

# Cerebral perfusion in the prodementia stages of Alzheimer's disease

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## Abstract

**Objectives** To investigate arterial spin-labelling (ASL) cerebral blood flow (CBF) changes in prodementia stages of Alzheimer's disease (AD).

**Methods** Data were obtained from 177 patients with subjective complaints, mild cognitive impairment and AD from the Amsterdam Dementia Cohort. AD stages were based on diagnosis and cerebrospinal fluid biomarkers amyloid- $\beta$  (A $\beta$ ) and total-tau (tau). General-linear-models were used to assess relationships between AD stages and total and regional CBF, correcting for age and sex.

**Results** Decreasing CBF was related to more advanced AD stages in all supratentorial regions (p for trend < 0.05). Post-

hoc testing revealed that CBF was lower in AD compared to controls and stage-1 prodementia patients (i.e. abnormal A $\beta$  and normal tau) in temporal and parietal regions, and compared to stage-2 prodementia patients (i.e. abnormal A $\beta$  and tau) in temporal regions. CBF values of stage-2 prodementia patients were numerically in between those of stage-1 prodementia patients and AD.

**Conclusion** The continuing decrease of CBF along the continuum of AD indicates the potential of ASL-CBF as a measure for disease progression.

## Key Points

- Decreasing CBF relates to more advanced AD stages in all supratentorial regions.
- The reduction of CBF does not reach a bottom level.
- ASL-CBF has potential as a measure for disease progression in AD.

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**Keywords** Arterial spin-labelling MRI · Cerebral blood flow/cerebral perfusion · Alzheimer's disease · Prodromal AD · Disease progression

## Abbreviations

A $\beta$	Amyloid beta
NGMV	Normalized grey matter volume
PCASL	Pseudo-continuous arterial spin-labelling
PPC	Precuneus and posterior cingulated
PVC	Partial volume corrected
P-tau	Phosphorylated-tau
SC	Subjective complaints
T-tau	Total-tau
WMH	White matter hyperintensities

## Introduction

The first pathological signs of Alzheimer's disease (AD), in the form of amyloid- $\beta_{1-42}$  ( $A\beta$ ) plaque deposition, can be detected up to decades before clinical symptoms first occur [1]. However, these very early pathological signs of AD do not correlate with disease progression in clinical AD [2]. Neuronal and synaptic function measures, such as CSF total-tau (tau) and  $^{18}\text{F}$ -fluorodeoxyglucose positron emission tomography (FDG-PET), are thought to start changing later on in the disease process. Moreover, they are thought to relate to cognitive performance, and to continue to change along the disease process, which might make them more suitable for monitoring disease progression [2–4].

Arterial spin-labelling (ASL) is a functional magnetic resonance imaging (MRI) technique that measures cerebral blood flow (CBF) by using magnetically labelled arterial blood that flows through the carotid and vertebral arteries as an endogenous contrast medium. Important advantages of ASL are its non-invasiveness and short acquisition time at higher magnetic field strengths, which allows routine clinical application in the workup of dementia when ASL is added to the standard dementia imaging protocol.

Recently, new diagnostic and scientific criteria for clinical AD and its predementia stages have been introduced by the National Institute on Aging and the Alzheimer's Association (NIA-AA) [5–7]. For scientific purposes, these criteria offer the possibility of subdividing subjects with normal cognition, such as patients with subjective complaints (SC), and patients with mild cognitive impairment (MCI) into different predementia AD stages using  $A\beta$  and neuronal injury biomarkers.

In a previous study we showed that ASL discriminated AD patients from controls, and that regional CBF values of MCI patients were numerically in between those of controls and AD patients [8]. In the current study we aimed to further investigate CBF along the course of AD, by studying CBF of patients in different predementia stages, based on the NIA-AA criteria [5–7].

## Methods

### Subjects

In this cross-sectional study we included 275 patients (107 AD patients, 64 MCI patients and 104 SC patients) from the memory clinic-based Amsterdam Dementia Cohort. All patients visited our memory clinic between October 2010 and June 2012, underwent brain MRI according to the dementia protocol described below, and had cerebrospinal fluid (CSF) available for analysis of CSF biomarkers. All patients underwent a standard dementia screening that included

medical history, physical and neurological examinations, screening laboratory tests, lumbar puncture, neuropsychological testing and brain MRI. Clinical diagnosis was established by consensus in a multidisciplinary team. AD patients met the NINCDS-ADRDA criteria for probable AD [9], and also met the core clinical criteria for probable AD proposed by the NIA-AA workgroup [6]. MCI patients fulfilled the Petersen criteria [10, 11]. Patients were considered to have subjective complaints when clinical investigations and neuropsychological test performance were normal. Patients with a history of head trauma or intracranial tumours were excluded. The local Institutional Review Board approved the study. All patients provided written informed consent.

