FULL METHODS

Patients and Study Design

This study was performed according to the principles of the Declaration of Helsinki and was approved by the Medical Ethical Committee of the University Medical Center Groningen, and registered at clinicaltrials.gov (NCT02272985). All patients gave written informed consent. The study was performed between March and November of 2015.

Patients receiving HD aged ≥65 years from our department with an arteriovenous fistula without significant recirculation were eligible for this study. Patients were studied during a regular dialysis session after the longest interdialytic interval (Monday or Tuesday). Patient characteristics were assessed at study entry and retrieved from the patients' medical history. Height was measured before, and weight before and after the PET-HD session. BP, heart rate, and tympanic temperature were measured before every PET-CT scan and every 30 minutes during the HD study session.

Hypertension was defined as predialysis systolic blood pressure >140 mmHg and/or diastolic blood pressure >90 mmHg or the use of anti-hypertensive drugs. UF rate was expressed in mL/h per kg body weight by dividing ultrafiltration volume by dialysis session length and postdialysis target weight. Equilibrated Kt/V was calculated according to the second-generation logarithmic Daugirdas.[1]

Based on the highly sensitive technique of [^{15}O]H_2O and based on former studies that mainly used TCD in which the number of HD patients varied between 12 and 27[2-7], we expected that a total of 14 patients would be sufficient, and aimed to include 14 patients. Additional inclusion criteria were a hemoglobin level between 6.2 and 8 mmol/L since at least 1 month before inclusion, because hemoglobin levels are associated with CBF. Patients with a history of dementia, hydrocephalus, cerebrovascular accident, raised intracranial pressure, end-stage liver disease, actively treated cancer, a known significant (>70%) internal carotid artery or major intracranial vessel stenosis, and patients with a contra-indication for MRI were excluded. After study-inclusion, routine duplex evaluation was performed to exclude subjects with an asymptomatic internal carotid artery stenosis

of more than 70% or major intracranial vessel stenosis, because this may interfere with the interpretation of CBF (change).

HD Study Session

All HD study sessions were performed in the afternoon in the PET-camera room. The ambient temperature of the room was kept constant at 20˚C, excluding an effect of outside temperature on cardiovascular stability during study sessions. After the first PET scan (T1), patients started dialysis still being in a horizontal position in the PET-camera. After the second PET scan (T2), which was performed within 30 minutes after the start of HD, patients were transferred to a hospital bed adjacent to the PET-camera to continue dialysis in a 30-45-degree supine position. Approximately 30 minutes before the start of the third PET scan (T3), which was performed in the final hour of the HD session, patients were transferred back to the PET.

A low-dose brain computed tomography was made before the first and third PET-scan to correct for attenuation of the PET data. A bolus injection of $[^{15}O]H_2O$ was administered intravenously at a constant rate through an indwelling peripheral venous catheter in the non-dialysis access arm. The injected dose of $[^{15}O]H_2O$ was 500 MBq per scan, with a total dose of 1500 MBq per patient for the whole study. During each PET-scan, arterial blood was sampled continuously from the dialysis line by a dedicated programmable blood-sampler to obtain the course of the radioactivity concentration in the blood during 5 minutes following the injection of $[^{15}O]H_2O$. To perform laboratory measurements, arterial blood was sampled from the arterial dialysis line just before each PET-scan.

Dialysis Settings

All patients were on bicarbonate dialysis with a low-flux polysulfone hollow-fiber dialyzer (F8; Fresenius Medical Care, Bad Homburg, Germany). Blood flow and dialysate flow rates were 200-300 and 500 mL/min, respectively. Dialysate temperature was 36.5°C in all patients. We used constant UF rate and dialysate conductivity. Dialysate composition was sodium 139 mmol/L, potassium 1.0 or 2.0

mmol/L depending on the prevailing plasma potassium, calcium 1.5 mmol/L, magnesium 0.5 mmol/L, chloride 108 mmol/L, bicarbonate 34 mmol/L, acetate 3.0 mmol/L, and glucose 1.0 g/L. The water for hemodialysis complied with the requirements of the European Pharmacopoeia (<100 colony-forming units/mL; <0.25 endotoxin units/mL).

