analysis were rather small (six HIV-positive versus ten HIV-negative subjects). When studying fluctuations in greater detail, it is important to consider the effect of different activation in different sub-groups[37], and that would require a larger study population. Taken together, the data from these four studies suggest no clear impact of HIV on the characteristics of the BOLD signal, indicating that differences in BOLD response between HIV-positive and –negative participants can be interpreted as a difference in brain activation, i.e. in the amount of neurons activated in a certain region.

### Whole brain and Regions Of Interest analysis

We analyzed 15 studies focusing on either whole-brain or Region Of Interest (ROI) analyses in HIV-positive and –negative subjects (tables 3 and 4).[14–17,20,21,23–25,27,29–31,33,34] While whole brain analyses are used to explore effects throughout the brain, ROI analyses focus on predefined regions, either anatomically or by a previous independent study, thereby reducing type I error. However, when regions are not previously specified, but rather defined based on whole-brain results from the same study, the chance of bias is drastically increased.[38] Neuropsychological studies have suggested that brain regions involved in attention, working memory, and episodic memory may be particularly affected in HIV-positive patients with HAND.[39–41] More recent neuroimaging studies center on fronto-striatal circuits.

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**TABLE 1.** Baseline characteristics

| Author | Correlated NPA | No | | Mean age (SD) | % male | Years of education (SD) | Impaired cognition | % substance abuse | Co-medica-tion | % on cART | Type of cART | Duration of cART | Mean current CD4 (IQR) | Mean nadir CD4 (IQR) | Duration infection | No sign. difference on; |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Caldwell 2013 | no | HIV+ | 34 | 46.1 (8.5) | 54 | 12.6(1.8) | NR | 0 | NR | 79 | NR | NR | 550 | 201 | 7.6 years | Age sex |
| | | HIV- | 28 | 44.9 (12.7) | 65 | 14.0(3.4) | NR | 0 | NR | | | | | | | |
| Thomas 2013 | yes | HIV+ | 52 | 41 (14) | 90 | 14(2) | 23% impairment | 25% | NR | 44 | NR | NR | 377 (291-616) | 260 (116-386) | NR | Age |
| | | HIV- | 52 | 44 (14) | 51 | 15(3) | NR | NA | NR | | | | | | | |
| Ances 2010 | yes | HIV+ | 6 | 30 (7) | 100 | 15(2) | GDS 0.34 | 16 | NR | 83 | NR | NR | 757 (424-900) | 588 (438-750) | NR | Age Sex Education |
| | | HIV- | 10 | 30 (6) | 60 | 18(3) | NR | 0 | NR | | | | | | | |
| Ernst 2009 | yes | HIV+ | 31 | 49.6 (8.4) | 97 | 15.5 (2.2) | NR | 0 | no neuro-impairing medication | 100 | NR | NR | 415 (40.4) | 152 (24) | NR | Age Sex Education Hematocrit |
| | | HIV- | 32 | 46.9 (13) | 88 | 15.5(2.3) | NR | 0 | No neuro-impairing medication | | | | | | | |
| Melrose 2008 | yes | HIV+ | 11 | 40.8 (7.1) | 100 | 16.3(1.5) | 9% mild impairment | 9% alcohol | 36.4% anti-depressant | 91 | NR | 9.9(5.4) years | 694.2 (197) | NR | 9.9 (5.4) years | Age Sex Education |
| | | HIV- | 11 | 40.9 (8.7) | 100 | 16.9(1.8) | 0 | 9% alcohol | 0 | | | | | | | |
| Chang 2013 | yes | HIV+ | 66 | 47.1 (8.6) | 100 | 14.6(2.3) | 43.90% HAND | 0 | NR | NR | NR | NR | 401.7 | 158,4 | 144.22 months | Age Sex Education Hematocrit |
| | | HIV- | 56 | 45.7 (12.7) | 100 | 14.8(2.2) | 0 | 0 | NR | | | | | | | |
| Ances 2008 | yes | HIV+ | 24 | 45.5 (6.9) | 71 | 14.5(3.5) | GDS 0.95 of 7 MND en 8 HAD | 0 | NR | 100 | *1 | 20mo | 368 | NR | >1 year | Age Sex Education |
| | | HIV- | 10 | 46 (12.6) | 60 | 14(3.2) | GDS 0.3 | 0 | NR | | | | | | | |

**TABLE 1.** Continued

| Author | Correlated NPA | No | | Mean age (SD) | % male | Years of education (SD) | Impaired cognition | % substance abuse | Co-medication | % on cART | Type of cART | Duration of cART | Mean current CD4 (IQR) | Mean nadir CD4 (IQR) | Duration infection | No sign. difference on; |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chang 2008 | yes | HIV+ | 24 | 40.15 (8.1) | 100 | 13.7(2.6) | GCD art+: 6.2(0.2) art-: 6.0(0.9) | 0 | no neuro-impairing medication | 50 | *2 | 134.8 (21.7) months | 467.5 | 218.5 | | Age Sex Education Hematocrit |
| | | HIV- | 18 | 39.82 (12.3) | 100 | 13.9(2.1) | GCD 2.7 (0.5) | 0 | No neuro-impairing medication | | | | | | 129.9 months | |
| Juengst 2007 | yes | HIV+ | 31 | 47.7 (15.7) | 90 | 14.5(2.7) | 35% MNCD or HAD | NR | NR | NR | NR | NR | 397.41 | NR | | none |
| | | HIV- | 16 | 42.3 (11.8) | 75 | 14.0(3.0) | NR | NR | NR | | | | | | NR | |
| Ernst 2002 | yes | HIV+ | 10 | 36.3 (7.9) | 100 | 14.8(2.0) | 0 | 20% smoking | NR | 90 | *3 | NR | 375 (187) | 241 (145) | | Age Sex Education |
| | | HIV- | 10 | 36.1 (6.8) | 100 | 15.6(2.6) | 0 | 20% smoking | NR | | | | | | NR | |
| Chang 2001 | no | HIV+ | 11 | 41 (4.8) | 100 | 14(2.1) | 36% MCMD 27% mild hiv-dementia | 0 | NR | 91 | *4 | NR | 329(197) | 170 (126) | | Age Sex Education |
| | | HIV- | 11 | 38 (4.8) | 100 | 16.4(3.3) | 0 | 0 | NR | | | | | | NR | |
| Maki 2009 | yes | HIV+ | 7 | 41.1 | 0 | 11.7 | NR | 29 | NR | 43 | NR | NR | NR | NR | | Age Sex Education |
| | | HIV- | 4 | 42.8 | 0 | 12.3 | NR | 50 | NR | | | | | | NR | |
| Chang 2004 | yes | HIV+ | 18 | 38.2 (7) | 78 | 14.2(1.6) | 55% MCMD 33% mild dementia | 0 | no chronic comedication | 83,3 | PI-regime | 27.7 weeks | 287(36) | 123 (37) | | Age Sex Education Hematocrit |
| | | HIV- | 18 | 38.0 (8.8) | 78 | 14.5(1.9) | 0 | 0 | No chronic comedication | | | | | | 91 (15) months | |
| Castelo 2006 | yes | HIV+ | 14 | 39 (9.1) | 100 | 14.8(2.0) | 0 | 0 | * 5 | 71 | NR | NR | 690(370) data from 10 out of 14 patients | NR | | Age Sex Education |
| | | HIV- | 14 | 40 (10.4) | 100 | 16(1.9) | 0 | 0 | * 5 | | | | | | NR | |

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**TABLE 1.** Continued

| Author | Correlated NPA | No | | No | Mean age (SD) | % male | Years of education (SD) | Impaired cognition | % substance abuse | Co-medica-tion | % on cART | Type of cART | Duration of cART | Mean current CD4 (IQR) | Mean nadir CD4 (IQR) | Duration infection | No sign. difference on; |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ances 2010 | no | HIV+ | 26 | | 39 | 77 | 16 | NR | 0 | NR | 60 | NR | at least 3 months | 486 | 278 | | Age Sex |
| | | HIV- | 25 | | 41 | 56 | 15 | NR | 0 | NR | | | | | | NR | Education |
| Schweinsburg 2012 | no | HIV+ | 11 | | 41.8 (6.1) | 82 | 13.9(2.4) | NR | 0 | NR | 91 | NR | NR | NR | 140(29-300) | | Age Sex |
| | | HIV- | 13 | | 42.5 (14.5) | 77 | 14.9(2.1) | NR | 0 | NR | | | | | | NR | Education |
| du Plessis 2015 | yes | HIV+ | 18 | | 32 (4.6) | 11 | 11(10-12)*[6] | GDS 0.21 | 0 | NR | 0 | NA | NA | 433 (199) | NR | | Age Sex |
| | | HIV- | 16 | | 28(5.2) | 6 | 12(11-12)*[6] | GDS 0.17 | 0 | NR | | | | | | NR | Education |
| Ipser et al 2015 | yes | HIV+ | 15 | | 40.6 (14.5) | 80 | 13.8(2.2) | GDS 0.32 (0.28) | Alcohol 13.3% | NR | 86 | NR | NR | 548 (255.29) | 310(193) | 80.4 mo | Age Sex Education |
| | | HIV- | 15 | | 39.7 (12.6) | 87 | 13.7(1.3) | GDS0.29 (0.26) | Alcohol 46.7% | NR | | | | | | | |
| Ortega et al 2015 | yes | HIV+ | 131 | | 39.0 | 72 | 13.1 | Z-score -0.39 | NR | NR | 63 | NR | NR | 551 | 281 | | Sex |
| | | HIV- | 45 | | 31.7 (10.9) | 58 | 13.4(2.7) | Z-score 0.14 | NR | NR | | | | | | NR | Education |

cART: combination AntiRetroviral Therapy, CD4: CD4-cell count, GCD: Global Cognitive Deficit, GDS: Global Deficit Score, HAD: HIV-Associated Dementia, HIV+: HIV-positive, HIV-: HIV-negative, IQR: InterQuartile Range, MCMD: Minor Cognitive Motor Disorder, NA: Not applicable, NPA: neuropsychological assessment, NR: Not Reported, SD: standard deviation

