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# CHAPTER 7

## **Changes in cerebral oxygenation and cerebral blood flow during hemodialysis - a simultaneous near-infrared spectroscopy and positron emission tomography study**

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## ABSTRACT

Near-infrared spectroscopy (NIRS) is used to monitor cerebral tissue oxygenation ( $rSO_2$ ) depending on cerebral blood flow (CBF), cerebral blood volume and blood oxygen content. We explored whether NIRS might be a more easily applicable proxy to [ $^{15}O$ ]H $_2$ O positron emission tomography (PET) for detecting CBF changes during hemodialysis. Furthermore, we compared potential determinants of  $rSO_2$  and CBF. In 12 patients aged  $\geq 65$  years, NIRS and PET were performed simultaneously: before (T1), early after start (T2), and at the end of hemodialysis (T3). Between T1 and T3, the relative change in frontal  $rSO_2$  ( $\Delta rSO_2$ ) was  $-8 \pm 9\%$  ( $P=0.001$ ) and  $-5 \pm 11\%$  ( $P=0.08$ ), whereas the relative change in frontal gray matter CBF ( $\Delta CBF$ ) was  $-11 \pm 18\%$  ( $P=0.009$ ) and  $-12 \pm 16\%$  ( $P=0.007$ ) for the left and right hemisphere, respectively.  $\Delta rSO_2$  and  $\Delta CBF$  were weakly correlated for the left ( $\rho 0.31$ ,  $P=0.4$ ), and moderately correlated for the right ( $\rho 0.69$ ,  $P=0.03$ ) hemisphere. The Bland-Altman plot suggested underestimation of  $\Delta CBF$  by NIRS. Divergent associations of pH,  $pCO_2$  and arterial oxygen content with  $rSO_2$  were found compared to corresponding associations with CBF. In conclusion, NIRS could be a proxy to PET to detect intradialytic CBF changes, although NIRS and PET capture different physiological parameters of the brain.

## INTRODUCTION

Cognitive impairment is highly common in patients with advanced chronic kidney disease (CKD).<sup>1</sup> Cognitive performance might be negatively affected by structural brain lesions that are often present in the CKD population, including lacunar infarctions,<sup>2</sup> microbleeds,<sup>3</sup> and loss of white matter integrity.<sup>4,5</sup> Besides the many risk factors for cognitive decline that are present in patients with CKD, the hemodialysis procedure itself might also induce brain injury. In patients with advanced CKD, the transition to dialysis has been associated with an accelerated decline of cognitive function and an increased incidence of strokes.<sup>6,7</sup> Hemodialysis involves repetitive fluid removal, thereby frequently resulting in alterations in blood pressure and volume status, which might induce circulatory stress.<sup>8</sup> The fluid removal during hemodialysis is accompanied by an increase of blood viscosity, and rapid shifts in electrolytes, acid-base balance, and uremic solutes.<sup>9,10</sup> Furthermore, exposure of the blood to the extracorporeal circuit during hemodialysis triggers an inflammatory response with complement activation, endothelial activation, and activation of coagulation pathways.<sup>11-13</sup> All these processes could theoretically affect the macro- and microvascular cerebral blood flow and cerebral oxygenation.

To unravel potential mechanisms that underlie the link between cognitive impairment and the hemodialysis procedure, we previously evaluated whether hemodialysis has a direct effect on cerebral blood flow (CBF). Using [<sup>15</sup>O]H<sub>2</sub>O positron emission tomography (PET), we found that hemodialysis induced a 10% decline in global and regional CBF in elderly hemodialysis patients.<sup>14</sup> This CBF decline does not automatically imply an impaired autoregulation, because the dynamic pressure-flow relationship may also be affected by alterations in cerebrovascular resistance apart from autoregulation, such as changes in pH, hematocrit and blood volume during hemodialysis. Second, we found that as hemodialysis-related factors a higher pH, higher tympanic temperature, and a larger ultrafiltration rate and volume were associated with a lower CBF. However, [<sup>15</sup>O]H<sub>2</sub>O PET-scanning involves radiation, requires an on-site cyclotron for nuclide generation, and is complicated to perform during a hemodialysis session. Therefore, there is a need for an alternative method that is easier to apply to monitor changes in cerebral perfusion during hemodialysis.<sup>15</sup>