PET Data Acquisition

For the $[^{15}O]H_2O$ PET-CT scans a Siemens Biograph 64-mCT (Siemens Medical Systems, TN) that acquires 109 planes over a total axial length of 216 mm was used. $[^{15}O]H_2O$ was produced by conversion of the $[^{15}O]O_2$ using a new designed IBA chemistry module (IBA RadioPharma Solutions, Belgium) and placed in a shielded class A foam hood located in the Good Manufacturing Practice laboratory. During preparation, ^{15}O gas flew from the cyclotron into the IBA chemistry module and was mixed with hydrogen gas and passed through a palladium column. The produced $[^{15}O]H_2O$ was collected in a sterile 0.9% saline solution to obtain a final product suitable for patient administration. The method was validated and met all pharmacopeia specifications. The practical production yield of ^{15}O labeled water using this method ranged between 1300-1700 MBq measured in the syringe. $[^{15}O]H_2O$ was produced one floor below the PET location facilitating rapid transport to the PET-camera room, which is important since its half-life is short (T½ 2.03 min).

First, a low-dose computed tomography scan was performed for attenuation and scatter correction. The dynamic PET acquisition (310 seconds) was started, followed after 10 seconds by an intravenous bolus injection of $[^{15}O]H_2O$. In total, the duration of every PET-CT scan was 5 minutes, which was uniform across all time points and all patients. Head movement was minimized with a head-restraining band. For CBF quantification, the arterial input function was obtained from arterial blood radioactivity, which was continuously monitored with an automated sampling system (Veenstra Instruments, Joure, the Netherlands). One extra blood sample was collected at 393±32 seconds after tracer injection to determine the amount of radioactivity in the blood using a γ-counter (Wizard2, Perkin Elmer, Waltham).

Three of the 36 scans could not be analyzed due to a technical problem with the automated sampling system during the measurement (patient identity 106 [T1], patient identity 107 [T2], patient- identity 102 [T3]).

MRI Data Acquisition

MRI was performed using a 1.5T whole body system (Aera, Siemens, Erlangen, Germany) on a non-dialysis day. The study MRI was performed median 3 days (range, -72 to +3 days) after the HD study session. The scan protocol (total scan time 30 minutes) included T1-weighted, T2-weighted, three-dimensional fluid-attenuated inversion recovery, diffusion-weighted imaging, susceptibility weighted imaging, and two-dimensional phase contrast sequences. No intravenous contrast was used. A neuroradiologist (PJvL) assessed white matter hyperintensities, and cortical atrophy, using the Fazekas scale and the global cortical atrophy scale, respectively. [8, 9] Microbleeds were scored on the susceptibility weighted imaging sequence.

Image Reconstruction and Preprocessing

Image processing and pharmacokinetic analysis were performed with PMOD 3.8 software (PMOD Technologies Ltd., Zurich, Switzerland). The average image (time-weighted) was used for rigid matching registration of the individual PET to the individual MRI.

The PET list-mode data were reconstructed using the 3D OSEM algorithm (3 iterations and 24 subsets), point spread function correction and time-of-flight, and reconstructed to 28 dynamic frames (1×10 sec, 12×5 sec, 6×10sec, and 9×20 sec). Data were corrected for attenuation, scatter and radioactivity decay. This resulted in images with a matrix of 400 × 400 × 111 of 2 mm voxels, smoothed with a 2 mm filter at full width at half maximum.

We used the 3D T2-FLAIR images for the registration process, because the 3D acquisition of the T1-weighted sequence was not available. Furthermore, several patients had marked brain atrophy and white matter lesions. Therefore, we used the population-based gray matter/white matter (GM/WM) maps to segment the cortical tissue, instead of using the subject probability maps. This means that

the cortical volumes of interest (VOIs) are slightly larger than when the individual maps for the subject are used. Since we did the modeling in the subject brain space (no deformations to adjust to the atlas) and the VOIs were based on the population-based GM/WM probabilities, the effect of the atrophy and lesions is expected to be minimal.

Predefined VOIs were transformed into the individual space, based on the Hammers atlas and limited to the gray matter tissue in the cortical regions (>30% gray matter probability based on standard probability).[10] After spatial registration, pharmacokinetic modeling was applied to the dynamic PET images to calculate the CBF, based on the implementation of the 1-tissue compartment model developed by E. Meyer.[11] Delay of the arterial input function and dispersion in the model were first calculated for the whole brain, and then these resulting values were fixed for the brain regions.