\* (lowCPE, highCPE) Protease Inhibitor 50%,42%. Nucleoside Reverse Transcriptase Inhibitor 66%,42%. Non-Nucleoside Reverse Transcriptase Inhibitor 33%,42%

° 8 tenofovir, 5 lamivudine, 3 zidovudine, 2 abacavir, 1 didanosine, 1 stavudine, 2 embtricitabine. 4 efavirenz, 2 nevirapine, 3 lopinavir/ritonavir, 2 ritonavir, 1 saquinavir, 1 atazanavir, 1 fosamprenavir, 1 nevirapine/lopinavir/ritonavir

Δ 3x d4t/lam/kaletra. 1x d4t/nelfinavir/nevirapine. 2x d4t/lam/nelfinavir. 1x d4t/rit/saquinavir. 1x azt/lam/capavirine. 1x azt/lam/indinavir

¤ 1x D4T/ddI. 1x D4T/lamivudine. 2x AZT/lamivudine/indinavir. 1x AZT/3TC/d4t. 1x D4T/nelfinavir/nevirapine. 1x D4T/ninavir/3TC. 1x ritonavir/saquinavir/D4T/3TC 3x ddI/ritonavir/indinavir/D4T/saquinavir

¥ 2 patients used medication that might affect cognition (effexor, celexa, ambien)

**TABLE 2.** BOLD-characteristics

| study | HIV/ SN | region | task | software | treshold | Correction M.C. | results |
|---|---|---|---|---|---|---|---|
| Ances (2010)[22] | 6/10 | visual cortex | Checker board | NR | p=0·05 | mask used | HIV + showed reduction in mean functional BOLD changes over time, and greater inter-subject variance in BOLD measures |
| Ances (2008)[26] | 24/10 | motor | Checker board +squeezing | voxbo | NA (amplitude BOLD signal) | NA | No significant difference in BOLD amplitude between HIV + and - |
| Juengst (2007)[28] | 31/16 | HRF | finger tapping | NR | NA (HRF) | NA | no significant difference in mean peak values between HIV + and HIV- |
| Ances (2010)[32] | 26/25 | visual cortex | checkerboard | AFNI | p=0·05 | yes, not specified | HIV+ reduced functional changes in BOLD signal |

AFNI: Analysis of Functional NeuroImages. BOLD: Blood Oxygenated Level Dependent. HIV: HIV-positive patients. HRF: Haemodynamic Response Function. M.C.: multiple comparisons. NA: not applicable. NR: not reported. SN: seronegative controls.

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**TABLE 3.** Whole brain analysis of difference BOLD signal HIV-/+ patients

| Study | HIV/SN | Network | Task | Software | Statistical tresholding | Result |
|---|---|---|---|---|---|---|
| Caldwell (2013)[20] | 34/28 | working memory | Sequential letter task | FEAT | FWE-corrected at voxel level p<0.05 Later relaxed (not specified) | HIV+ greater activation on the simpler attention task but less activation on the working memory task |
| Ernst (2009)[23] | 31/32 | attention | tracking balls | SPM2 | FWE-corrected at voxel level p<0.05 | HIV+ more activation in right prefrontal region only with most difficult task. |
| Melrose (2008)[24] | 11/11 | semantic event sequencing | picture sequencing task + object discrimination control | SPM2 | Voxel threshold 0.001 uncorrected, small volume correction | HIV+ less signal change in frontal regions and left caudate, more signal changes in postcentral/supramarginal gyrus. Functional connectivity: dysfunction withing the basal ganglia and prefrontal cortex, and within interactions between these regions |
| Chang (2013)[25] | 66/56 | attention | tracking balls | SPM8 | FWE-corrected at cluster level p<0.05 | HIV+ has load dependent decreased activation in right temporal region, while HIV- showed load dependent increase |
| Chang (2008)[27] | 24/10 | attention | tracking balls | SPM2 | FWE-corrected at cluster level p<0.05 used various tresholds | HIV+ has greater load dependent activation in right frontal and cingulate regions. |
| Ernst (2002)[14] | 10/10 | working memory | sequential letter task | SPM99b | Voxel treshold 0.001 uncorrected | HIV+ has more BOLD activation in the lateral prefrontal cortex on all tasks. |
| Chang (2001)[15] | 11/11 | working memory | sequential letter+number task | SPM99b | Voxel treshold 0.001 uncorrected | HIV+ has greater activation in parietal regions and frontal lobes(lateral prefrontal cortex and supplementary motor area) |
| Maki (2009)[29] | 7/4 | memory | encoding task, recognition task | SPM2 | Cluster corrected (min size >30) uncorrected treshold p<0.05 | encoding: HIV- more activation in hippocampal and temporal/ frontal cortical structures. recognition: HIV+ more in left superior temporal gyrus, hippocampus and right insular cortex. |
| Chang (2004)[30] | 18/18 | l attention | tracking balls | SPM99b | Cluster corrected for m.c. (not specified) | HIV+ decreased activation in the normal visual attention network, and increased activation in adjacent/contralateral structures |
| Castelo (2006)[31] | 14/14 | memory | encoding + recognition task | SPM99b | NR | Encoding: no difference. Recognition: HIV+ less activity in right posterior hippocampus, right inferior frontal gyrus, left lingual gyrus, and more activity in lateral frontal and posterior parietal regions |
| Schweinsburg (2012)[16] | 11/13 | fronto-striatal | mental rotation task | AFNI | cluster corrected multiple tresholds/cluster size | HIV+ had increased activation in areas of the PPC-striato-frontal pathway, and in left insular and right occipital cortex, less activation in the anterior cingulate |
| du Plessis (2015)[17] | 18/16 | ventral-striatal | reward task | SPM8 | FWE-corrected cluster level p=0.05 | No between group differences |

AFNI: Analysis of Functional NeuroImages. BOLD: Blood Oxygen Level Dependent. FEAT: fMRI experts Analysis Tool. FWE: Family Wise Error. M.C.: Multiple Comparisons. NR: Not Reported. SPM: Statistical Parametric Mapping. PPC: PosteroParietal Cortex

**TABLE 4.** ROI analysis of differences BOLD signal HIV+/- patients

| Study | HIV/SN | Network | Task | Software | Corrected for MC? | Pre-specified ROI? | Result |
|---|---|---|---|---|---|---|---|
| Thomas (2013)[21] | 52/52 | Functional connectivity 5 domains | resting state | NR | FDR corrected p<0.05 | yes | HIV+ had less intra- and internetwork correlations in several functional brain networks |
| Chang (2008)[27] | 24/18 | visual attention | tracking balls | SPM2 | uncorrected p=0.05 | no | HIV+ load-dependent increase in frontal regions when HIV- have load-dependent decrease |
| Maki (2009)[29] | 7/4 | episodic encoding | encoding task, recognition task | SPM2 | Cluster corrected | yes | HIV+ decreased hippocampal activity during encoding and increase hippocampal activation during recognition. |
| Castelo (2006)[31] | 14/14 | episodic encoding | encoding task, recognition task | SPM99b | Not reported | both | HIV+ had attenuated activation of brain regions known to support episodic encoding (right posterior hippocampus, left and right lingual gyrus, right inferior frontal gyrus), and recruited additional cortical regions. |
| | | | | | hippocampal activation; no | yes | HIV+ less activation in bilateral hippocampus |
| DuPlessis (2015)[17] | 18/16 | Ventral-striatal reward | Reward task | SPM8 | no | yes | HIV+ decrease in activation in ventral striatum for anticipating neutral and rewarding cues |
| Ortega (2015)[33] | 132/49 | Functional connectivity 4 domains | Resting state | FS-FAST | FDR corrected p=<0.05 | yes | HIV+ had lower cortico-striatal functional connectivity. HIV+ cART+ had higher connectivity then HIV+ cART- |
| Ipser (2015)[34] | 15/15 | Functional connectivity 3 domains | Resting state | AFNI | Not reported | yes | HIV+ had reductions in connectivity in fronto-striatal regions. |

AFNI: Analysis of Functional NeuroImages. FDR: False Discovery Rate. FS-FAST: FreeSurfer functional analysis stream. MC: multiple comparisons. NR: not reported. ROI: region of interest. SPM: statistical parametric mapping.

Four of the 15 fMRI studies focused on attention deficits using visual attention tasks.[23,25,27,30] In these tasks, subjects had to track a certain number of balls among other moving balls. Overall, studies reported an increase in activation in the attention network (right (pre)frontal and cingulate regions) and/or adjacent structures when attentional load increased. HIV-positive subjects patients performed at the same behavioral level (test accuracy and reaction time) as HIV-negative subjects up until the most difficult tasks. Taken together, these data suggest that HIV-positive subjects show hyperactivation of brain regions and/or recruit adjacent regions to achieve the same behavioral results, up onto the point where functional brain activation falls short and behavioral results are affected. Apparently, more neural activation is needed in the HIV-positive individuals. These analyses suggest that in HIV-positive subjects, an attention deficit is present which can, to a certain degree, be counter balanced by the use of brain reserve capacity.[42]

Three studies employed working memory paradigms. Working memory was tested using a sequential number task, in which a series of numbers is presented and subjects were instructed to press a button when the number shown is the same as *n* items before. Ernst et al. (2002) and Chang et al. (2001) found an increase in activation in the lateral prefrontal cortex and/or parietal regions in the HIV-positive group.[14,15] Caldwel et al. (2013) found that HIV-infected subjects had more activation but similar accuracy on the simpler tasks, but less activation and diminished accuracy on the more difficult tasks, when compared to HIV-negative controls.[20]

In addition to the studies investigating attention and working memory, there were two studies investigating memory (encoding and recall).[29,31] Maki et al. (2009) and Castelo et al. (2006) used comparable tasks, in which subjects were instructed to remember either words or pictures, and recall them later. Whole brain as well as ROI-analyses revealed differences for HIV-positive patients in activation in hippocampal and/or temporal/frontal cortical structures. Castelo et al. (2006) found no difference in activity during encoding and less activity during recognition for HIV-positive patients, while Maki et al. (2009) found less activity during encoding and more activity during recognition in HIV-positive patients. These conflicting results could possibly be due to the small sample size of both studies (n=11[29] and n=28[31]) and/or the fact that the task used differed slightly. Furthermore, Castelo et al. (2006) did not provide insight in the statistical inference used, which makes it more difficult to interpret their outcomes. In all, despite the limitations, all memory studies do suggest a dysfunction of hippocampal-prefrontal regions in HIV-positive subjects, possibly underlying memory deficits.