A technique that has been proposed to monitor the adequacy of cerebral perfusion is non-invasive near-infrared spectroscopy (NIRS) by measuring frontal cerebral tissue oxygenation.<sup>16</sup> During hemodialysis, relative drops of more than 15% in frontal cerebral tissue oxygenation (rSO<sub>2</sub>) were associated with decreased executive cognitive function one year after the start of hemodialysis.<sup>17</sup> Changes in frontal rSO<sub>2</sub> are commonly considered to

reflect changes in (frontal) CBF,<sup>18,19</sup> but whether an intradialytic decline in frontal rSO<sub>2</sub> reflects a simultaneous and similar fall in frontal CBF is unknown.

In this study, we aimed to evaluate whether changes in frontal cerebral oxygenation can identify changes in frontal CBF during hemodialysis. In detail, we investigated (i) the correlation and agreement between intradialytic changes in frontal rSO<sub>2</sub> and frontal gray matter CBF, and (ii) how hemodialysis and oxygenation-related factors and markers of inflammation and endothelial activation are associated with changes in rSO<sub>2</sub>, as compared to CBF.

## **MATERIALS AND METHODS**

### **Ethics**

The 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.

### **Study design and patient recruitment**

The study was performed between March and November 2015 and comprised two objectives: (i) to evaluate the effect of hemodialysis on cerebral perfusion, which was published recently,<sup>14</sup> and (ii) to study the correlation between changes in frontal rSO<sub>2</sub> and changes in frontal and global CBF, as measured by [<sup>15</sup>O]H<sub>2</sub>O PET-CT.

Hemodialysis patients aged ≥65 years from the department of Nephrology of the University Medical Center Groningen and from the Dialysis Center Groningen with an arteriovenous fistula without significant recirculation were eligible for this study. 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 contraindication 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. Patient characteristics were assessed at study entry and retrieved from the patients' medical history. Based on the highly sensitive technique of [<sup>15</sup>O]H<sub>2</sub>O and based on former studies that mainly used transcranial Doppler in which the number of hemodialysis patients varied between 12 and 27,<sup>20-25</sup> we expected that a total of 14 patients would be sufficient, and aimed to include 14 patients.

## **Setting**

NIRS monitoring and three PET-CT scans were performed simultaneously during a single, regular hemodialysis session after the longest interdialytic interval (Monday or Tuesday). All hemodialysis study sessions were performed in the afternoon in the PET-CT camera room, with a constant ambient room temperature of 20°C, excluding an effect of outside temperature on cardiovascular stability during the study sessions.

First, NIRS monitoring was started. Next, the first PET-CT scan was performed (T1), after which patients started hemodialysis still being in a horizontal position in the PET-CT camera. After the second PET-CT scan (T2), which was performed at a mean of 21 minutes (range 13-29) after the start of hemodialysis, patients were transferred to a hospital bed adjacent to the PET-CT camera to continue dialysis in a 30-45 degrees supine position. Before the third PET-CT scan (T3), which was performed at the end of the hemodialysis session at mean 209 minutes (range 168-223 minutes) after the start of hemodialysis, patients were transferred back to the PET-CT camera. Prior to each PET-CT measurement, patients rested in the supine position for at least 20 minutes, thereby reducing the influence of postural change on both NIRS and PET measurements.<sup>26, 27</sup> Blood pressure, heart rate, and tympanic temperature were measured every 30 minutes and before every PET-CT scan. Blood pressure was measured using an automated blood pressure monitor. Cerebrovascular resistance was calculated as the mean arterial pressure (MAP) divided by the CBF of the frontal gray matter. For the dialysis settings, see Supplemental file.