### MRI acquisition

MRI examinations were performed on a 3T whole body MR system (SignaHDxt, GE Medical Systems Milwaukee, WI, USA) using an 8-channel head coil. Structural images included a sagittal 3D T1-weighted sequence (IR-FSPGR, echo time=3.0ms, repetition time=7.8ms, inversion time=450ms, flip angle=12°, matrix 256×256, 176 slices, voxel size 1×0.9×0.9mm) for anatomical information, and a sagittal 3D fluid attenuated inversion recovery (FLAIR) sequence (CUBE, echo time=123.6 ms, repetition time=8000 ms, inversion time=2351 ms, echo-train length=230, acquisition matrix 224×224, reconstruction matrix 256×256, 132 slices, voxel size 1.2×1×1mm) to determine the severity of white matter hyperintensities (WMH) using the Fazekas scale [12]. Pseudo-continuous ASL (PCASL) [13, 14] perfusion images (3D-FSE acquisition with background suppression, post-label delay 2.0s, echo time=9 ms, repetition time=4.8 s, spiral read-out eight arms × 512 samples; 36×5.0mm axial slices, 3.2×3.2mm in-plane resolution, reconstructed pixel size 1.7×1.7mm, acquisition time 4 min) were calculated using a single-compartment model [15] after the subtraction of labelled from control images. More details are provided in an earlier study by Binnewijzend et al. [8].

### Pre-processing and MRI data analysis

Both T1-weighted and PCASL images were corrected for gradient non-linearities in all three directions. Further data analyses were carried out using FSL (version 4.1; <http://www.fmrib.ox.ac.uk/fsl>). Pre-processing of T1 images consisted of non-brain tissue removal [16], linear registration to standard space [17] and tissue segmentation [18] yielding partial volume estimates. Gray matter volumes, normalized for subject head size (NGMV), were calculated with the SIENAX software tool [16]. The CBF maps [8] were linearly registered to the brain-extracted T1 images. The brain mask was used to calculate uncorrected mean whole brain CBF. Partial volume estimates were transformed to the ASL data space and used in

a regression algorithm [19], using a Gaussian kernel of 9.5-mm full width at half maximum, to create partial volume corrected (PVC) cortical CBF maps. Partial volume estimates were subsequently used as a weighting factor to calculate mean cortical CBF. The MNI152 atlas and the Harvard-Oxford cortical atlas (both part of FSL) were used to create regions of interest (ROIs) of the frontal, temporal, parietal, precuneus and posterior cingulate (PPC), occipital and cerebellar brain areas, to extract mean regional uncorrected and PVC cortical CBF values.

### CSF analysis

CSF was obtained by lumbar puncture of the L3/L4 or L4/L5 intervertebral space, using a 25-gauge needle, and collected via a syringe in 12-ml polypropylene tubes (Sarstedt). A small amount of CSF was used for routine analysis, including total cells (leucocytes and erythrocytes), total protein and glucose. Within 2 hours, CSF samples were centrifuged at  $2100 \times g$  for 10 min at  $4^{\circ}\text{C}$  and the supernatant was transferred into a second polypropylene tube (Sarstedt) and stored at  $-20^{\circ}\text{C}$  until AD biomarker analysis. CSF amyloid- $\beta_{1-42}$  and total-tau were measured with Innostest (Innogenetics) sandwich enzyme-linked immunosorbent assay as described previously [20]. The team involved in the CSF analysis was not aware of the clinical diagnosis.

### Definition of AD stages

Patients were divided into AD stages based on the combination of both syndrome diagnosis and CSF biomarker values, considering  $A\beta$  below 550 ng/L, and tau above 375 ng/L as abnormal (i.e. positive) [20]. For AD, only patients with a high likelihood of having an AD pathophysiology were included. AD patients with normal  $A\beta$  and/or tau values ( $n=41$ ) were excluded. For predementia stages, we collapsed MCI and preclinical stages to overall predementia AD stages to ensure sufficient power. Only patients fitting in the biomarker stages were included. Therefore, MCI patients with normal  $A\beta$  values ( $n=42$ ) and SC patients with normal  $A\beta$  and abnormal tau values ( $n=13$ ) were excluded.