Four studies centered on the fronto-striatal network. This is important as frequently occurring symptoms in HAND like changes in executive functioning suggest a dysfunction in this circuit.[40,43,44] Moreover, a recent meta-analysis found evidence for hyperactivation in

the fronto-striatal circuit in HIV-positive subjects.[18] Melrose et al. (2008) used a semantic event sequencing task, during which subjects had to arrange semantic events in the right order.[24] They found more activation in the right postcentral/supramarginal gyrus for the HIV-positive group, while the HIV-negative groups showed more activation in frontal regions. Functional connectivity analyses on resting state data by Melrose et al. (2008), Thomas et al. (2013) , Ortega et al (2015) and Ipser et al (2015) suggested dysfunction between basal ganglia and other (frontal) regions, and less intra- and internetwork correlations in certain prespecified brain networks.[21,24,33,34] This means that even without using a task, a disturbance could be found between networks in HIV-positive subjects compared to seronegatives. Schweinsburg et al. (2012) studied the effect of HIV on mental rotation, because it is part of the fronto-striatal circuit.[45] They found increased activation in areas of the postero-parietal cortex pathway and in left insular and right occipital cortex, together with less activation in the anterior cingulate in HIV-positive subjects.[16] Reaction times and accuracy on the fMRI tasks did not differ between the two groups. Finally, a study by du Plessis et al. (2014) on fronto-striatal reward processing included only cART naïve subjects. Using a whole brain analysis, the study found no significant difference between cART naïve HIV-negative and –positive subjects. However, a ROI analysis revealed significant less activation in the ventral striatum during anticipating neutral or rewarding cues in the latter group. They did not report differences in frontal function.

**Effect of cART**
Only two studies compared functional data between patients with and without cART[27,33], and one investigated differences in BOLD signal for different kinds of cART.[26] The two papers studying attention both found a significant difference in BOLD activation with a greater attentional load-dependent increase in brain activation for patients on cART and lower accuracy on the performance of the most difficult task.[26,27] Ances et al. (2008) found an increase in the BOLD response for patients on low CNS penetration effectiveness drugs.[26] Ortega et al. (2015) found higher functional connections in HIV-patients with cART then HIV-patients without cART in fronto-striatal networks using a functional connectivity analysis.

**Effect of aging**
Finally, six studies report on the effect of aging on brain function in HIV-positive and HIV-negative subjects (table 5).[21,23,25,28,32,34] Since HIV-patients are aging, it is important to investigate if aging has an interaction with HIV-status on functional data because both HIV and aging have a degenerative effect on the brain and functional brain regions. Two studies investigated the effect of HIV and aging on characteristics of the BOLD signal, and found no interactions.[28,32] Thomas et al. (2013) and Ipser et al. (2015) calculated functional connectivity during resting state to evaluate regional interactions between prespecified functional networks.

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**TABLE 5.** BOLD-signal differences HIV+/- patients combined with aging effect

| Study | HIV/SN | Network | Task | Software | Wb/roi | Statistical inference | Results |
|---|---|---|---|---|---|---|---|
| Thomas (2013)[21] | 52/52 | Functional connectivity 5 domains | resting state functional connectivity | NR | ROI | FDR corrected treshold of 0.05 | Aging causes decrease in intranetwork correlations in DMN and SAL and internetwork correlations between DMN-SAL. No interaction HIV and aging. |
| Ernst (2009)[23] | 31/32 | visual attention | tracking balls | SPM2 | ROI | FWE-corrected at voxel level p<0.05 | After 1 year, HIV+ more BOLD signal in right prefrontal and posterior parietal cortices and cerebellum bilaterally. HIV- less BOLD signal after 1 year. |
| Chang (2013)[25] | 66/56 | Visual attention | tracking balls | SPM8 | WB | FWE corrected at cluster p<0.05 | HIV+ had greater age-related increases in brain activation in right parietal, cingulate and paracentral regions, cerebellar vermis, left frontal, temporal and occipital regions. |
| Juengst (2007)[28] | 31/16 | HRF | finger tapping | NR | WB | NA (HRF) | no effect or interaction with HIV status for age in mean BOLD peak value |
| Ances (2010)[32] | 26/25 | visual cortex | checkerboard | AFNI | VOI | p=0.05 corrected for m.c. (not specified) | HIV and increasing age independently caused decreases in functional BOLD signal, no interaction |
| Ipser (2015)[34] | 15/15 | Functional connectivity 3 domains | Resting state | AFNI | ROI | Not reported | Reduction in connectivity in individuals over 50 years, no interaction between age and HIV. |

AFNI: Analysis of Functional NeuroImages. BOLD: Blood Oxygen Level Dependent. DMN: Default Mode Network. FDR: False discovery Rate. FWE: Family Wise Error. HRF: Haemodynamic Response Function. M.C.: Multiple Comparisons. NR: Not Reported. ROI: region of interest analysis. SAL: Salience network. SPM: Statistical Parametric Mapping. VOI: Volume Of Interest analysis. WB: whole brain analysis.

They found similar decreases in correlations between networks with aging and HIV-infection, but there was no interaction between HIV and aging.[21,34] Ernst et al. (2009) and Chang et al. (2013) used the same visual attention task.[23,25] The longitudinal study by Ernst et al. (2009) found that after 1 year follow-up, HIV-positive subjects had more activation in the right prefrontal and posterior parietal cortices and bilateral cerebellum than HIV-negative subjecst.[23] A possible explanation is a learning effect in the HIV-negative group, or an effect of ongoing brain injury in the HIV-positive group. Chang et al. (2013) also reported interactions between age and HIV status with greater age-related increase in activation in various regions.[25] Noting that from the five studies, only one found an interaction with HIV and aging, the limited evidence seems to point towards there being no interaction between these parameters. We therefore decided to regard them as independent factors.

## DISCUSSION

This systematic review of 17 studies describes the effect of HIV-infection on brain function as measured by BOLD fMRI. Overall, HIV does not seem to alter BOLD-characteristics. This is important, as this finding suggests that the coupling between neural activation and the BOLD response itself is not necessarily different in HIV-positive patients. A difference in BOLD response is therefore attributable to a difference in the amount of neural activation. The majority of studies found that for completing the same task, HIV-positive patients showed more activation or recruited more regions when compared to HIV-negative controls. Although there is a large variety in study design, studied populations, and levels of statistic inferences, most evidence seem to point to affected fronto-striatal function. There appears to be no or limited interaction between HIV-status and aging on functional neuroimaging data, although there are few longitudinal studies. Finally, the effect of cART on brain function is not yet been adequately addressed.

Since its introduction in the 1990s, fMRI has been proven to be a very sensitive instrument, with an even greater ability to detect functional brain abnormalities than neuropsychological assessment.[10–12] Neuropsychological studies have shown that HIV seems to predominantly affect the fronto-striatal network.[41,46,47] This network consists of neural pathways that connect frontal regions with the basal ganglia, and these circuits are, amongst other things, involved in executive functioning.[18,41,48] This systematic review confirms these neuro-psychological test observations by showing that impairment of the fronto-striatal system was more pronounced in HIV-positive versus HIV- negative patients. This is consistent with previous literature.[18] It is important to note that studies in this review suggest that, even without clinical symptoms or neuropsychological abnormalities, a functional impairment exists in the brains of HIV-patients. One

explanation for this occurrence is the so-called brain-reserve theory, where patients use a hyperactivation or activation of adjacent structures, thus more neural effort, to achieve the same behavioral results.[49] Compared to controls, HIV-positive participants show an overall comparable behavioral performance though performance in behavioral outcomes is poor for the more difficult tasks.

It appears that HIV-patients use hyperactivation of brain regions and recruitment of additional brain regions to maintain the same behavioral score, but this mechanism falls short when performing the more difficult tasks. It appears that this hyperactivation is inefficient, possibly due to interfering processes related to the HIV-infection. There are several theories of how HIV infection results in functional impairment: first of all, the virus itself, which enters the central nervous system (CNS) within days after infection.[50,51] There is no evidence that HIV actually infects nor damages neurons, but due to specific viral proteins produced by infected cells such as gp120, Tat, or Vpr subsequent local damage can be done.[52] The neurotoxicity theory by HIV is supported by the fact that starting combination antiretroviral therapy often greatly improves the cognitive ability of patients suffering from HAD.[53] However, even in patients receiving adequate antiretroviral therapy, cognitive decline can still occur.[54] Perhaps the compartmentalization of HIV in the CNS and the accompanying local ongoing neuro-inflammation, or the sensitizing of the immune system by the virus might be an explanation for this observation.[55,56] Furthermore, the effect of the virus has been compared to the neurodegenerative process seen in aging. However, the four papers in this systematic review investigating aging and HIV suggest that there is no or limited interaction between HIV-status and aging, and that they are independent factors to consider.

Antiretroviral drugs are another potential important cause for cognitive disorders in HIV-positive patients. With the recently published INSIGHT START study in mind, it is recommended to start cART even in patients with CD4-counts above 500 cells per cubic millimeter.[57] Subsequently, this will lead to more patients on therapy and therefore it thus remains of importance to investigate the (sub)clinical and possible long-term consequences of continual antiretroviral therapy on cognition. One of the drugs often implicated in decreased cognitive functioning, is Efavirenz.[58] For example, a recent study on treatment interruption found an improvement in cognition as measured by NPA after cessation of therapy.[59] Additionally, the authors found a difference in improvement after discontinuing different cART regimes, with cessation of Efavirenz containing regimes giving the most effect. Indeed, the effect of cART on cognitive performance has been described before, with Efavirenz as the most significant example.[60] Studies in this review showed that patients on cART use more of their brain reserve, and the type of cART affects the BOLD response. This suggests a possible effect of medication and the type of medication on cognition in HIV patients. Based on the results of this systematic review,

functional MRI appears to be an appropriate tool to detect subtle cognitive changes. There are, however, very few studies investigating the effects of chronic cART on the CNS. Recently, another South-African study in cART naïve HIV-positive patients investigating the fronto-striatal network using an inhibition task was published, showing subcortical dysfunction.[61] Currently, a randomized longitudinal study is underway utilizing fMRI to estimate the effect of Efavirenz on cognition (clinicaltrials.gov NCT02308332).