## **NIRS monitoring and analysis**

For NIRS monitoring an In Vivo Optical Spectroscopy device (INVOS™ 5100C Cerebral/Somatic Oximeter | Covidien – Medtronic, Minneapolis, USA) was used, with sensors placed bilaterally on the patient's forehead according to the manufacturer's recommendations. The adhesive optodes were connected to the NIRS device and the sampling rate was 0.2 Hz. For the analysis of rSO<sub>2</sub>, we excluded values of zero and excluded rSO<sub>2</sub> values with a quality score <4, to increase accuracy and exclude movement artifacts. Mean rSO<sub>2</sub> values were calculated for the 5-minute time periods during which the three [<sup>15</sup>O]H<sub>2</sub>O PET-CT scans were performed.

## **PET and MRI acquisition**

For the [<sup>15</sup>O]H<sub>2</sub>O PET-CT a Siemens Biograph 64-mCT (Siemens) Medical Systems, Tennessee, USA) was used. After performing a low-dose CT scan for attenuation and scatter correction, the dynamic PET acquisition (310 sec) was started, followed after 10 sec by an intravenous bolus injection of [<sup>15</sup>O]H<sub>2</sub>O. The injected dose of [<sup>15</sup>O]H<sub>2</sub>O was 500 MBq per scan, and 1500 MBq per patient for the study in total. Three of the 36 scans could not

be analyzed due to a technical problem with the automated sampling system (patient-identity 106 [T1], patient-identity 107 [T2], patient-identity 102 [T3]).

To define regional CBF, we also performed magnetic resonance imaging (MRI) in all patients using a 1.5T whole body system (Aera, Siemens, Erlangen, Germany) on a separate day. The scan protocol 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.

### **Image reconstruction and processing**

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. The volumes of interest 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).<sup>28</sup> 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.<sup>29</sup> 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 volumes of interest. For additional information on PET processing, see the Supplemental Methods.

### **Laboratory measurements**

For the laboratory measurements, including hemoglobin, hematocrit, pO<sub>2</sub>, pCO<sub>2</sub>, SaO<sub>2</sub>, and pH, arterial blood was sampled from the arterial dialysis line just before each PET-CT scan. Arterial O<sub>2</sub> content (CaO<sub>2</sub>) was calculated using the following equation:

$$CaO_2 \text{ (mL/dL)} = 1.34 \times Hb \times (SaO_2/100) + (0.0031 \times pO_2),^{30}$$

where Hb represents the hemoglobin concentration (converted to g/dL), SaO<sub>2</sub> represents the oxygen saturation (%), and pO<sub>2</sub> represents the oxygen pressure (converted to mmHg).

Markers of inflammation included high sensitive C-reactive protein (CRP), and pentraxin-3. Pentraxin-3 responds rapidly to inflammatory stimuli and is considered an appropriate marker for the intradialytic inflammatory response.<sup>31</sup>

Markers of endothelial activation included angiotensin-1, angiotensin-2, the angiotensin 2:1 (AP 2:1) ratio, and von Willebrand factor (vWF).<sup>32-34</sup>

### **Statistical analyses**

First, absolute changes in  $rSO_2$ , CBF, and clinical and laboratory characteristics were studied using linear mixed models (LMM), which allowed for individual random effects. The likelihood ratio test was used to determine whether the LMM including a random intercept and slope statistically better fitted the data as compared to including a random intercept only.

For the primary study objective, the relative change in  $rSO_2$  ( $\Delta rSO_2$ ) was compared with the relative change in frontal gray matter CBF ( $\Delta CBF$ ) during hemodialysis. We decided to study the correlation between *relative* changes instead of *absolute* changes, because NIRS and PET measure different physiological parameters (oxygenation vs. perfusion) and have different units. Besides, we chose the frontal gray matter CBF instead of the total (gray and white) frontal lobe CBF, because it has been estimated that approximately 85% of  $rSO_2$  is derived from more superficial cortical cerebral tissue, thus not including frontal white matter.<sup>18</sup> First,  $\Delta rSO_2$  and  $\Delta CBF$  were calculated as the mean of the individual percent change between T1 and T3 using descriptive statistics, reported as mean (%)  $\pm$  SD. Second, Pearson or Spearman correlation tests, whether appropriate, were used to evaluate the correlation between  $\Delta rSO_2$  and  $\Delta CBF$ . Finally, we created a Bland-Altman plot with the difference between  $\Delta CBF$  and  $\Delta rSO_2$  as a function of  $\Delta CBF$ , since PET is considered the reference method.<sup>35,36</sup> 95% levels of agreement were calculated as the mean of the differences  $\pm 1.96 \times SD$ . Subsequently, we checked for fixed and proportional bias using a T-test and linear regression model, respectively. In an additional analysis, we tested the correlation and agreement of  $\Delta rSO_2$  and  $\Delta CBF$  between T2 (as a second baseline shortly after the start of hemodialysis) and T3, and between T1 and T2. Furthermore, we additionally calculated cerebrovascular resistance (CVR) as  $MAP / CBF$ .