Subsequently, MR images of all patients were visually inspected. One AD patient was excluded because registration of the ASL images to T1 images failed, possibly due to sub-optimal quality of the ASL images. One other AD patient was excluded because of haemochromatosis.

This resulted in the following four study groups: 80 SC patients with normal CSF  $A\beta$  and tau (controls, stage 0), 13 predementia patients (six SC, seven MCI) with abnormal CSF  $A\beta$  and normal CSF tau (stage 1), 20 predementia patients (five SC, 15 MCI) with abnormal CSF  $A\beta$  and tau (stage 2) and 64 clinically demented AD patients with abnormal CSF  $A\beta$  and tau (stage 3).

### Statistics

Statistical analyses were performed using IBM SPSS Statistics for Mac (Version 19.0., IBM Corp., Armonk, NY, USA). For continuous measures, differences between groups were assessed using one-way analyses of variance (ANOVA) with post-hoc Bonferroni tests to correct for multiple comparisons. A chi-squared test was used to compare frequency distributions of sex in all study groups, and syndrome diagnosis (i.e. SC or MCI) in the predementia groups. General linear models (GLMs) were used to assess the relationships between AD stages (entered as continuous measure to assess the dose-response effect) and total and regional uncorrected and PVC cortical CBF, correcting for the effect of age and sex. Post-hoc, univariate GLMs (total CBF) and multivariate GLMs (regional CBF) with Bonferroni tests were performed to compare CBF values of different AD stages, correcting for the effect of age and sex (model 1), and additionally for WMH (model 2). Linear regression analyses were performed across groups and within the study groups to assess relationships between CBF (independent variable) and cognition, using MMSE-scores (dependent variable). Age and sex, and additionally WMH, were entered into the model as covariates.

### Results

Demographics, MRI findings and CSF results are presented in Table 1. Stage-2 predementia patients and stage-3 AD dementia patients were older than controls. Patients with AD dementia had lower MMSE-scores compared to all the other groups. Stage-2 predementia patients and AD dementia patients had more WMH than controls. NGMV was lower in AD dementia patients compared to controls and stage-1 predementia patients, and NGMV was lower in stage-2 predementia patients compared to controls. Finally, by definition, CSF  $A\beta$  was lower in all groups compared to controls, and CSF tau was higher in AD dementia patients and stage-2 predementia patients compared to controls and stage-1 predementia patients.

### Total CBF differences

With increasing AD stage, both uncorrected whole brain and PVC cortical CBF decreased ( $p$  for trend  $< 0.01$ ) (Table 2). Post-hoc tests showed that AD dementia patients had lower uncorrected whole brain CBF compared to controls and stage-1 predementia patients, and lower PVC cortical CBF compared to controls. There were no differences in CBF between predementia patients and controls. Additional correction for WMH did not change the group differences.

**Table 1** Patient characteristics, magnetic resonance imaging (MRI) findings and cerebrospinal fluid (CSF) data

	Control	Predementia	AD	
	Stage 0	Stage 1	Stage 2	Stage 3
Number	80	13	20	64
Age (y) <sup>a</sup>	58±9	64±7	66±5*	66±7*
Females, no. (%)	31 (39)	5 (39)	9 (45)	34 (53)
Diagnosis (MCI), no. (%)	-	7 (54)	15 (75)	-
MMSE <sup>a,b</sup>	28±2	28±2	26±2	21±4* <sup>‡,§</sup>
WMH <sup>a</sup>	0.6±0.7	1.2±0.9	1.2±0.8*	1.1±0.8*
NGMV (mL) <sup>a</sup>	780±53	770±55	733±63*	711±46* <sup>‡</sup>
CSF Aβ <sub>1-42</sub> (ng/L) <sup>a</sup>	986±188	444±89*	442±70*	459±68*
CSF tau (ng/L) <sup>a</sup>	220±73	292±75	782±370* <sup>‡</sup>	796±340* <sup>‡</sup>

<sup>a</sup> Data are given as mean ± standard deviation

<sup>b</sup> Mini Mental State Examination (MMSE) scores of two controls were excluded because they were obtained by means of an interpreter