Another important consideration in this review is the statistical and analytical methods used in the various studies. First, most studies included only small numbers of patients sometimes hampering firm conclusions. Another problem is statistical inference. Following improvements in fMRI analysis techniques and software, statistical and methodological issues have become less of a problem during recent years. For example, the earliest studies did not properly correct for multiple comparisons[23,30,31] or proper tresholding[27,31] while more recently published studies tend to have better methodological quality.[17,21,25] Another limitation is that studies included in this review all described a very 'clean' population; i.e. dominantly male participants, with ages between 30 and 50, and lacking information on comorbidities, co-infections or previous cART regimes. Therefore, no conclusions could be drawn regarding the effect of these factors on functional brain imaging. It is important for future fMRI studies to include younger patients or those with comorbidities or co-medication. Furthermore, different tasks used in these studies make generalizability of results more difficult and needs to be addressed in future study designs. The use of longitudinal studies is mandatory since they can aid in exploring the use of fMRi in detecting early changes before clinical symptoms.

Summarizing, when compared with HIV-negative subjects, HIV-positive patients showed a hyperactivation of brain regions, suggesting a so-called brain-reserve theory, when investigating regions involved in attention, (working) memory, and executive functioning, with the most evidence pointing to defects in fronto-striatal pathways. Increasing age has a comparable effect on brain function, but it does not interact with HIV-status. Limited data points to an effect of cART on brain function. Further research is needed to confirm this effect.

In conclusion, fMRI is a sensitive instrument to detect changes in brain activation associated with subtle cognitive changes in HIV-patients.

Review of Functional MRI in HIV: effects of aging and medication

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# PART 4

Discussion and Summary

# CHAPTER **8**

Summary and general discussion

# INTRODUCTION

This thesis focusses on cognitive decline in HIV infection, of which the different forms are summarized under the term HIV-Associated Neurocognitive Disorder (HAND). The different parts of the thesis discuss separate aspects of HAND, like the effect of combination antiretroviral therapy (cART) on its etiology, the prevalence of HAND in Western and resource-limited settings, how to screen for HAND, and challenges in diagnosing it. Finally, the use of a novel diagnostic instrument, blood oxygenated level dependent functional MRI (BOLD fMRI) is investigated. The main findings of this thesis are that some types of antiretroviral therapy, and then especially Efavirenz, have a negative effect on cognition even in asymptomatic patients. Moreover, discontinuing this drug can significantly improve cognitive functioning. Furthermore, we found a high prevalence of cognitive decline in a Dutch HIV-positive outpatient population as well as in a South-African urban population. We tried to find a suitable screening tool for the latter, but were unsuccessful. However, we were successful in developing a protocol for which steps to take after a positive screening, and evaluated a possible treatment for HAND; cognitive rehabilitation. Finally, we summarized the research on BOLD fMRI and HIV in a systematic review. In the following chapter, the results of the studies mentioned above will be summarized and discussed. Furthermore, ideas for future research, screening approaches and treatments will be shared, together with views on researching cognition in resource-limited settings.

# PART 1:
# EFFECT OF CART ON NEUROCOGNITIVE DECLINE IN HIV PATIENTS

### Efavirenz: the notorious ART

The fact that Efavirenz has neuropsychiatric side effects was already known from when it was first registered as an antiretroviral drug.[1] But because Atripla (containing Efavirenz) had long been the only single-tablet regimen (STR) on the market, it was therefore the first choice in most, if not all HIV-treatment centers. Even after the development of other STRs, Atripla remained popular because of its low costs. If patients would complain about cognitive decline after starting Efavirenz, they would simply switch to another regimen. However, it was never properly investigated if those patients that stayed on Efavirenz did not also have cognitive issues that they didn't report or didn't recognize as a side effect of their medication. In our outpatient clinic, it was not uncommon for these so-called asymptomatic patients to switch to other regimens at some point, for instance because of drug-drug interactions with other medications. A substantial portion of these asymptomatic patients reported positive effects on their cognition only

after discontinuing Efavirenz, prompting us to investigate the matter. In **chapter 2**, we describe the results of the ESCAPE study in which we investigated just that; the effect of discontinuing Efavirenz on cognition in asymptomatic patients. We found that switching to a different, non-Efavirenz-containing regimen caused an improved performance on neuropsychological assessment (NPA), especially in the domains 'attention' and 'speed of information processing'; domains that mostly rely on the frontostriatal network. Interestingly, the effect was the greatest on the most difficult tests of the NPA. Furthermore, after the study finished most patients preferred to remain on an Efavirenz-free regimen because of a subjective improvement.

### Frontostriatal functioning and the brain reserve theory

The frontostriatal system is an umbrella term for different neural pathways that connect regions in the frontal lobe of the brain to the striatum, one of the basal ganglia.[2] These frontostriatal pathways are involved in executive functioning and other behavioral aspects such as inhibition and reward processing.[3] Executive functioning regulates, inter alia: working memory, managing complex tasks, planning ahead, processing of new information and the ability to shift behavior according to environmental stimuli. Furthermore, patients with impaired executive functioning often experience problems with attention tasks.[3] In the ESCAPE trial, we found the greatest effect of Efavirenz on the domains 'attention' and 'speed of information processing'; domains that rely on the integrity of the frontostriatal system, thus suggesting Efavirenz has an unfavorable effect on these pathways. Interestingly, earlier studies on the effect of HIV infection itself have also found this system to be most affected.[4,5] These results are consistent with findings on autopsy in AIDS patients where the virus itself was mostly located in the basal ganglia.[6] Taken together, this could mean that the mechanism of neurotoxicity of Efavirenz is similar to that of HIV infection itself, or that the two could enhance each other. Furthermore, in the ESCAPE study, we found that the effects of Efavirenz were most apparent in the most difficult tasks of the NPA. This fits a certain theory that has been proposed in neuropsychological research called the brain reserve theory. This theory provides that everyone has a certain amount of 'brain reserve'; i.e. a spare brain capacity that can be recruited when there is a higher demand for cognitive functions. A problem with a person's brain reserve would therefore not be very evident in everyday life where there is normally not much need for it. The fact that the effect of Efavirenz was most obvious in the most difficult tasks, aligns with this brain reserve theory. This could also explain the fact that the patients in the ESCAPE study found themselves to be asymptomatic, i.e. they had no cognitive complaints, while it turned out their cognition improved when they discontinued Efavirenz. Moreover, most of the patients participating in the ESCAPE trial had a high level of education and high functioning jobs. This could be because of inclusion bias as patients with higher levels of education are more prone to participate in clinical research. However, it is interesting in the light of the

brain reserve theory that maybe patients with the largest brain reserve (i.e. the patients with higher intelligence capacities) are the ones that can stay asymptomatic on Efavirenz and thus were eligible to participate in the ESCAPE study.

### Financial considerations

As stated above, Efavirenz is a component of the first STR, namely Atripla. However, since Atripla® was marketed in 2006, more antiretrovirals have been developed, as well as other STRs. A considerable number of these regimens do not have such overt neurotoxic side effects as Efavirenz-containing regimens. Therefore, the question could rise as to why it is still important to investigate the side effects of Efavirenz. Isn't it more efficient to just choose one of the many alternatives? First of all, even if no one uses Efavirenz anymore, it would still be interesting to investigate its effect on cognition, because it could shed some light on the etiology of HAND. Secondly, there is a substantial number of patients who actually still use Efavirenz, because it is a component of the oldest STR and as such, also the first STR to lose its license and become generically available, and thus cheaper. This means Atripla, and therefore Efavirenz, is the drug of first choice in environments where funds are sparse, for instance resource-limited settings. Illustratively, in several very recently published articles in resource-limited settings (sub-Saharan Africa, Peru), approximately half of all HIV patients are on an Efavirenz-containing regimen.[7–11] In the Western world, these numbers aren't as high (9.4% of all HIV-infected patients in the Netherlands in 2018)[12]. However, Atripla's financial advantage makes it an attractive choice for the ones paying the medication bills, i.e. the patient or the health insurance company. Objectively proving Efavirenz has a negative effect on cognition even in seemingly asymptomatic patients will make it more evident that other, possibly more expensive antiretrovirals should be the first choice.

### Drug levels in relation to cognitive outcome – less is more?

To strengthen our hypothesis that Efavirenz is neurotoxic, we evaluated Efavirenz concentrations in relation to cognition in the ESCAPE trial in **chapter 3.** In this drug level study, we found that having a higher Efavirenz concentration was associated with worse performance on NPA. Moreover, patients that entered the trial with a higher concentration had more benefit from discontinuing Efavirenz. These results do suggest that Efavirenz is neurotoxic. In the latest WHO guidelines, published in 2019, Efavirenz is mentioned as an alternative first-line choice, but in a lower dose; i.e. 400mg instead of 600mg.[13] This is interesting in the light of the findings of our study that a higher Efavirenz concentration in blood is associated with a worse performance on an NPA. Several studies found that Efavirenz 400mg was non-inferior to Efavirenz 600mg.[14] This lower dosage is to be preferred over the higher one, for toxicity as well as financial reasons. I would therefore advise lowering the dose of Efavirenz in the current generic Emtricitabine/Tenofovir/Efavirenz STR to 400mg.

Moreover, in chapter 3 we additionally tested the usefulness of neurofilament light (NfL) in plasma as a biomarker for cognitive decline. NfL in cerebrospinal fluid (CSF) is a biomarker for cognitive decline that has been used successfully in the past, so we had high hopes for NfL in plasma. This would be the preferable outcome, because sampling plasma is a lot easier than sampling CSF, which would make it a considerably more convenient biomarker. However, it turned out that plasma NfL could not predict NPA outcome, and therefore, based on our study, cannot be used as a biomarker. A possible explanation for this outcome is that NfL is a very specific marker for a certain type of brain damage, namely axonal damage. NfL is a structural component of axons and, as such, is released into CSF and blood when axons are damaged.[15] It is possible that the cognitive effects of Efavirenz have a different pathological mechanism then axonal damage, for instance damage to dendritic cells.[16,17] The fact that NfL in CSF was high in patients with HIV dementia in earlier studies could also mean that NfL is more sensitive to more serious forms of cognitive decline, or that plasma NfL is less sensitive than NfL in CSF, a fact that has been suggested in animal studies.[18,19] All in all, I would advise against using plasma NfL as a biomarker for HIV-associated and/or cART-associated cognitive decline. More research should be done on other possible biomarkers.