For the secondary study objective, associations of hemodialysis and oxygenation-related factors and of markers of inflammation and endothelial activation with  $rSO_2$  and frontal gray matter CBF were explored. For this objective, we studied *absolute* instead of *relative*  $rSO_2$  and CBF values at all time points using LMM, thereby increasing power since this enabled us to use all 33 and 36 measurements of CBF and  $rSO_2$ , respectively, instead of 10 and 12 measurements of  $\Delta CBF$  and  $\Delta rSO_2$ , respectively. Furthermore,  $rSO_2$  and CBF values of the left and right hemisphere were merged for the analyses, because  $rSO_2$  and CBF changes did not differ significantly between the left and the right hemisphere. The hemodialysis-related factors were defined previously based on literature and included mean arterial pressure (MAP),<sup>17</sup>  $pCO_2$ ,<sup>37</sup> pH,<sup>37</sup> tympanic temperature,<sup>38</sup> hematocrit,<sup>20-22, 39, 40</sup>



and ultrafiltration (UF) volume and rate.<sup>21,22</sup> For this study, we additionally studied the relation of oxygenation-related factors (CaO<sub>2</sub> and pO<sub>2</sub>) and markers of inflammation (CRP and pentraxin-3) and endothelial activation (angiopoietin-1, angiopoietin-2, AP 2:1 ratio and vWF) with both rSO<sub>2</sub> and frontal gray matter CBF change. All the hemodialysis and oxygenation-related factors and markers of inflammation and endothelial activation were studied univariately using LMM, checking the significance of interactions with scan-order. We did not perform adjusting for multiple testing, because the hemodialysis and oxygenation-related factors and markers of inflammation and endothelial activation were selected beforehand.

In supplementary analyses we repeated the main analyses after excluding one outlier. Second, we tested the correlation between the absolute rSO<sub>2</sub> and CBF values at all time points. Third, we tested the correlation of  $\Delta$ mean frontal-rSO<sub>2</sub> (mean of left and right rSO<sub>2</sub>) with  $\Delta$ global-CBF (from a volume of interest covering the whole brain).

Two-sided  $P < 0.05$  was considered statistically significant. Statistical analyses were performed with SPSS, version 23 (SPSS Inc, IBM company, USA), Stata/Se 14.2 (StataCorp LLC, USA), and GraphPad Prism version 5.0 (GraphPad Software, USA).