Stage 0: Aβ-/tau- patients with subjective complaints (SC), Stage 1: Aβ+/tau- patients with SC and mild cognitive impairment (MCI), Stage 2: Aβ+/tau+ patients with SC and MCI, Stage 3: Aβ+/tau+ patients with AD

\*p<0.05 compared to controls, <sup>‡</sup> p<0.05 compared to stage-1 predementia patients, <sup>§</sup> p<0.05 compared to stage-2 predementia patients

AD Alzheimer’s disease, WMH white matter hyperintensities (based on Fazekas-score), NGMV normalized grey matter volume

**Table 2** Region-of-interest based cerebral blood flow (CBF) values

	Control	Predementia	AD	
	Stage 0	Stage 1	Stage 2	Stage 3
Uncorrected whole brain CBF***	32±5	32±5	30±5	28±5 <sup>a,b</sup>
PVC cortical CBF**	48±8	48±7	45±8	43±8 <sup>a</sup>
Regional uncorrected cortical CBF				
Frontal*	22±5	21±5	20±4	19±4
Temporal***	26±5	26±5	24±4	21±4 <sup>a,b,c</sup>
Parietal***	29±5	28±5	26±5	24±5 <sup>a,d</sup>
PPC***	38±7	37±7	34±6	31±6 <sup>a,b</sup>
Occipital***	35±7	34±6	32±7	29±7 <sup>a,d</sup>
Cerebellum	26±5	26±6	25±6	25±5
Regional PVC cortical CBF				
Frontal*	49±9	49±8	47±9	43±9
Temporal**	44±7	44±6	42±8	39±7 <sup>a,d</sup>
Parietal***	55±9	55±9	51±10	47±10 <sup>a,d</sup>
PPC***	63±11	62±10	58±11	55±11 <sup>a,d</sup>
Occipital**	55±9	54±9	51±12	49±10 <sup>a</sup>
Cerebellum	40±9	41±0	36±8	38±10

Results are given as mean ± standard deviation. If analysis of variance was p<0.05 a post-hoc Bonferroni test was performed. Shown results are corrected for age and sex

\*p<0.05, \*\*p<0.01 and \*\*\*p<0.001

<sup>a</sup> p <0.05 compared to controls

<sup>b</sup> p<0.05 compared to stage-1 predementia patients

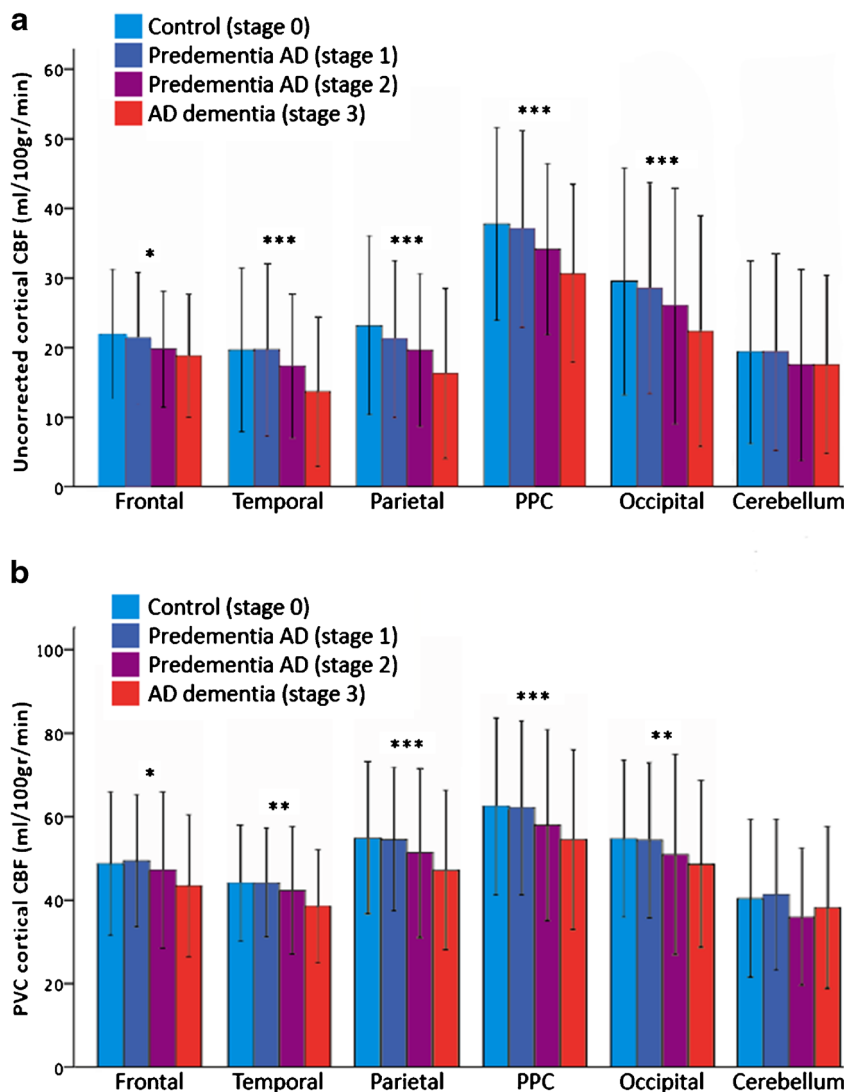
<sup>c</sup> p<0.05 compared to stage-2 predementia patients