Neuropsychological Tests

A neuropsychological assessment battery was performed to characterize the study population and included all major cognitive domains. The battery included the Mini Mental State Examination (MMSE; measuring global cognitive function), digit span (measuring attention), Trail Making Test A and B (TMT A and B; measuring attention, executive function including set shifting, and motor speed), clock drawing (measuring executive function, visuospatial skills), verbal fluency (measuring executive function, language), Dutch version of the RAVLT (measuring verbal memory, immediate and delayed recall, and recognition) and the Hospital Anxiety and Depression Scale (HADS) for identifying depression and anxiety symptoms. The order of the tests was fixed and cognitive testing was performed on a non-dialysis day. It took approximately 45-60 min per subject to complete the tests. The neuropsychological assessment was performed median 95 days (range, -196 to -33 days) before the PET-HD session.

Statistical Analyses

Intradialytic changes in levels of the HD-related characteristics were studied using repeated measures ANOVA (with a Greenhouse-Geisser correction in case of non-sphericity), with a Bonferroni correction.

For the primary study objective, global and regional CBF changes were analyzed by LMM, which allowed for individual random intercepts and slopes of CBF over time. The random slopes were on the basis of the actual scan times per patient. Relative CBF change was calculated as the mean of the individual percentual change between T1 and T3 using descriptive statistics, and is reported as mean±SD (%).

For the secondary study objective, associations of HD treatment-related factors, which might potentially explain CBF change, with CBF were studied. Those factors included MAP, pCO_2, pH, tympanic temperature, hematocrit, and UF volume and were selected based on literature. [2-4, 12-16] The factors were studied univariately using LMM, checking the significance of interactions with scan-order. Because UF volume was associated with CBF, the association between UF rate and CBF was evaluated as well.

In additional analyses, associations of cognitive test scores and structural brain characteristics with CBF were explored. To this end, we first tested correlations with baseline CBF using Pearson or Spearman correlation, if appropriate. Subsequently, we studied the associations including all CBF measurements in a LMM. For these analyses, the cognitive test scores were converted to Z scores.

Several sensitivity analyses were performed. First, regional CBF change was also calculated for the left and right hemisphere separately. Second, in order to eliminate a possible effect of HD on the arterial sampling from the arteriovenous fistula, CBF change between T2 and T3 was calculated. Third, CBF change in only the gray matter of each VOI was studied instead of the sum of gray and white matter of the corresponding region.

Two-sided $P<0.05$ was considered statistically significant. Statistical analyses were performed with SPSS, version 23 (SPSS Inc., IBM company), GraphPad Prism version 5.0 (GraphPad Software, San Diego), and R version 3.4.0 (R Core Team, 2017).

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SUPPLEMENTARY TABLES

Table S1. Intradialytic BP trajectories of the individual study participants.

Study identity	Before start HD		Nadir during HD		Interval between the start of HD and nadir
	SBP (mmHg)	MAP (mmHg)	SBP (mmHg)	MAP (mmHg)	(minutes)
101	166	112	169	122	145
102	164	103	146	105	163
103	166	N.A.	N.A.	N.A.	N.A.
105	151	102	105	90	70
106	107	77	108	77	158
107	136	96	105	84	6
108	191	113	158	102	152
109	143	102	131	100	167
110	130	88	108	67	260
112	156	99	158	97	138
114	144	102	106	79	253
115	173	116	140[#]	103[#]	132[#]

[#] *This patient (identity 115) lost consciousness due to dialysis-induced hypotension shortly after the third scan (T3). BP was not measured during this event; the next measured BP after regaining consciousness was 155/90. Thus, the nadir presented in this table is not the nadir at the moment of the dialysis-hypotension episode.*

SBP, systolic blood pressure; HD, hemodialysis; MAP, mean arterial pressure; N.A., not available.

Table S2. Correlations of cognitive function and structural markers of brain lesions with baseline CBF.