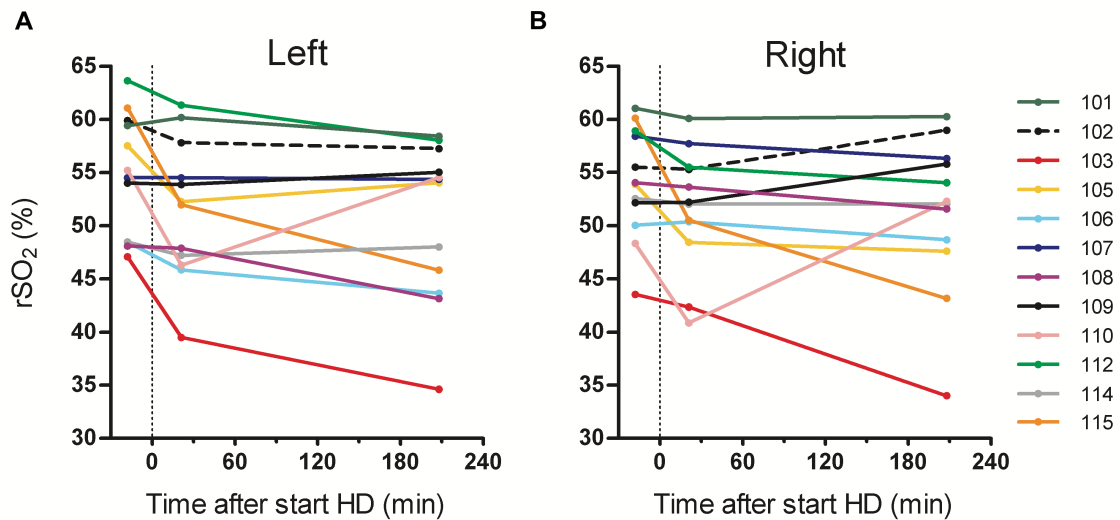
## RESULTS

### Patient enrolment and characteristics

Of the 15 patients who gave written informed consent, 12 patients completed the study (Table 1). Three patients withdrew from the study, because of a kidney transplantation, hip fracture, and withdrawal of consent, respectively. None of the patients had to be excluded because of a significant carotid artery stenosis.

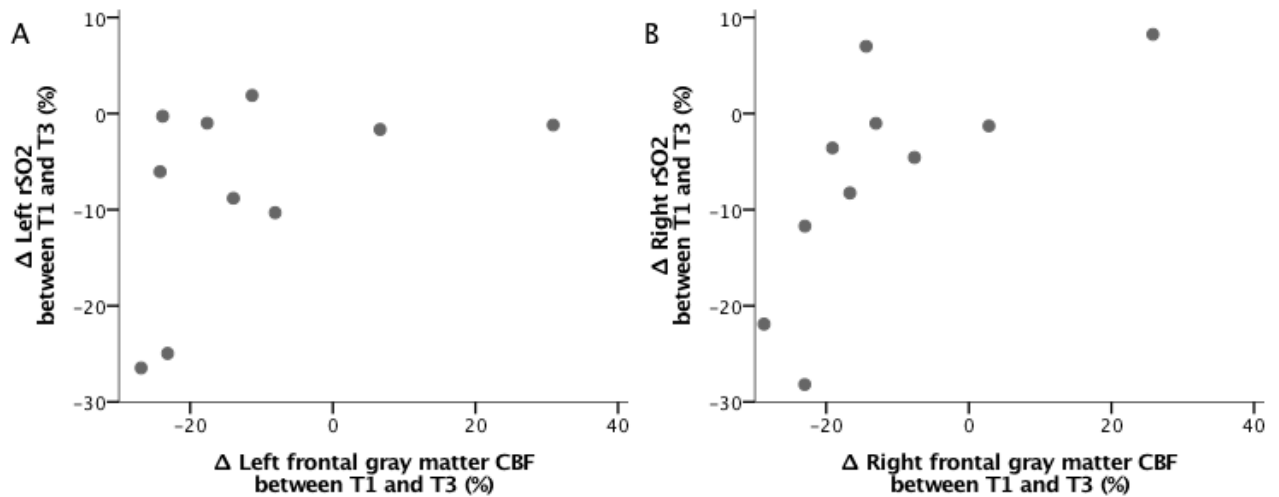
### Intradialytic NIRS-rSO<sub>2</sub> and frontal gray matter PET-CBF changes

Raw individual rSO<sub>2</sub> levels during hemodialysis are shown in Figure 1 for the left and right hemisphere. Using LMM, left rSO<sub>2</sub> declined from 54.8±5.7% to 51.2±7.3% (absolute difference -4.2% [95% CI, -6.6; -1.8];  $P=0.001$ ), whereas right rSO<sub>2</sub> declined from 54.1±5.2% before hemodialysis (T1) to 50.6±7.5% (absolute difference -3.1% [95% CI, -6.4; 0.3];  $P=0.08$ ) at the end of hemodialysis (T3) (Table 2). Frontal gray matter CBF declined from 44.1±7.8 mL/100g/min at T1 to 38.3±5.4 mL/100g/min at T3 (absolute difference -5.9 mL/100g/min [95% CI, -10.4; -1.5];  $P=0.009$ ) in the left hemisphere, and from 44.7±7.7 mL/100g/min at T1 to 38.8±5.1 mL/100g/min at T3 (absolute difference -6.2 mL/100g/min [95% CI, -10.6; -1.7];  $P=0.007$  in the right hemisphere (Table 2).