<sup>d</sup> p<0.1 compared to stage-1 predementia patients

Stage 0: Aβ-/tau- patients with subjective complaints (SC), Stage 1: Aβ+/tau- patients with SC and mild cognitive impairment (MCI), Stage 2: Aβ+/tau+ patients with SC and MCI, Stage 3: Aβ+/tau+ patients with AD

AD Alzheimer’s disease, PPC precuneus and posterior cingulate, PVC partial volume corrected

**Fig. 1** Lower regional cerebral blood flow (CBF) is associated with advancing Alzheimer's disease (AD) stage. Bar graphs display mean regional uncorrected CBF (a) and mean partial volume corrected (PVC) cortical CBF (b) for the different AD stages, per brain region. Error bars represent standard deviations ( $\pm 2SD$ ). Dose-response relationships between CBF and AD stage were detected using general-linear models (AD stage entered as a continuous measure), resulting in a  $p$  for trend  $< 0.05$  in all brain regions except the cerebellum. The most prominent relationships between decreasing CBF and advancing AD stage were found in the posterior supratentorial brain regions ( $p$  for trend  $< 0.001$ ). *PPC* precuneus and posterior cingulate cortex. \* $p$  for trend  $< 0.05$ , \*\* $p$  for trend  $< 0.01$ , \*\*\* $p$  for trend  $< 0.001$



### Differences in regional CBF patterns

As expected, regional PVC cortical CBF values were much higher than regional uncorrected cortical CBF values, as the latter also contains cerebrospinal fluid and maybe even some edges of white matter (Table 2). A more advanced AD stage was related to lower regional CBF in all regions except the cerebellum. Relationships were most prominent in the temporal, parietal, PPC and occipital regions for uncorrected cortical CBF, and in the parietal and PPC regions for PVC cortical CBF ( $p$  for trend  $< 0.001$ ; Fig. 1). Post-hoc, uncorrected cortical and PVC cortical CBF were lower in temporal, parietal, PPC and occipital regions in AD dementia patients compared to controls (Table 2). Furthermore, uncorrected cortical CBF was lower in AD dementia patients compared to stage-1 predementia patients in the temporal, parietal (trend), PPC and occipital (trend) regions, and compared to

stage-2 predementia patients in the temporal lobes. AD dementia patients showed a trend towards lower PVC cortical CBF values compared to stage-1 predementia patients in the temporal, parietal, PPC and occipital regions. No between-group CBF differences were found in the frontal lobes and the cerebellum, and there were no CBF differences between stage-2 predementia patients, stage-1 predementia patients and controls. Additional correction for WMH did not change the group differences.

### Relationship between CBF and cognition

Across groups, uncorrected whole brain CBF and PVC cortical CBF were related to cognitive performance in all brain regions, except the cerebellum (Table 3). When we investigated correlations within each stage separately, in AD dementia patients this relationship was seen in the temporal and parietal regions (uncorrected cortical