**Other antiretrovirals at risk**
In the ESCAPE study, we focused on the neurocognitive effects of Efavirenz, mainly because this specific antiretroviral is best known for its negative effects on the brain. However, as stated above, there are other antiretrovirals and other STRs available as suitable alternatives. For instance, in the newest WHO guidelines, the integrase inhibitor Dolutegravir replaced Efavirenz as the first-line treatment of first choice. Even so, more recently developed antiretrovirals can also have substantial negative effects on the brain. Sometimes this only becomes clear after the medication has been in use for a while. A good example of this is Dolutegravir. At the time of registration, hopes were high for this specific medicine, because in phase II and III trials, minimal side effects were reported; insomnia was reported 5-11%, fatigue in 4% and psychiatric disorders in 2%.[20–24] However, in clinical practice, patients started reporting more and more side effects, and preliminary studies in small cohorts saw high rates of discontinuation because of neurotoxicity. To investigate if this was also true in the Dutch population, we examined these rates using the nationwide database of the Athena cohort in **chapter 4**. To make sure this was not a class effect, we compared discontinuation rates due to neurotoxicity of Dolutegravir with the rates for Elvitegravir, another integrase inhibitor. It turned out that 12.7% of all patients discontinued Dolutegravir or Elvitegravir, and the main reason for discontinuing was (neuropsychiatric) toxicity. Since then, several studies have proposed mechanisms of action for the negative effect of Dolutegravir on the brain, e.g. dysregulated autophagy through microglia damage, the forming of stress-related oxidative metabolites, or an effect on the blood-brain barrier.[25–27] The story of Dolutegravir is a cautionary tale for

multiple reasons. First, it showed that neurocognitive decline is not only a problem of older antiretroviral regimens. Second, it taught us to curb our enthusiasm for new therapies until real-world data is available.

In order to investigate the effect of integrase inhibitors on cognition and mood in more detail, a study mirroring the ESCAPE study but with Dolutegravir and Bictegravir is on its way: the INSTINCT (effect of INSTegrase Inhibitors on NeuroCogniTion and sleep disorders) study. In this study, NPA and fMRI will be used to evaluate cognitive functioning when switching from a protease-inhibitor-containing regimen to either Dolutegravir or Bictegravir. When designing this study, lessons can be learned from the ESCAPE study. For instance, I would advise using other questionnaires to evaluate quality of life and mood. In the ESCAPE study, we used questionnaires designed to screen for abnormalities and/or disorders. However, we recruited asymptomatic patients. This meant that almost all of them scored top marks on the questionnaires at the start of the trial, and there was no room for improvement. However, lots of patients told me after switching that their mood and quality of sleep was significantly improved, and therefore their quality of life. However, this is not reflected in the analysis of the questionnaires. It is probably wiser to use subjective measures that focus on the change in perceived quality of life-related outcomes, or that measure the higher regions of functioning, in order to evaluate improvement in asymptomatic patients. Second, it could be beneficial to evaluate the chosen time period between the two measurements. In the ESCAPE study, we opted for 12 weeks. For fMRI outcomes, this is an adequate interval due to its sensitive nature. However, for NPA outcomes, this interval might be too short, and a duration of 24 weeks might be more prudent. Even better would be three measurements; at baseline, at 12 weeks, and at 24. An added bonus would be that the measurements of the ESCAPE study and the INSTINCT study could be more easily pooled because they would both have a measurement at 12 weeks. Furthermore, where the progress of the ESCAPE study was hampered by lower-than-expected inclusion rates, this would probably be less of a problem in the INSTINCT study. The main reason for patients not wanting to participate in the ESCAPE study, was not that they did not want to switch, but that they wanted to switch to regimens that were not Eviplera. At the time of recruiting for the ESCAPE study, the STR Triumeq (Abacavir/Lamivudine/Dolutegravir) had just come available and, as stated above, patients and healthcare providers had high hopes for this antiretroviral. This meant that patients on Atripla that were willing to switch their cART and were contacted by us to participate in the ESCAPE study, declined in order to be able to switch to Triumeq. In the INSTINCT study set-up, patients will be switched to either one of two new regimens (including Triumeq), which would probably mean patients are more susceptible to participating in this trial.

# PART 2:
# OCCURRENCE AND DETECTION OF
# NEUROCOGNITIVE DECLINE IN HIV PATIENTS

**Screening for cognitive decline and steps to take**

An NPA is the gold standard for diagnosing HAND, but because of its time-consuming nature, cognitive screening tests are commonly used in clinical practice. In **chapter 5**, we describe a study that investigated the results of cognitive screening tests in the HIV-positive outpatient-clinic population in our hospital. In this study, we found that around half of these patients had subjective and/or objective cognitive complaints. Moreover, 40% of the population had depression and/or anxiety complaints. Furthermore, in 59% of these cases, a possible underlying cause could be defined, but only for one out of five patients, subsequent action was taken to eliminate that cause.

Other studies conducted after the publication of our study present slightly lower numbers of patients with cognitive impairment, i.e. around 35%.[28–31] This can be explained by the fact that in our study, we tested for objective and subjective complaints. The studies reporting lower numbers investigated only objective complaints using screening tests or only subjective complaints using questionnaires. Interestingly, three studies also investigated the consequences of cognitive decline and found a negative effect on activities of daily living (ADL) and frailty score.[31–33] This means cognitive impairment in HIV occurs frequently and has detrimental effects on patients, and therefore should be addressed and treated where possible. There are several international guidelines available on clinical care for HIV patients.[34–36] In Europe, the guidelines of the European AIDS Clinical Society (EACS) are most frequently used. The guideline provides some guidance on how to manage cognitive impairment, for instance by advising to eliminate other, treatable causes for cognitive decline such as hypothyroidism and syphilis. In our study, we developed an easy-to-follow and complete flow chart to make it easier for treating physicians to see which steps they should take after a positive screening test. Our flow chart has been recommended in at least one study since its publication.[37] In the flowchart we also advise switching from known neurotoxic cART and screening for depression. This is consistent with the latest version of the EACS guidelines, published in 2018, which included a statement that advised avoiding treating patients with cognitive complaints with Efavirenz, and screening patients for depression every 1-2 years.

**Tackling cognitive decline and mood disorders**

Although guidelines advise screening for cognitive decline in HIV patients, they do not offer any treatment options, which raises the question what treatment options actually are available for cognitive decline at the moment. I believe the most important ones are cognitive rehabilitation, cognitive training, physical therapy and serious gaming.

In our study we evaluated a cognitive rehabilitation program as a possible treatment for cognitive decline in HIV. These kind of programs have proved useful in other conditions such as acquired brain injury.[38] They focus on compensatory interventions to reduce interference of the cognitive deficits with daily life by substituting different latent skills or by acquiring new skills.[39] The rehabilitation program we developed specifically for HIV patients was very successful; patients reported an improvement of 2.3 points out of 10 on predefined goals for cognitive challenges in everyday life. However, we only tested it in a very small group (5 patients). It would be interesting to investigate the effect of such a program in a larger group of affected patients. Moreover, the nature of a rehabilitation program, with frequent hospital visits and repeated exercises, makes it time-intensive and thus expensive and less patient-friendly. In this light, it may be interesting to explore new innovative approaches, such as serious gaming. A serious game is not designed to entertain, but to use game mechanics and characteristics in order to get the user to achieve real-life goals.[40,41] This means that a serious game uses the positive aspect of a game (e.g. challenges, short-term goals, fantasy elements) to motivate and engage the user into learning and/or training skills that will help them in managing the negative effects of their condition, i.e. a game that encourages behavioral changes. A recent study in the Netherlands tested a serious game for use in cognitive rehabilitation in patients with acquired brain injury and found it to be useful and positively appreciated.[42] One way of making such a treatment approach readily available and easy to use, is to integrate it in a mobile phone app.

A recent review describes a beneficial effect of cognitive training in HIV-infected patients with cognitive decline.[43] Cognitive training is different from cognitive rehabilitation since it uses neurocognitive exercises to improve or maintain cognitive functioning, mostly focused on a specific cognitive domain. Although the review found promising results of cognitive training, the authors also concluded that there are no official protocols and/or guidelines to implement this as a treatment now. Further research is therefore needed, according to the authors. Another possible treatment modality for cognitive decline is implementing more physical activity. This has been shown to have a positive effect on cognition in other populations.[44,45] One study found a beneficial effect of physical activity on cognition in HIV patients.[46] Although it is unlikely that physical activity alone can alleviate all problems with cognitive decline, it might be a good addition to other therapies.