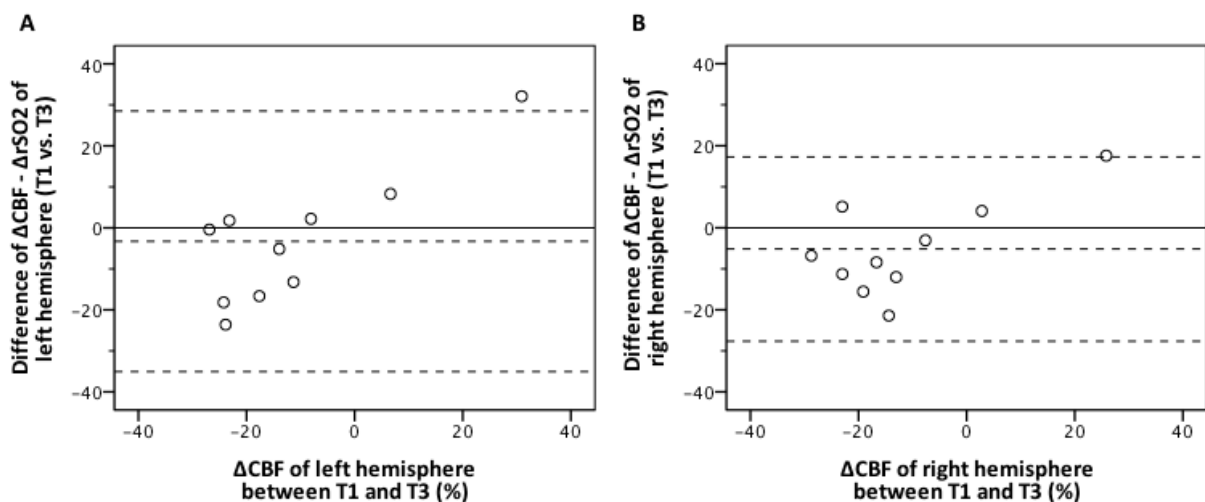
**Fig 1.** Individual  $rSO_2$  trajectories during hemodialysis of the left (1A) and right (1B) hemisphere. NIRS/PET measurement 1 was performed at a mean of 18 min (range 15-31 min) before the start of hemodialysis. Hemodialysis is regarded as baseline ( $t=0$ ). Measurement 2 and 3 were performed at a mean of 21 minutes (range 13-29 min) and 209 minutes (range 168-223 min) after the start of hemodialysis, respectively. Each line represents one patient.

The relative change in  $rSO_2$  between T1 and T3 ( $\Delta rSO_2$ ) was mean  $-8 \pm 9\%$  for the left and  $-5 \pm 11\%$  for the right hemisphere, respectively. The relative change in frontal gray matter CBF between T1 and T3 ( $\Delta CBF$ ) was  $-11 \pm 18\%$  for the left and  $-12 \pm 16\%$  for the right hemisphere, respectively. The individual relative changes in  $\Delta MAP$ ,  $\Delta CBF$ ,  $\Delta rSO_2$  and  $\Delta CVR$  per patient between T1 and T3 are shown in Table S1.  $\Delta rSO_2$  and  $\Delta CBF$  were moderately correlated for the right hemisphere ( $\rho$  0.69,  $P=0.03$ ), but weakly correlated for the left hemisphere ( $\rho$  0.31,  $P=0.4$ ) (Figure 2).



**Fig 2.** Correlation between  $\Delta$  rSO<sub>2</sub> and  $\Delta$  Frontal gray matter Cerebral Blood Flow of the left (2A) and right (2B) hemisphere, calculated between T3 and T1. Correlation coefficient for the left hemisphere:  $\rho$  0.31 ( $P=0.4$ ), and the right hemisphere:  $\rho$  0.69 ( $P=0.03$ ).

