Cognitive impairment and MRI-findings in patients with HIV on antiretroviral treatment
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GENERAL DISCUSSION, CLINICAL IMPLICATIONS AND FUTURE PERSPECTIVES
GENERAL DISCUSSION

There are a limited number of studies available that assess human immunodeficiency virus (HIV) related effects on cognitive function, brain structure and physiological alterations within the context of systemically well-controlled infection by combination antiretroviral therapy (cART). These data are particularly sparse for the aging HIV-infected population receiving long-term effective cART. The research from this thesis is therefore a significant contribution to the current available scientific literature on this topic. It has lead to the insight that only a limited proportion of middle-aged aviremic HIV-infected patients on long-term cART appears to be cognitively impaired, which is characterized by a wide range of subtle cognitive deficits. In addition, subtle brain structure and physiological alterations exist in this population of adequately treated HIV-infected patients. Several determinants, including measures related to past immune deficiency, vascular and metabolic risk factors, were found to be associated with HIV-associated cognitive dysfunction, brain structure and physiological alterations, providing support for a multifactorial etiology. In addition to confirming commonly known factors associated with HIV infection (i.e. viral toxicity, immune activation and inflammation), there is increasing evidence to suggest a relationship between HIV and vascular disease. Such an association may play a more important role for patients receiving long-term cART with well-controlled HIV-infection. The research in this thesis provides support to start cART treatment at an early stage, to prevent HIV and vascular disease progression. In this chapter, the major findings of this thesis are discussed in relation to relevant literature findings, clinical implications, and the implications for future research.

HIV-associated cognitive impairment

The current number of available studies on cognitive impairment within the HIV-infected population receiving long-term cART with systemically well-controlled infection is limited. The number of patients investigated within each study is relatively small; the total number of patients in all studies combined is 520.[1–7] Therefore, the inclusion of 103 patients in our neuroimaging sub-study of the AGEhIV cohort contributes significantly to the currently available literature.[8]

HIV-infected patients in our study had poorer scores for all cognitive domains compared to HIV-uninfected controls. Statistically significant group differences were observed for the domains of attention, executive function and information processing speed. Cognitive impairment, as defined by Frascati criteria, was highly prevalent in HIV-infected patients but nearly equally so in HIV-uninfected controls (48% vs 36%). The multivariate normative comparison (MNC) is a statistical method specifically designed to control the false positive rate while retaining sensitivity. Applying MNC, a lower prevalence of 17% of HIV-associated cognitive impairment was found in HIV-infected men with suppressed viremia on cART.[8]

Most available studies have applied the Frascati criteria for HIV-associated neurocognitive disorders (HAND) to define cognitive impairment. In addition, a homogenous population of HIV-infected patients was carefully selected i.e. patients with similar disease and treatment status were included and comorbid conditions known to affect cognition were excluded. However, the reported prevalence of cognitive impairment remained high, ranging from 22-41%.[2,3,5–7] Two studies have reported the prevalence of cognitive impairment in the control group, which varied from 13-31%.[6,7]
These results show that the prevalence of cognitive impairment as defined by the Frascati criteria in HIV-uninfected controls is nearly as high as for the HIV-infected patient group.[6–8] This illustrates the low specificity and supports the raised concern of overestimating HIV-associated cognitive impairment using Frascati criteria.[9] These findings underline the importance to include a (representative) control group to enable demonstration of such shortcomings.

There are two major shortcomings of the Frascati criteria for HAND that may explain the high risk of false positive findings and the low specificity of these criteria. Firstly, given the low threshold to classify cognitive impairment i.e. with a cut-off of 1 standard deviation (SD) below the normative mean, implies that by definition 16% of the general population meets this criteria on a given test, assuming that test scores are normally distributed.[9,10] Secondly, the multiple univariate comparisons performed (i.e. at least two measures per domain for a total of 7 domains is recommended) increase the probability of making one or more false discoveries, the so-called family-wise error.[9,10] The MNC method enables more reliable detection of cognitive impairment. With its multivariate nature, the sensitivity to detect milder forms of HIV associated cognitive impairment can be maintained, while the number of false positives can be kept low.