In our study population, we also found a high percentage of mood complaints. Depression and anxiety are common comorbidities in other chronic diseases as well.[47–49] A recent meta-analysis conducted in Canada using a large cohort consisting of almost one million patients with varying chronic diseases, including diabetes, asthma and COPD, found a prevalence of 16% for mental health disorders. They estimated the added cost

of these mental health disorders on 16.000 dollar per patient per 3 years.[50] Furthermore, studies found that patients with HIV that suffer from depression have less adherence to drugs, and a higher change of negative disease outcomes such as an increase in HIV viral load and a higher mortality.[51,52] This means it is important to be on the lookout for mood disorders in HIV-infected patients and other chronic conditions, and treat it where possible. Several studies found a positive effect of cognitive behavioral therapy (CBT), alone or in small groups, on mood disorders in for instance diabetes and epilepsy.[53–55] Even in HIV patients, CBT has been found to aid in adherence and to alleviate mood complaints.[56,57] In order to facilitate the delivery of this treatment, e-health can be used. This would make interventions available for a larger group of people, and might be more cost-effective than conventional interventions. CBT over the telephone was beneficial in bariatric surgery patients, and CBT trough an app alleviated symptoms in cancer patients.[58,59] A recently published randomized controlled trial in HIV-infected patients conducted in hospitals in the Netherlands found a positive effect of an internet-based self-help intervention on mood complaints.[60] Another treatment approach to mood complaints tested in other chronic conditions is, again, the use of physical activity. Several studies in patients with chronic diseases found less depressive complaints in patients with a higher level of physical activity.[61,62] A recent meta-analysis found a positive effect of physical activity interventions on mood, depression and even cognition in patients with various brain disorders.[44]

These results show that we have several options for optimizing the care for HIV-infected patients with cognitive decline and/or mood complaints. Ideally, this should be administered through an e-health solution to make it easily accessible, low-cost and patient-friendly. In my opinion, it would be a combination of the abovementioned therapies: a program that integrates strategies to minimize the effect of cognitive decline on everyday life (cognitive rehabilitation) with exercises to train and/or preserve specific cognitive domains - preferably executive functioning, speed of information processing, and attention – (cognitive training) and offers cognitive behavioral therapy - possibly in a small group with other patients to add the benefit of buddy/fellow-sufferer contact. And of course, it should include a physical activity module.

**Screening and diagnosing HAND in challenging settings**
Although screening for cognitive decline in HIV patients is strongly advised, this can be challenging in different settings. This is especially problematic because the majority of HIV patients resides in resource-limited settings. In **chapter 6**, we evaluate a new screening tool, the MoCA basic, for use in sub-Saharan Africa. In order to appraise the screening tool, we also conducted a (concise) NPA in the population. The study found that the MoCA basic was not a good screening tool for cognitive decline in these settings. Another important finding of this study was that HAND could be diagnosed in a large number of patients

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(66%) by using the NPA. Interestingly, there was quite a big difference in prevalence numbers when using different normative data to calculate Z-scores.

By performing this study in South Africa, we learned first-hand the challenges and difficulties of screening and diagnosing cognitive decline in a resource-limited, rural and poor area. Furthermore, the fact that South Africa has 11 official languages complicated the process even further. This meant we had to translate the NPA tests as well as the screening test in at least two languages. Moreover, in this poor and rural area, the majority of our population did not have a proper formal education, possibly resulting in a limited vocabulary, underdeveloped calculating skills, and a lack of general knowledge. This has an effect on a lot of cognitive tests, for instance on verbal fluency, where patients had to sum up as much words in a specific category or starting with a specific letter, or tests that use mathematical concepts such as adding and subtracting or spatial drawing. But we found that even the concept of sitting on a desk and using a pen-and-paper test, or performing an assignment in a timed matter, concepts that seem natural and straightforward to us, are complex and unfamiliar to someone who has only had a few years of non-Western education. In our study, we finally chose the MoCA basic as the screening tool to use, because a pilot with the normal MoCA was unsuccessful. The patients had trouble with spatial drawing when copying the cube, could not name all the animals in the fluency task because most of them had never heard of or seen a camel before, and had trouble remembering the list of words in the memory task because they didn't know what velvet meant.

In the MoCA basic, we found a suitable alternative, because it did not use any language tasks, and complex tasks were rewritten to represent problems of everyday life. For instance: in the MoCA, there is a test called 'serial 7' where a patient has to subtract 7 from 100, and again subtract 7 from that outcome, etc. In the MoCA basis, this test was replaced by a test where patients had to find three ways to use different coins and/ or bills to pay for an item that costs 13 dollars. The animals in the fluency task, and the words in the memory task were changed to more widely known animals and words. Thus, the MoCA basic was specially designed for challenging settings, and prior studies, mainly conducted in Asia, have found it to be useful.[63,64] It therefore came as a surprise that it was not useful as a screening test in our study. The previously published studies referred to above were conducted in Asia, not Africa, and in an HIV-negative population, which could explain the difference in the results. Another reason could be that the MoCA basic does not test the domain ´speed of information processing´, and in our NPA, we found this particular cognitive domain to be most impaired. Consequently, for future use adding a speed test would improve the validity and usefulness of the MoCA basic. Given the fact that the color trail test and the symbol digit modalities test, both speed tests, could be conducted in our NPA, they could be suitable additions to the MoCA basic.

Otherwise, depending on the resources available, a concise NPA would also be an option for screening for cognitive disorders.

The limitations referred to above apply equally to NPA tests when studying cognition in resource-limited settings. Illiteracy and lack of education greatly limit the number of cognitive tests we can use. There are a few tests available that are neutral when it comes to culture and language, but these are barely enough to conduct a full NPA, while ideally, five different domains should be tested using at least two different tests per domain. Furthermore, an NPA is scored by calculating a Z-score using normative data; i.e. test results from a comparable population. Given the fact that we also included a HIV-negative group in our study, we were able to calculate Z-scores by using a highly comparable group. On top of this, we calculated Z-scores by using normative data from other, comparable populations. These data were obtained from researchers who had conducted neuropsychological studies in rural sub-Saharan Africa and were kind enough to share the results from their control group. These studies were performed in rural and urban populations in Uganda and South Africa. Although these populations should have been comparable, the two different methods produced different normative data sets. This illustrates the fact that the use of the correct normative data is important, but also that finding the correct normative data is troublesome in resource-limited settings. For instance, in South Africa, there is a big difference between rural and urban populations, employed or unemployed patients, and there are many different languages. Fixing this problem will not be an easy task. First, I believe it is required that any researcher that studies cognition in sub-Saharan Africa should find as much normative data as possible; ideally by including a control group in the study, or otherwise by contacting other researchers who have studied cognition in the same area or setting and asking them for their raw normative data. It would be even better if there was a database where all researchers could share their raw normative data, in accordance with the FAIR principle to make data Findable, Accessible, Interoperable and Reusable.[65] This could not only aid research, but also neuropsychologists performing NPAs in sub-Saharan Africa. Researchers on HAND in Africa have cooperated with each other before, at the neuroAIDS in Africa conference in 2009, where the need for the development of appropriate normative data was already discussed.[66]

# PART 3:
# VALUE OF FUNCTIONAL MRI IN DIAGNOSING HAND

**The BOLD and the beautiful**
In **chapter 7,** we describe a systematic review of all the research conducted with Blood Oxygenated Level Dependent (BOLD) fMRI in HIV patients up to June 2015. The aim for

this review was not only to list the available articles, but also to comment on the quality of the research, and in particular the quality of the statistical analyses used. Although using sound statistical inference is always important in clinical research, in fMRI research, it is absolutely vital. Never has this been better illustrated than by Bennett et al. in 2010 when they published the results of an experiment where a dead salmon was scanned in an MRI 'performing' a mentalizing task, and uncorrected analyses showed activation in the medial brain cavity and the upper spinal column of the salmon.[67] This unusual study, although amusing, also teaches an important lesson. Because of the nature of the BOLD fMRI technique, tens of thousands of statistical tests need to be performed per contrast over multiple contrasts. This dramatically increases the chance of a false positive result; as is demonstrated in the finding of neuronal activity in the brain of the aforementioned salmon. That is why we specifically reported if a proper correction for multiple comparisons was done for the studies included in our review. Most of the studies did control for multiple comparisons, but a few did not.

When describing the studies in the review, we chose to group them according to the cognitive domain tested. Interestingly, it seemed as though the more recent studies focussed more on more complex domains such as executive functioning, while the older studies tested domains such as 'attention' or 'working memory'. One possible explanation could be that it took more time to develop tasks for complex domains, than for example a simple attention task. However, as it turned out, those higher domains, and specifically executive functioning, became the main region of interest in HAND research, thus further explaining the shift towards tasks investigating those domains. A recent meta-analysis analyzed the results of trials using BOLD fMRI to investigate executive functioning in HIV-positive patients, and found evidence for frontostriatal dysfunction.[5] The review lists all studies using BOLD fMRI in HIV patients, but there was one subgroup of studies that was of particular interest to us because of the other research we were conducting: studies evaluating cART using fMRI. In the review, we could only find three studies investigating the effect of different medications on fMRI outcomes. These studies found a difference in brain activation between patients with and without cART (greater increase in brain activation with more difficult attention tasks for patients with cART), and even a behavioral effect (lower accuracy on attention task for patients with cART), suggesting a negative effect of cART on brain functioning that can be measured with BOLD fMRI. Since the publication of our review, only a handful of studies on cART and fMRI have been published. Two studies used functional connectivity to assess the effect of medication; a study comparing HIV patients with and without medication found no differences between them.[68] Another study evaluated the effect of switching antiretrovirals; they found an increased connectivity in the dorsal attention network in patients switching from Efavirenz to Rilpivirine, and in patients switching from Raltegravir to Dolutegravir.[69] The authors also performed an fMRI inhibition task, but no significant

changes were found in either study group. However, the study included only 22 subjects (10 in the Efavirenz group, and 12 in the Raltegravir group), which seems like a small number to detect such subtle changes. A more recent study used a larger group of 39 patients on Efavirenz, and compared their activity patterns in an inhibition task with 27 HIV patients on a non-Efavirenz-containing regimen and 20 HIV-negative controls.[70] This task uses STOP and GO trials to test for reactive inhibition (can one inhibit the response to press a button if a STOP trial is given instead of a GO trial?) and proactive inhibition (how do one's reactions change when the probability that a STOP sign will appear is announced on beforehand?). They found a difference between the patients on Efavirenz and the other two groups in behavioral outcomes, i.e. the Efavirenz group's response time did not decrease in accordance with higher chances of STOP signals. Furthermore, on fMRI outcomes a significant effect was also demonstrated; the Efavirenz group showed less activation in frontostriatal pathways associated with proactive inhibition. These results are promising for fMRI research in medication effects. In the aforementioned INSTINCT study, we will use the same inhibition task to assess the effect of switching a protein-inhibitor-based regimen to an INSTI regimen. The lessons learned from our systematic review, i.e. using the proper statistical inference, and a large study group will be very useful in making sure this research will have an impact.