	Global CBF		Regional CBF											
			Frontal lobe		Parietal lobe		Temporal lobe		Occipital lobe		Cerebellum		Thalamus	
	r	*P*	r	*P*	r	*P*	r	*P*	r	*P*	r	*P*	r	*P*
Cognitive tests:														
zMMSE	-0.03	0.9	-0.05	0.9	-0.04	0.9	0.05	0.9	0.09	0.8	0.05	0.9	0.02	1.0
zDigit Span forward	0.20	0.6	0.20	0.5	0.16	0.7	0.39	0.2	0.39	0.2	0.28	0.4	0.23	0.5
zDigit Span backward	-0.28	0.4	-0.22	0.5	-0.28	0.4	-0.08	0.8	-0.30	0.4	-0.43	0.2	-0.06	0.9
zRAVLT delayed recall	0.09	0.8	0.04	0.9	0.05	0.9	0.12	0.7	-0.02	1.0	0.01	1.0	0.20	0.6
zTMT A	0.06	0.9	0.01	0.9	-0.02	0.9	-0.01	1.0	0.10	0.8	0.37	0.3	-0.10	0.8
zTMT B	0.16	0.6	0.14	0.7	0.16	0.7	0.04	0.9	0.29	0.4	0.17	0.6	-0.28	0.4
TMT B/A ratio	0.11	0.8	0.08	0.8	0.08	0.8	0.06	0.9	0.23	0.5	0.12	0.7	-0.16	0.7
zVerbal fluency	-0.17	0.6	-0.24	0.5	-0.23	0.5	-0.04	0.5	-0.16	0.6	-0.14	0.7	-0.19	0.6
zClock drawing	-0.14	0.7	-0.10	0.8	-0.08	0.8	-0.11	0.8	-0.13	0.7	-0.28	0.4	0.12	0.7
Structural markers of brain lesions:														
Microbleeds	-0.19	0.6	-0.13	0.7	-0.09	0.8	-0.22	0.5	-0.36	0.3	-0.32	0.4	0.03	0.9
Fazekas score	-0.13	0.7	0.01	1.0	0	1.0	-0.18	0.6	-0.37	0.3	-0.38	0.3	0.12	0.7

Correlations were calculated using Pearson or Spearman correlation, if appropriate. The cognitive tests scores were converted to Z scores for the analyses. For regional CBF, the mean CBF of the left and right hemisphere was used for the analyses.
MMSE, Mini Mental State Examination; r, correlation coefficient; RAVLT, Ray Auditory Verbal Learning Test; P, P value indicating significance level; TMT, trail making test.

Table S3. Associations of cognitive function and structural markers of brain lesions with CBF. [1]

Region	zTMT B [a]		TMT B/A ratio [b]		Fazekas score [c]		Microbleeds	
	Estimated effect on CBF (mL/100g per minute)		Estimated effect on CBF (mL/100g per minute)		Estimated effect on CBF (mL/100g per minute)		Estimated effect on CBF (mL/100g per minute)	
	zTMT B	zTMT B*T2	TMT B/A	TMT B/A*T2	Fazekas	Fazekas*T2	MB	MB*T2
Interaction with scan-order[d]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Global	NS	NS	0.6 (-2.9 to 4.1)	-1.9 (-4.1 to 0.2)	-1.0 (-4.6 to 2.5)	2.0 (-0.1 to 3.8)	NS	NS
Regional:								
Frontal lobe	NS	NS	0.6 (-4.3 to 5.5)	-2.2 (-3.9 to 0.6)*	-0.6 (-5.0 to 3.7)	1.3 (-0.1 to 2.7)	NS	NS
Parietal lobe	0.5 (-4.2 to 5.2)	-1.5 (-2.9 to -0.1)*	0.9 (-4.4 to 6.1)	-2.5 (-4.1 to -0.9)**	-0.5 (-5.1 to 3.9)	1.7 (0.4 to 3.0)*	NS	NS
Temporal lobe	NS	NS	0.5 (-3.5 to 4.4)	-2.2 (-3.6 to -0.8)**	-1.3 (-4.9 to 2.3)	2.5 (1.4 to 3.5)***	1.4 (-7.5 to -4.9)	2.5 (0.2 to 4.8)*
Occipital lobe	NS	NS	1.5 (-2.8 to 5.7)	-3.0 (-5.3 to -0.7)*	-2.4 (-6.4 to 1.6)	4.1 (2.5 to 5.7)***	NS	NS
Cerebellum	1.9 (-4.1 to 0.2)	-2.0 (-3.7 to -0.3)*	0.9 (-3.9 to 5.7)	-3.0 (-4.9 to -1.0)**	-3.2 (-7.8 to 1.5)	4.2 (2.7 to 5.7)***	-3.2 (-11.0 to -4.9)	5.4 (-1.5 to 11.9)*
Thalamus	NS	NS	-1.1 (-6.1 to 3.9)	-2.9 (-5.4 to -0.3)*	0.5 (-4.6 to 5.6)	3.5 (1.3 to 5.8)**	NS	NS