The MNC method compares the neuropsychological test performances of each individual HIV-infected patient to the performances of the entire group of HIV-uninfected otherwise similar controls (i.e. the reference). It enables the detection of small deviations from the reference across the complete spectrum of cognitive performances within one statistical comparison, thereby controlling the family-wise error.[11] The false positive rate, i.e. erroneously concluding that an individual is impaired, is set at 5% (one tailed). In addition, multiple univariate comparisons are more likely to miss small deviations from the reference due to the large variability in cognitive performance that exist within the normal population.[12] The variability in performances across multiple tasks has been suggested to be a more sensitive predictor of impairment of everyday functioning, as compared to averaged composite measures of cognitive performance.[13]

Applying the MNC method, using the HIV-uninfected controls from the AGEhIV cohort as the normative group, the prevalence of HIV-associated cognitive impairment in our patient population was reduced from 48% (according to Frascati criteria) to 17%.[8] This value is more in line with the estimated prevalence based on observations from current clinical practice.[9] Similar findings were reported in a study by Janssen and colleagues in which the prevalence of HIV-associated cognitive impairment was reduced from 41% (according to Frascati criteria) to 11% (according to MNC).[7] Therefore, the MNC method is a more reliable method compared to the Frascati criteria to detect cognitive impairment among HIV-infected patients on effective cART.

Generally, HIV-infected patients show similar neuropsychological performances compared to HIV-uninfected controls,[14] however a small but still substantial proportion of patients show worse performances. As a result, HIV-infected patients perform, on average, slightly worse than HIV-uninfected controls.[7,8] Within our study, the patients classified as cognitively impaired showed mild cognitive deficits across a wide range of cognitive domains (Figure 1, Chapter 2). This finding corresponds with the previously reported complaints by cognitively impaired HIV-infected patients, namely difficulty with completing tasks involving serial steps, having trouble with focusing on details for long periods or memorizing a list of words.[15,16]
Risk factors

In our study, decreased cognitive performance (as determined by MNC) in HIV-infected patients was found to be associated with lower nadir CD4 counts, history of prior cardiovascular disease, diabetes mellitus type 2, impaired renal function and cannabis use.[17] Although several mechanisms have been proposed to contribute to HIV-associated cognitive impairment in those with systemically well-controlled infection by cART, studies on this topic are sparse. However, cognitive impairment has been associated with measures of immune deficiency (i.e. current and nadir CD4 and having experienced prior clinical acquired immune deficiency syndrome (AIDS)-related conditions), as is the case for our research as described in this thesis.[2,6,17] This provides support for the hypothesis that current or past immune deficiency remains a risk factor of cognitive impairment in the context of long-term effective cART.

Furthermore, increased levels of systemic markers of inflammation and immune activation (i.e. TNF-α, β-2-microglobulin; chronically activated and senescent CD8 cells and apoptotic CD4 cells) are reported in HIV-infected patients, despite suppressed HIV on long-term cART, compared to HIV-uninfected controls.[6] Weak associations between such markers of inflammation and immune activation (i.e. IL-6 and chronically activated CD8 cells) and the performance on a single cognitive domain were reported.[6] However, the authors suggest that systemic inflammation and immune activation are not major drivers of cognitive decline in HIV. They emphasize that markers of inflammation and immune activation in the cerebral spinal fluid (CSF) are expected to be more strongly associated with cognitive dysfunction. This was confirmed by another study, which found that in the context of effective suppression of HIV by long-term cART, HIV-associated cognitive impairment is associated with immune activation (i.e. neopterin levels) within the CSF.[18] This suggests that neuro-inflammation persists, despite systemically well-controlled HIV by cART.[19] However, within this study HIV-uninfected controls were lacking. Therefore, it remains unclear if such an association is specific to chronically suppressed HIV-infection. The effect of intrathecal immune-activation in patients on effective cART is poorly understood and future studies on this topic are warranted and currently being conducted by our group.