## CONCLUSION

There are several lessons to be learned from the research conducted in this thesis. First of all: it is now clear that Efavirenz has a negative effect on cognition, even in asymptomatic patients. I would therefore propose that physicians try to avoid using Efavirenz, or at the very least use the lower dosage of 400mg instead of 600mg. The fact that even asymptomatic patients have shown to suffer from a negative effect of an antiretroviral drug that is known for neuropsychiatric side effects, means that this could also very much be the case with other antiretrovirals where neurological or psychiatric side effects are common. That is why I believe it is important to properly investigate an effect on cognition in old and new antiretrovirals where real-world data shows a relatively high number of those side effects, such as Dolutegravir. Currently, an NPA is the only way to do this. In accordance with the findings in the ESCAPE study and the brain reserve theory, such an NPA should incorporate difficult tasks, and plenty of tasks on executive functioning. Our screening study showed that subjective and objective screening methods don't always correlate, and a biochemical biomarker for cognitive decline in HIV patients still hasn't been found, as our NfL study showed. However, preliminary data shows a promising role for BOLD fMRI as a diagnostic tool in investigating subtle cognitive changes. In the INSTINCT study, we will try to further prove the usefulness of fMRI in this specific diagnostic dilemma. As is evident from NPA and fMRI research

alike, the frontostriatal pathway is the main region of interest that should be used in fMRI analyses in HIV patients, and as is displayed in our systematic review, using correct statistical inference is vital in analyzing fMRI data. I suspect a growth in fMRI studies in HIV patients the coming years, seeing the developments in the field. Studies incorporating an HIV-negative or a cART-naïve control group will be of most use. However, I believe it will be a while before this new player in the field will have deserved a firm position on the team.

Secondly, as is shown in our screening studies in Dutch and South African populations, the prevalence of HAND is still high, and there is often a link with mood disorders. There are currently no treatment modalities available for cognitive decline, but our screening study offers a step-by-step protocol for actions to be taken after a patient fails a screening test. Furthermore, from our results on the cognitive rehabilitation program and the results of other researchers on other treatment options, there are indications that a multi-faceted treatment program incorporating cognitive rehabilitation and training, cognitive behavioral therapy in small groups, and a physical activity module, preferably administered through e-health solutions (apps, telephone, serious game) could be a treatment option for this group.

Lastly, screening and diagnosing HAND in resource-limited settings proved to be challenging due to a limited number of useful cognitive tests because of high rates of illiteracy and lack of education, but mostly because of the shortage in applicable normative data. As our South African screening study showed, the use of inappropriate normative data can yield very different results. A database where researchers could share their raw normative data would be highly useful.

The days of countless young people suffering from HIV dementia are luckily behind us. Let's try and make an improved cognitive health for people living with HIV a reality for days to come.

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**8**

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Summary and general discussion

8

# CHAPTER 9

Summary in Dutch
Publications
Acknowledgments
Curriculum Vitae

## SUMMARY IN DUTCH / NEDERLANDSE SAMENVATTING

### Introductie

In de jaren 80 van de vorige eeuw maakte de wereld kennis met een onbekend en ernstig ziektebeeld onder jonge patiënten gekenmerkt door een sterk verminderde afweer; het Acquired Immunodeficiency Syndrome (AIDS). Later bleek dat dit veroorzaakt werd door het Humaan Immunodeficiëntie Virus (HIV). In het begin van de HIV-pandemie was het ziekteverloop snel en bijna altijd fataal, en de schrijnende casuïstiek zorgde voor een tot dan toe ongekend snelle ontwikkeling van mogelijke medicijnen. Maar ook al kwam al in 1987 het eerste anti-HIV middel op de markt, het duurde tot 1995 totdat duidelijk werd dat een combinatie van ten minste drie verschillende middelen als enige geschikt was om het virus te onderdrukken. Deze behandeling wordt combinatie antiretrovirale therapie (cART) of highly active antiretrovirale therapie (HAART) genoemd. Ondertussen is, mede dankzij de ontwikkeling van nieuwere en betere antiretrovirale middelen, de levensverwachting van HIV-patiënten gelijk aan die van mensen zonder HIV. Helaas betekent dat niet dat alle problemen over zijn. Omdat cART het virus alleen onderdrukt en niet geneest, is het van belang dat een patiënt de rest van zijn leven deze medicijnen blijft slikken, met mogelijke bijwerkingen en toxiciteit tot gevolg. Bovendien is gebleken dat HIV-patiënten een hogere kans hebben op het ontwikkelen van bijkomende ziekten (comorbiditeiten) zoals hart- en vaatziekten, botontkalking, cognitieve achteruitgang en bepaalde vormen van kanker. Inzicht krijgen in de mechanismen en behandelingen van deze comorbiditeiten is de nieuwste uitdaging binnen de HIV-zorg.

Dit proefschrift gaat over HIV-geassocieerde cognitieve achteruitgang, een veel voorkomende comorbiditeit van HIV-infectie, en een aandoening die zich op veel verschillende manieren kan manifesteren. Voor de komst van cART was dat met name in de meest ernstige vorm (HIV-dementie), tegenwoordig zijn het met name mildere vormen waar patiënten onder lijden. De verschillende vormen en uitingen worden in de internationale literatuur samengevat onder de noemer HIV Associated Neurocognitive Disorders (HAND).

Het feit dat HIV een beschadigend effect heeft op het brein was al bekend sinds het begin van de HIV-pandemie, toen fulminante dementie vaak voorkwam bij vergevorderde AIDS. Sinds cART overlijden HIV-patiënten bijna nooit meer aan AIDS en leven ze langer. Daarom wordt het steeds belangrijker om de mechanismen achter comorbiditeiten zoals HAND te ontrafelen en zo hopelijk tot een behandeling te komen. Een van die mogelijke mechanismen is een potentieel toxisch effect van cART op het brein. Er zijn hier aanwijzingen voor uit eerdere klinische en laboratoriumstudies. Ook het feit dat patiënten soms heftige cognitieve bijwerkingen van hun cART rapporteren steunt

deze theorie. Dit is het meest bekend bij het middel Efavirenz. Efavirenz is onderdeel van Atripla, de eerste combinatiepil die alle drie de antiretrovirale middelen van een cART regime in één pil verenigde. Atripla was vanwege het gebruikersgemak heel lang de eerste keus voor de behandeling van HIV. Tegenwoordig wordt het met name veel gebruikt in niet-Westerse landen. In de praktijk werd vaak gezien dat een groep patiënten meteen veel bijwerkingen ondervond en daarom stopte met Atripla, wat betekent dat de patiënten die er mee door zijn gegaan weinig tot geen bijwerkingen ervaarden.

Om te onderzoeken of Efavirenz ook vermindering van cognitie geeft bij patiënten die dat zelf niet merken (asymptomatisch patiënten) wordt in hoofdstuk 2 een studie beschreven waarbij patiënten op Atripla overgingen naar een ander middel en cognitietesten werden afgenomen. Ook de concentratie van het geneesmiddel in het bloed werd bepaald en vergeleken met cognitie en een nieuwe biomarker in hoofdstuk 3. In een landelijke database is verder onderzoek gedaan naar cognitieve bijwerkingen van nieuwere antiretrovirale medicijnen, zoals wordt beschreven in hoofdstuk 4.

Wat onderzoek naar HAND lastig maakt, is dat er (nog) geen overeenstemming bestaat over de manier waarop HAND vastgesteld kan of moet worden. Een neuropsychologisch onderzoek (NPO) is de gouden standaard, maar er is discussie danwel onzekerheid over de afkapwaarden, de vorm en de grootte van het NPO, en de benodigde normatieve data. En omdat een NPO een paar uur in beslag neemt en door getraind personeel afgenomen moet worden, is er een grote vraag naar simpele, korte screeningstesten. In hoofdstuk 5 worden de uitkomsten van een aantal screeningstesten in de polipopulatie van het UMC Utrecht beschreven, en de vervolgstappen bij een afwijkende screening belicht. Daarnaast wordt in hoofdstuk 6 een cultuur- en taalneutrale screeningstest in een rurale populatie in Zuid-Afrika getest.

Naast screeningstesten zijn biomarkers ook een mogelijke oplossing voor het diagnostische dilemma van HAND. Een nieuwe onderzoeksmethode is functionele MRI (fMRI), waarbij de activatie van verschillende hersengebieden tijdens het uitvoeren van een taak in kaart wordt gebracht. Blood Oxygenated Level Dependent (BOLD) fMRI is een techniek die het verschil tussen zuurstofrijk en zuurstofarm bloed gebruikt om hersenactivatie in kaart te brengen. BOLD fMRI wordt al veel gebruikt in (neurologische) aandoeningen zoals MS en schizofrenie, maar of het een rol kan spelen bij HAND is nog niet duidelijk. Daarom wordt in hoofdstuk 7 een review beschreven waarin op een systematische manier studies met BOLD fMRI in HIV-patiënten worden samengevat.

### Resultaten

Deel 1 van dit proefschrift is met name gericht op het effect van cART op het ontwikkelen van neurocognitieve achteruitgang. Hoofdstuk 2 beschrijft de bevindingen van de

ESCAPE studie waarbij asymptomatische patiënten op Atripla wisselden naar Eviplera; een combinatietablet zónder Efavirenz. Met een uitgebreid NPO werd hun cognitie in kaart gebracht vóór en 12 weken na de switch. Ook was er een controlegroep die Atripla bleef gebruiken. Na 12 weken bleek de Eviplera-groep het significant beter te doen op het NPO dan de controlegroep, en dan met name op de domeinen aandacht en snelheid van informatieverwerking. Dit is interessant, omdat deze domeinen gebruik maken van het frontostriatale netwerk. Dit is een term voor verschillende neuronale verbindingen die de frontaalkwab verbinden met het striatum, één van de diepe hersenkernen. Deze verbindingen zijn met name betrokken bij executief functioneren en andere gedragsmatige aspecten zoals inhibitie en reactie op beloning. In andere studies die het effect van HIV op het brein onderzochten, kwam ook vaak schade aan het frontostriatale systeem aan het licht. Mogelijk is het effect van Efavirenz op het brein vergelijkbaar met dat van de HIV-infectie zelf, of versterken ze elkaar. Wat verder opviel, was dat het effect van de switch het grootste was op de moeilijkste taken van het NPO. Dit past binnen de zogeheten 'brain reserve theory' waarbij er van uit wordt gegaan dat iedereen een reserve brein capaciteit heeft, wat kan worden aangesproken op het moment dat er een hogere vraag is naar cognitieve functies. Een probleem met de brein reserve zou men dan tijdens gewone dagelijkse bezigheden (bijna) niet merken, en dat zou ook kunnen verklaren dat de patiënten in de ESCAPE studie asymptomatisch waren; i.e. dat ze geen klachten ervaarden van hun cognitie. Wat daarbij nog belangrijk is om op te merken, is dat bijna alle patiënten die waren gewisseld van medicijn niet meer terug wilden naar Atripla, omdat ze zich stukken beter voelden zonder Efavirenz.