Associations were studied using linear mixed effects models including a random intercept and slope. The estimated effect (95%CI) of the individual characteristics on CBF is presented. The zMMSE, zDigit span backwards and forwards, zFluency, zTMT A, zRAVLT delayed recall, and zClock drawing test scores were not associated with CBF. [a,b]A higher zTMT B score or TMT B/A ratio indicates worse executive function. [c]The Fazekas score was entered to the model as a continuous covariate. [d]The interaction models could be interpreted by adding the effect of the single term and the interaction term, e.g. yielding an association of a higher zTMT B score with a lower CBF at T2 as compared with T1.
MB, microbleeds; NS, not significant; TMT, trail making test.
**P<0.05, **P<0.01, ***P<0.001*

[1] Note to the reader: the associations presented in this table can only be viewed as hypothesis generating since the relatively small sample size militates against definitive conclusions. The cognitive test results were not corrected for age and education due to the small sample size. Furthermore, we were not able to analyze the Fazekas score (which is an ordinal variable including 4 categories) also as a categorical variable due to the small sample size

Table S4. Intradialytic changes in regional CBF, specified per hemisphere.

	Before start HD	After start HD	At the end of HD
Brain region	T1	T2	T3
Frontal lobe L	39.9 ± 6.9	38.6 ± 5.5	34.8 ± 4.9
Frontal lobe R	40.4 ± 6.9	39.2 ± 5.7	35.2 ± 4.6
Parietal lobe L	37.1 ± 7.1	36.0 ± 6.3	32.3 ± 5.3
Parietal lobe R	37.7 ± 6.9	36.7 ± 6.2	32.9 ± 4.9
Temporal lobe L	35.5 ± 5.3	35.4 ± 5.6	31.5 ± 4.6
Temporal lobe R	36.2 ± 4.9	36.0 ± 5.7	32.1 ± 4.8
Occipital lobe L	41.4 ± 5.5	41.3 ± 7.3	37.3 ± 5.4
Occipital lobe R	42.3 ± 4.8	41.9 ± 6.7	38.1 ± 6.0
Cerebellum L	43.3 ± 6.7	44.7 ± 7.3	38.4 ± 5.8
Cerebellum R	43.2 ± 7.0	44.9 ± 7.6	38.5 ± 6.5
Thalamus L	47.2 ± 7.8	48.1 ± 9.1	41.7 ± 9.0
Thalamus R	47.3 ± 7.3	48.2 ± 8.2	41.7 ± 7.9

*CBF data (mL/100g per minute) are presented as unadjusted means **±** SD. [a] Scan 2 (T2) and 3 (T3) were performed at mean 21 and 209 minutes after start of HD (T1), respectively.*
L, left; R, right.

Table S5. Intradialytic changes in regional CBF, of the gray matter only.

	Before start HD	After start HD	At the end of HD	Dialysis treatment effect	
Brain region	T1	T2	T3	T1 vs. T3	T2 vs. T3
Frontal lobe gray matter	44.4 ± 7.7	43.1 ± 6.4	38.5 ± 5.2	-5.8 (-10.8 to -0.9) *	-4.7 (-8.9 to -0.5) *
Parietal lobe gray matter	42.2 ± 8.1	41.0 ± 7.5	36.7 ± 5.7	-5.6 (-10.1 to -1.0) *	-4.8 (-8.7 to -0.9) *
Temporal lobe gray matter	38.7 ± 5.5	38.4 ± 6.2	34.2 ± 5.1	-4.5 (-8.2 to -0.7) *	-4.4 (7.6 to -1.2) **
Occipital lobe gray matter	43.8 ± 5.4	43.5 ± 7.5	39.4 ± 5.9	-4.6 (-9.0 to -0.3) *	-4.8 (-8.7 to -1.0) **
Cerebellum lobe gray matter	43.9 ± 6.9	45.4 ± 7.5	38.9 ± 6.3	-5.1 (-9.3 to -0.8) *	-6.4 (-10.2 to -2.7) ***

*CBF data (mL/100g per minute) are presented as unadjusted means **±** SD. Scan 2 (T2) and 3 (T3) were performed at mean 21 and 208 minutes after start of HD (T1), respectively. Dialysis treatment effects are obtained from linear mixed effects models including a random intercept and slope, and presented as mean difference (95% CI).*
**P<0.05, **P<0.01, ***P<0.001*