In our study, a wide range of vascular and metabolic risk factors (i.e. past cardiovascular disease, impaired renal function and diabetes mellitus) were found to be associated with decreased cognitive performance in HIV-infected patients.[17] Such vascular and metabolic risk factors are known to impact cognitive function in HIV-uninfected controls.[20] The current findings suggest these factors may become of increasing importance in HIV-infected patients on long-term effective cART as drivers of cognitive decline.

Other risk factors for HIV-associated cognitive impairment include age, lower premorbid IQ, psychological distress (i.e. anxiety, depression, somatization, sleep problems, subjective cognitive complaints, health distress, social functioning, energy level and general health perceptions) and lifestyle factors (i.e. frequent cannabis use).[7,17] These findings are supported by our research in this thesis.

The HIV-infected population is aging, the proportion of older HIV-infected patients continues to increase, therefore the subject of cognitive impairment in aviremic HIV-infected
patients on cART may become an increasingly important topic of research and clinical care. A further understanding of the risk factors for cognitive impairment in HIV-infected patients with systemically well-controlled infection on long-term cART is necessary to facilitate the development of preventative strategies and treatments.

**Brain alterations**

Brain alterations found in HIV-infected patients in our study consist of macrostructural brain alterations of lower grey matter (GM) volume and increased load of white matter hyperintensities (WMH) of presumed vascular origin.[21] In addition, microstructural white matter (WM) alterations were present, which consisted of a diffuse pattern of lower fractional anisotropy (FA) and higher mean diffusivity (MD) throughout the brain.[22] Finally, hemodynamic brain alterations of lower cerebral blood flow (CBF) were found.[23] Amongst this spectrum of brain structure and physiological alterations, only the extent of WMH of presumed vascular origin was found to be associated with decreased cognitive performance in HIV-infected men with suppressed viremia on cART.[21]

The number of studies on macrostructural and microstructural brain alterations as well as hemodynamic changes in the HIV-infected population receiving long-term cART with systemically well-controlled infections is also limited. Lower brain volumes have been reported in HIV-infected patients compared to HIV-uninfected controls.[7] Likewise, in our study, lower GM volumes were found in HIV-infected patients compared to controls.[21] Lower GM volumes in the medial and superior frontal gyrus and the thalamus in HIV-infected patients in comparison to HIV-uninfected controls have been reported in other studies.[7,14] In addition, lower brain volumes have been associated with decreased information processing speed and motor function in HIV-infected patients.[7] Our study was the first to examine WMH of presumed vascular origin in middle-aged HIV-infected patients with durable viral suppression on cART. Such WMH were more extensive in HIV-infected patients compared to HIV-uninfected, otherwise similar controls, and associated with HIV-associated cognitive deficits.[21]

In contrast to the results reported on macrostructural brain alterations, results across diffusion tensor imaging (DTI) studies on exclusively aviremic HIV-infected populations on long-term cART are less consistent. Two studies reported no microstructural WM alterations in HIV-infected patients in comparison to HIV-uninfected controls.[14,24] In contrast, HIV-infected patients showed WM structure alterations in comparison to HIV-uninfected, otherwise similar controls in our study.[22] This consisted of a diffuse pattern of lower FA and higher MD throughout the brain. Towgood and colleagues assessed brain physiology by means of arterial spin labeling (ASL) within their cohort of HIV-infected patients on successful treatment and they reported no group differences between HIV-infected patients and HIV-uninfected controls with regard to cerebral blood flow (CBF).[25] However, a decreased CBF (after adjusting for age, ecstasy use and waist circumference) was found among HIV-infected patients compared to HIV-uninfected controls in our study.[23] Such discrepant findings on microstructural WM and hemodynamic alterations could be explained by the sample size of these studies. The extent of microstructural WM alterations and hemodynamic alterations found in our study were subtle and widespread. This subtlety of the HIV-effect on microstructural WM alterations, hemodynamic...
alterations and cognitive function, suggests that a possible relationship would likely be subtle as well. This may explain the lack between such microstructural and hemodynamic alterations and HIV-associated cognitive decline.