Tijdens de ESCAPE studie zijn ook de concentraties van Efavirenz gemeten en vergeleken met NPO-uitslagen. De uitkomsten van deze analyse staan beschreven in hoofdstuk 3. Het bleek dat een hogere Efavirenz concentratie geassocieerd was met een slechtere score op het NPO, én dat patiënten die de studie ingingen met een hogere concentratie ook meer profijt hadden van de switch. Deze uitkomsten versterken de hypothese dat Efavirenz toxisch is voor het brein. Verder wordt in hoofdstuk 3 ook de bruikbaarheid beschreven van een nieuwe biomarker; neurofilament light (NfL). NfL is een afbraakproduct van axonen/zenuwvezels en een verhoogde concentratie hiervan in hersenvocht is eerder al gelinkt aan HIV-gerelateerde schade aan het brein. Recentelijk is het ook mogelijk geworden om NfL in bloedplasma te meten, wat beduidend makkelijker te verkrijgen is dan hersenvocht. Plasmaconcentraties van NfL van de deelnemers van de ESCAPE werden vergeleken met hun NPO-uitkomsten, maar er werd geen correlatie gevonden. Op basis van deze studie is plasma NfL dus geen goede biomarker voor HIV-geassocieerde cognitieve achteruitgang gebleken.

De ESCAPE studie was gericht op Efavirenz, maar dit is ondertussen al een wat ouder geneesmiddel. Nieuwere middelen beloven over het algemeen minder bijwerkingen.

Een recent middel, Dolutegravir, had hoge verwachtingen omdat uit de eerste studies bleek dat het maar weinig bijwerkingen had, en dat maar 2-3% van de patiënten stopten met het middel als ze er eenmaal mee waren gestart. Helaas bleek na de introductie in de praktijk dat toch best veel patiënten bijwerkingen ervaarden, en ook zeker op het gebied van cognitie/neurologie. In hoofdstuk 4 hebben we daarom gekeken naar de hoeveelheid patiënten op Dolutegravir die stopten met dit medicijn, en de redenen om te stoppen. We keken ook meteen naar Elvitegravir, een middel uit dezelfde klasse (integrase remmers). Om een zo compleet mogelijk beeld te krijgen, is gebruik gemaakt van de database van de stichting HIV monitoring (SHM). Deze database houdt de gegevens bij van alle HIV-patiënten in Nederland en geeft zo dus een heel compleet beeld van een volledige populatie. Het bleek dat 12,7% van alle patiënten die startten met één van beide medicijnen uiteindelijk er weer mee stopte. De belangrijkste reden om te stoppen was (neuropsychiatrische) toxiciteit. Deze studie laat twee belangrijke zaken zien; dat ook bij de nieuwe medicijnen neuropsychiatrische bijwerkingen optreden, en dat we bij nieuwe medicatie voorzichtig moeten zijn met grote beloftes, zeker tot de real-world data bekend is.

In deel 2 van dit proefschrift wordt meer gekeken naar het voorkomen en het opsporen van HAND. In hoofdstuk 5 wordt data beschreven van de jaarlijkse cognitieve screening van patiënten op de HIV-poli van het UMC Utrecht. Alle HIV-patiënten die onder behandeling zijn in het UMC Utrecht wordt jaarlijks een cognitieve screening aangeboden. Hierbij worden twee objectieve screeningstesten gedaan; de HIV Dementia Scale (HDS) en de Montreal Cognitive Assessment (MoCA). Daarnaast worden vragenlijsten over stemmingsstoornissen en participatie afgenomen, en worden patiënten gevraagd naar subjectieve veranderingen in cognitie. Van de bijna 300 patiënten die zijn meegenomen in deze studie bleek ongeveer de helft subjectieve klachten te hebben, en/of onder de afkapwaarde van een van de screeningstesten te scoren. Daarnaast bleken angst/stemmings-klachten aanwezig bij 40% van de patiënten. Verder bleek bij 59% van de patiënten een mogelijke onderliggende oorzaak voor de klachten te spelen, zoals bijvoorbeeld een depressie, neurotoxische medicatie, of een traag werkende schildklier. Helaas werd maar bij 20% van de patiënten ook echt actie ondernomen om die oorzaak weg te nemen. Dit is de reden dat we in de studie een voorstel doen voor een stapsgewijs protocol om naar deze oorzaken te kijken en ze waar mogelijk aan te pakken. Voor de patiënten waarbij geen andere oorzaak weg te nemen was, werd een cognitief revalidatieprogramma getest en beschreven in de studie. Bij cognitieve revalidatie wordt er gefocust op het ontwikkelen van strategieën om in het dagelijks leven om te gaan met cognitieve beperkingen. Hoewel de onderzoeksgroep erg klein was (5 patiënten), waren de resultaten van het revalidatietraject veelbelovend. Patiënten rapporteerden een stijging in de mate van slagen van tevoren opgestelde doelen van 2.3 punten op een schaal van 10, een behoorlijke verbetering.

correctie voor multipele vergelijkingen hadden gedaan. In de review waren ook een aantal studies die keken naar een interactie tussen HIV-status en leeftijd, maar hier waren geen aanwijzingen voor. Er waren maar drie studies die het effect van HIV-medicatie op fMRI uitkomsten onderzochten. Deze studies vonden dat patiënten met medicatie meer breinactivatie lieten zien bij moeilijkere taken, en soms zelfs de taken ook minder goed uitvoerden dan patiënten zonder medicatie. Al met al laten de studies in deze review zien dat BOLD fMRI een veelbelovend instrument kan zijn voor het onderzoek naar HAND en het effect van medicatie hierop. Hierbij moet wel altijd zeer goed gekeken worden naar de juiste methodologie en statistische analyses.

**Conclusie**

Samenvattend zijn er een aantal conclusies te trekken danwel lessen te leren van het onderzoek uit dit proefschrift. Ten eerste is het nu duidelijk dat Efavirenz een negatief effect heeft op cognitie, zelfs in asymptomatische patiënten. Hierbij speelt de concentratie in het bloed en dus mogelijk de dosering ook een rol. Het feit dat medicatie zelfs zonder duidelijke subjectieve bijwerkingen wel een effect kan hebben op cognitie, samen met de bevinding dat ook de nieuwere medicijnen klachten op neurologisch en cognitief gebied kunnen geven, laat ons zien dat het nodig is om altijd uitvoerig onderzoek te doen naar deze bijwerkingen bij zowel oudere als meer recente antiretrovirale medicijnen. Belangrijk is dan om objectieve meetmethoden te gebruiken, zoals een NPO. Vanuit de resultaten van de ESCAPE studie en in lijn met de brain reserve theory zou dit NPO dan idealiter veel uitdagende taken moeten bevatten, die de brein reserve aanspreken, en genoeg taken die executief functioneren testen. Een biochemische biomarker voor cognitieve achteruitgang in HIV-infectie is er nog niet, zoals de NfL-studie laat zien. Wel zijn er hoopgevende signalen over het gebruik van BOLD fMRI als diagnosticum. Hierbij is het frontostriatale systeem de regio waar de meeste focus op zal komen te liggen. Ten tweede is bewezen dat HAND vaak voorkomt, en dat er vaak een link bestaat met stemmingsstoornissen. We hebben een duidelijk stapsgewijs protocol ontwikkeld voor wat er moet gebeuren als iemand bij een screening uitvalt. Ook bleek een cognitief revalidatieprogramma behulpzaam. Als laatste bleek dat het een uitdaging is taal en cultuur neutrale screeningstesten te vinden voor niet-Westerse landen, en dat in deze populatie het gebruik van passende normatieve data in het bijzonder belangrijk is.

HAND is een veel voorkomende aandoening bij HIV-patiënten, en heeft een impact op bijna alle onderdelen van hun leven. Het is ontzettend belangrijk dat er meer onderzoek komt naar het opsporen en behandelen van deze comorbiditeit.

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# CURRICULUM VITAE

Charlotte Hakkers was born on 25 November 1986 in Sleeuwijk, the Netherlands. After graduating from the Gymnasium Camphusianum in Gorinchem, she started medical school at Maastricht University. A technical error in assigning elective courses in her second year had her somewhat reluctantly placed in a medical microbiology elective under supervision of Prof. Dr Annelies Verbon. This turned out to be the onset of her interest and enthusiasm for infectious diseases and HIV in particular. Furthermore, during her time at medical school she completed clinical rotations in Uganda and South Africa and did a scientific internship at the department of medical microbiology in Maastricht, under supervision of Dr Petra Wolffs and Dr Judith Beuving. After obtaining her medical degree in 2012, she worked as a resident (ANIOS) internal medicine at the Isala hospital in Zwolle under supervision of Dr P.H.P. Groeneveld and Dr H.P.E. Peters. In June 2013, she was accepted for a residency in internal medicine at the UMCG in Groningen, the first three years of which she was to continue at the Isala in Zwolle. In September 2014, she got the opportunity to start a PhD project at the department of infectious diseases at UMC Utrecht, where she was supervised by Prof. Dr A.I.M. Hoepelman and Dr J.E. Arends. In January 2018, she decided to resume her residency at the UMC Utrecht under supervision of Prof. Dr K. Kaasjager and Dr J.J. Oosterheert instead of returning to Groningen. From January 2019 to September 2020, she did a part of her residency at the Diakonessenhuis in Groningen under supervision of Dr G.A.J. van Boekel, before returning to UMC Utrecht. She lives in Zeist with Aldert Bergsma and their two children, Noor and Sil.