**Table 3** Relationship between cerebral blood flow (CBF) and cognition (Mini-Mental State Examination scores)

	Across groups	Control	Predementia	AD	
		Stage 0	Stage 1	Stage 2	Stage 3
Uncorrected whole brain CBF	0.33**	−0.01	0.22	−0.20	0.21 <sup>+</sup>
PVC cortical CBF	0.27**	−0.02	0.30	−0.21	0.19
Regional uncorrected cortical CBF					
Frontal	0.23*	−0.06	−0.03	0.05	0.18
Temporal	0.38**	0.02	0.22	−0.03	0.21 <sup>+</sup>
Parietal	0.40	−0.01	0.22	0.16	0.24 <sup>+</sup>
PPC	0.40**	0.03	0.28	−0.17	0.17
Occipital	0.34**	0.12	0.25 <sup>§</sup>	−0.35 <sup>+</sup>	0.18
Cerebellum	0.09	−0.05	0.24	−0.30	−0.04
Regional PVC cortical CBF					
Frontal	0.24*	−0.07	0.18	−0.21	0.23 <sup>+</sup>
Temporal	0.31**	−0.02	0.26	−0.13	0.22 <sup>+</sup>
Parietal	0.32**	−0.02	0.41	−0.18	0.21 <sup>+</sup>
PPC	0.29**	0.01	0.46	−0.28	0.16
Occipital	0.23*	0.04	0.44	−0.30	0.11
Cerebellum	0.08	−0.04	0.13	−0.05	0.02

\* $p < 0.01$ , \*\* $p < 0.001$ , <sup>+</sup> $p < 0.1$ ; adjustment for age and sex (model 1)

<sup>§</sup> A relationship between cognition and uncorrected CBF in the occipital lobes (standardized beta 0.70;  $p < 0.05$ ) was detected after additional adjustment for white matter hyperintensities (Fazekas-scores; model 2)

Stage 0: A $\beta$ -/tau- patients with subjective complaints (SC), Stage 1: A $\beta$ +/-tau- patients with SC and mild cognitive impairment (MCI), Stage 2: A $\beta$ +/-tau+ patients with SC and MCI, Stage 3: A $\beta$ +/-tau+ patients with AD

AD Alzheimer's disease, PPC precuneus and posterior cingulate, PVC partial volume corrected

and PVC cortical CBF; trends), and in the frontal lobes (PVC cortical CBF; trend). Stage-2 predementia patients showed a relationship between uncorrected CBF and cognition in the occipital lobes (trend). After additional adjustment for WMH (model 2), the essence of the results remained unchanged, with the addition that in stage-1 predementia patients a relationship between cognitive performance and uncorrected CBF in the occipital lobes reached significance (standardized beta 0.70,  $p < 0.05$ ).

## Discussion

The main finding of this study was that lower CBF was observed with more advancing stages of AD across the spectrum from normal cognition to AD dementia. Furthermore, CBF was related to cognitive performance across AD stages and within the stage-3 AD group.

To date, several ASL studies have detected CBF decreases in AD, most prominently in the bilateral parietal cortex and the precuneus and posterior cingulate cortex [8, 21, 22]. Based on the currently widely supported suggestion that neuronal dysfunction occurs prior to the stage of clinical AD, the aim

of this study was to investigate ASL-measured CBF of A $\beta$  positive non-demented patients in different disease stages [5, 7]. This resulted in the finding that, compared to A $\beta$  and tau negative controls, ASL-CBF was decreased in AD dementia patients, but not in predementia AD patients. Temporoparietal CBF was lower in AD dementia patients than in stage-1 predementia patients, in particular for uncorrected cortical CBF, but to a lesser extent also for PVC cortical CBF. Stage-2 predementia patients showed no decreased CBF compared to controls, but their CBF did not differ from AD dementia patients either. In fact, quantitative CBF values of stage-2 predementia patients were numerically in between the CBF values of stage-1 predementia patients and stage-3 AD dementia patients. These findings are in accordance with the idea that CBF alterations, as a sign of neuronal dysfunction, occur further along the disease process than the accumulation of A $\beta$ , but before the stage of clinical dementia.

This is the first study comparing ASL-measured CBF of predementia AD stages with study groups based on CSF biomarker profiles [5–7]. Previous ASL studies investigating cerebral perfusion in MCI detected parietal and medial temporal hypoperfusion in a less prominent and widespread pattern than the hypoperfusion found in AD compared to controls [8, 21–26]. However, none of these studies reported amyloid

status of the MCI patients. This leaves room for the possibility that other CBF-decreasing non-AD pathology caused regional CBF changes in MCI, such as DLB, which has previously been shown to also be related to severe CBF decreases [27, 28]. In our study, we therefore used biomarkers to define our study groups, allowing us to examine changes in CBF across the entire spectrum of AD.