In conclusion, these findings suggest that besides subtle cognitive deficits, macrostructural, microstructural and hemodynamic brain alterations exist in the era of long-term effective cART. The successful treatment of HIV and exclusion of otherwise confounding factors may explain the subtlety of the observed alterations. In addition, it could explain the variability in associations found between brain alterations and HIV-associated cognitive impairment.

Pathogenesis

Of the few neuroimaging studies available on the HIV-infected population with systemically well-controlled infection on long-term cART, a minority explored potential determinants of brain alterations, and most were unable to detect any significant determinants.[7,14,24,25] A major strength of our study is that we have applied several MRI techniques and that we have explored potential determinants for each individual technique. A HIV-effect was found in each neuroimaging technique that we have studied and different determinants were identified (Table 1). The findings suggest that several pathogenic mechanisms may underlie HIV-associated brain alterations in the context of sustained suppressed HIV viral load on effective cART.

HIV-specific factors such as duration of exposure to immune deficiency (i.e. the number of years spent with CD4 cell counts < 500 cells/mm³) and having experienced prior clinical AIDS

<table>
<thead>
<tr>
<th>Determinants</th>
<th>WMH</th>
<th>FA/MD</th>
<th>CBF</th>
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<tbody>
<tr>
<td>HIV-seropositive status¹</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Age</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Brain volume</td>
<td>-</td>
<td>+</td>
<td></td>
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<tr>
<td>Years spent with low CD4 cell count²</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Having had a previous AIDS defining condition</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Number of antihypertensives used</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>+</td>
<td>-</td>
<td>-</td>
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<tr>
<td>D-dimer</td>
<td>+</td>
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<tr>
<td>LDL-cholesterol</td>
<td>-</td>
<td>+</td>
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<tr>
<td>Tryglycerides</td>
<td>-</td>
<td>-</td>
<td>+</td>
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<tr>
<td>Waist circumference</td>
<td>-</td>
<td>-</td>
<td>+</td>
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</tbody>
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Abbreviations: WMH=white matter hyperintensities, FA=fractional anisotropy, MD=mean diffusivity, CBF=cerebral blood flow

+ p<0.05
~p<0.10
- not significant

1 Multiple regression model including data from HIV-infected patients and HIV-uninfected controls
2 CD4 < 500 cells/mm³
were found to be associated with microstructural WM alterations, WMH of probable ischemic consequences of cerebral small vessel disease (cSVD), and hemodynamic alterations. Moreover, such HIV-associated brain alterations were also found to be associated with vascular and metabolic risk factors (i.e. number of anti-hypertensives used, diastolic blood pressure, D-dimer, low density lipoprotein (LDL) cholesterol, triglyceride levels and waist circumference, summarized in Table 1).

These findings suggest that part of such HIV-associated brain alterations may have occurred during periods of immune deficiency, when viral toxicity and host-derived proinflammatory factors were at their peak, and reflect irreversible damage. Associations found between the extent of brain alterations and older age within HIV (although an interaction effect of HIV by age was not found) provides further support. It may suggest a so-called legacy effect; that older HIV-infected patients are infected for a longer period and have been exposed to host and viral factors associated with long-term survival in the absence of treatment, or have been treated with more toxic antiretroviral agents which did not result in durable suppression of HIV replication.[16] Toxicity and proinflammatory responses induced by host and viral factors (Figure 2, Chapter 1) may induce structural and chemical changes, affecting synaptic function and possibly leading to widespread synapto-dendritic neuronal injury.[26] Besides neuronal injury, vessel wall damage may have occurred during previous periods of immune deficiency, by directly infecting the vessel walls and indirectly by HIV-mediated pro-inflammatory responses of the vessel wall. Moreover, such vessel wall inflammation may be further exacerbated by direct toxicity of cART or indirectly by cART-related metabolic complications. The umbrella term of HIV-associated vasculopathy was proposed to cover the pathological mechanisms of direct and indirect effects of HIV-infection and cART. Such pathogenic mechanisms are illustrated in Figure 3, Chapter 1.[27]

In addition, the associations found between HIV-associated brain alterations and the vascular and metabolic risk factors provide support for potential ongoing vessel wall damage or current HIV-associated vasculopathy, despite systemically effective cART.[27] Particular cART regimes have been associated with direct vessel wall damage as well as vascular and metabolic risk factors (i.e. hypertension, dyslipidemia and insulin resistance) impacting vessel wall function indirectly. Moreover, since increased immune activation, a known complication of HIV, remains prevalent even in HIV-infected patients on long-term effective cART (i.e. higher levels of soluble CD14 and lower CD4/CD8 ratio), vessel walls may be affected by ongoing immune activation and inflammation.[28–31]

In the HIV-infected population, age-related comorbidities are increasingly recognized. While no interaction effect for HIV status by age has been found, the associations between the extent of brain alterations and older age may suggest premature or accelerated (vascular) aging in HIV-infected patients. In younger HIV patients, brain alternations appear early but progress in a similar pace as during normal aging. In older HIV patients, brain alternations appear to progress at a faster rate than in normal aging.[32,33] Moreover, macro-structural grey matter volume decreases and microstructural WM alterations have been associated with MRI-findings of vascular pathology before.[34–36]

In conclusion, different pathogenic mechanisms (i.e. synapto-dendritic injury and endothelial dysfunction) may exist simultaneously within the brain of HIV-infected patients with systemically well-controlled infection by cART. Such pathogenesis may become more prominent with advancing age of the HIV-infected population,[37] and eventually result in cognitive deterioration.[20]
Limitations
Although several systemic markers of inflammation and immune activation were explored, we did not have access to such markers in the CSF. The presence of HIV-associated cognitive impairment despite systemically well-controlled infection on long-term cART may be explained by ongoing immune activation and low-grade viral replication within the central nervous system (CNS). This may be explained by the ability of cART to cross the BBB.[38] A recent pilot study of cART intensification with maraviroc, a CCR5 antagonist with a good CNS penetration and anti-inflammatory properties, provided support for improvement of HIV-associated cognitive impairment in virally suppressed (in blood and CSF) HIV-infected patients.[39] This suggests that ongoing viral activity with inflammation and immune activation in the CNS may exist in aviremic HIV-infected patients on cART. However, despite improvement of cognitive function, no changes were detected in CSF biomarkers, which may be explained by the small sample size.

Clinical implications
The research of this thesis has resulted in several insights and clinical implications. It underlines the importance of preventing prolonged immune deficiency, given its association with brain alterations and cognitive impairment. In addition, it provides support for the importance of monitoring cardiovascular risk factors and promoting healthy life styles in HIV-infected patients. Moreover, it revealed the importance of reliable detection of HIV-infected patients with subtle cognitive deficits. This has contributed to the insight that the underlying etiology is multifactorial. Therefore, strategies to improve cognitive function in HIV should promote strict medication adherence and diminish risk factors for vascular disease.

Future perspectives
Further research on larger samples of HIV-infected patients with cognitive impairment is warranted to provide further insights to their risk factors. Moreover, additional research on measures of ongoing low grade viral replication, immune activation and inflammation (particularly within the CNS) is of importance to provide further understanding of the pathogenesis of HIV-associated cognitive impairment in adequately treated HIV-infected patients. In addition, longitudinal follow-up, particularly of older patients (> 60 years), will provide insight on the progression of such cognitive deficits, brain structure and physiological alterations and reveal potential amplifying effects of age (i.e. accelerated vascular aging). Other measures of vascular pathology could also be explored (e.g. measures of carotid media thickness) which may serve as a more feasible biomarker for HIV-associated cognitive impairment. Finally, the effects of a wide range of potential pharmacological (i.e. cART with a high ability to cross the BBB, so called neuro-cART e.g. maraviroc)[40] or psychological and behavioral (i.e. improving psychological well-being or cognitive strategies, or promoting lifestyle factors) interventions could be investigated. This may enable successful treatment of milder forms of HIV-associated cognitive impairment in the near future. Preventing cognitive impairment is of high importance; milder forms of HIV-associated cognitive impairment are difficult to improve via cART but they are functionally significant, impacting ability to work, cART adherence and quality of life.
REFERENCES