In the current hypothetical biomarker models for AD, FDG-PET is considered the main functional neuroimaging tool to assess neuronal injury in terms of hypometabolism in AD [3, 4, 29]. Since glucose metabolism and perfusion are closely related, ASL is a potential alternative for the visualization of cerebral and neuronal function [30–32]. However, the relationship between perfusion and metabolism has not yet been thoroughly investigated in the predementia stages of AD. One PET study by Drzezga et al. [33] found lower regional glucose metabolism in A $\beta$ -positive healthy controls compared to A $\beta$ -negative controls, suggesting a relationship between A $\beta$  and glucose metabolism. This is not consistent with our finding that ASL-measured CBF did not change with the presence of amyloid pathology in a very early stage of AD. Direct comparisons of FDG-PET measured glucose metabolism and ASL-measured cerebral perfusion in predementia AD are needed to clarify whether glucose metabolism and cerebral perfusion are directly related or consecutive processes.

In a previous study we showed that cognitive performance was related to ASL-measured CBF in AD [8]. In the current study we also found this relationship within the AD dementia group, albeit in fewer regions and less strong. This discrepancy may be due to the fact that in the current study, AD patients were selected based on CSF biomarkers in addition to clinical diagnosis, possibly causing less variability in both cognition and CBF within the AD dementia group compared to our previous study, in which the AD population also contained subjects with normal or borderline normal CSF A $\beta$  and tau values. Besides a trend towards a significant relationship between occipital CBF and cognition in the stage-2 predementia group (in the unexpected direction), no relationships between CBF and cognition were found in the A $\beta$  positive predementia groups. However, it is not inconceivable that a lack of power contributed to this negative result. After adjustment for WMH, a relationship between uncorrected CBF in the occipital lobes and cognition did appear within the stage-1 predementia group.

This study has several strengths and limitations. First, the study groups were selected based on both clinical diagnosis and CSF biomarkers. This limited our sample sizes, but at the same time resulted in quite homogeneous study groups, strongly reducing the effect of perfusion changes caused by for example neurodegeneration of non-AD origin. Since the criteria for clinical stages in preclinical and prodromal AD show strong similarities, and implementing all stages in a sequential manner is not easily done, we chose to reduce the

number of study groups by combining preclinical and prodromal patient groups with identical biomarker profiles. A quantitative 3D pseudo-continuous ASL sequence with whole-brain coverage was used to study CBF. Furthermore, both CBF without correction for partial volume effects (representative for clinical practice) and PVC cortical CBF (actual gray matter CBF) were studied. The inclusion of subjects with subjective complaints instead of healthy controls might be considered a limitation. However, controls were only included if they had normal biomarkers, strongly decreasing the likelihood that they would have preclinical AD. Additional potential limitations related to image technique include the fact that ASL scan quality could not be assured completely. Although the perfusion images were visually of good quality, the labelling efficiency was not assessed formally. Furthermore, we did not scan with several delay times to account for differences in travel times between groups. However, in line with recently published recommendations [14], a delay time of 2.0 s (instead of 1.5 s) was used to alleviate the effect of delayed blood arrival in the brain in our study groups [34].

In conclusion, we found lower CBF with advancing NIA-AA-based stages of AD. A $\beta$ -positive predementia patients with normal CSF tau levels showed no reduction in ASL-measured CBF, endorsing the idea that CBF changes are not directly related to A $\beta$  deposition. Furthermore, the continuing decrease along the continuum of AD shows that the reduction of CBF does not reach a bottom level, but is associated with decline severity, suggesting that ASL-measured CBF may be used as a measure for disease progression.

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a) Binnewijzend MA, Kuijjer JP, Benedictus MR, van der Flier WM, Wink AM, Wattjes MP et al. Cerebral blood flow measured with 3D pseudocontinuous arterial spin-labeling mr imaging in alzheimer disease and mild cognitive impairment: a marker for disease severity. *Radiology* 2013;267:221-30.

b) Binnewijzend MA, Kuijjer JP, van der Flier WM, Benedictus MR, Moller CM, Pijnenburg YA et al. Distinct perfusion patterns in Alzheimer's disease, frontotemporal dementia and dementia with Lewy bodies. *Eur Radiol* 2014;24:2326-33.

Methodology: retrospective, cross-sectional study, performed at one institution.

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