The Bland-Altman plot showed moderate agreement (Figure 3). The overall bias was  $-3 \pm 16\%$  ( $P=0.5$ ) for the left and  $-5 \pm 12\%$  ( $P=0.2$ ) for the right hemisphere (Figure 3). Furthermore, linear regression suggested a proportional bias, indicating underestimation of  $\Delta$ CBF by NIRS with larger CBF increases or decreases.



**Fig 3.** Bland-Altman plot of % changes in rSO<sub>2</sub> ( $\Delta$ rSO<sub>2</sub>) and in CBF ( $\Delta$ CBF) between T1 (before hemodialysis) and T3 (at the end of hemodialysis), displayed for the left (3A) and right (3B) hemisphere. The X-axis represents  $\Delta$ CBF (%), while the Y-axis represents the difference between  $\Delta$ CBF and  $\Delta$ rSO<sub>2</sub>. The central solid line represents zero bias. The central dashed line indicates overall bias, which is  $-3\%$  for the left, and  $-5\%$  for the right hemisphere, calculated as the mean of the differences, while the lower and upper dashed lines represent limits of agreement:  $-35\%$  and  $29\%$  for the left, and  $-28\%$  and  $17\%$  for the right hemisphere, respectively. Linear regression suggested the presence of proportional bias (left hemisphere:  $P=0.003$ , regression equation:  $Y= 5.1$

+ 0.8x; right hemisphere:  $P=0.03$ , regression equation:  $Y= 0.6 + 0.5x$ . This indicates that NIRS increasingly underestimated  $\Delta\text{CBF}$  with large(r) changes in CBF.

One patient showed a large increase in CBF (patient-id 110) and could be regarded as an outlier, although it is unknown whether a 30-40% increase in CBF is physiologically implausible during hemodialysis. After removal of this outlier, the correlation coefficients were almost similar (correlation  $\Delta r\text{SO}_2$  and  $\Delta\text{CBF}$  left hemisphere:  $\rho 0.30$  ( $P=0.4$ ), and right hemisphere:  $\rho 0.64$  ( $P=0.06$ ). However, removal of this outlier changed the Bland-Altman analysis yielding an (almost) significant fixed bias now, instead of a proportional bias (left hemisphere: fixed bias -7% ( $P=0.09$ ), lower and upper limits of agreement -29% and 14%; right hemisphere: fixed bias -8% ( $P=0.03$ ), lower and upper limits of agreement -25% and 9%).

Additionally, we studied  $\Delta r\text{SO}_2$  and  $\Delta\text{CBF}$  between T2 and T3, and between T1 and T2. Between T2 and T3, the correlation between  $\Delta r\text{SO}_2$  and  $\Delta\text{CBF}$  was moderate for the left ( $\rho 0.64$ ,  $P=0.048$ ) and strong for the right hemisphere ( $\rho 0.76$ ,  $P=0.01$ ) (Figure S1). The agreement plot was almost similar as for T1 vs. T3, and linear regression again suggested proportional bias (Figure S2). Between T1 and T2,  $\Delta r\text{SO}_2$  and  $\Delta\text{CBF}$  did not correlate for both hemispheres (Figure S3), and the agreement plot again suggested proportional bias (Figure S4).

Supplementary analyses showed no significant correlation between  $\Delta\text{MAP}$  and  $\Delta r\text{SO}_2$  (Figure S5), or between  $\Delta\text{MAP}$  and  $\Delta\text{CBF}$  (Figure S6). Second, using LMM, CVR did not change significantly during hemodialysis, and no significant correlation between  $\Delta\text{CVR}$  and  $\Delta r\text{SO}_2$  was found (Figure S7). Third, no significant correlation between the absolute  $r\text{SO}_2$  and absolute CBF values at any time point was found (Figure S8). Finally, defined as T1 versus T3,  $\Delta\text{mean-frontal } r\text{SO}_2$  (mean of left and right  $\Delta r\text{SO}_2$ ) and  $\Delta\text{global-CBF}$  (the whole brain as region of interest) were non-significantly correlated ( $\rho 0.51$ ,  $P=0.1$ ). Defined as T2 versus T3,  $\Delta\text{mean-frontal } r\text{SO}_2$  and  $\Delta\text{global-CBF}$  showed a strong correlation ( $\rho 0.72$ ,  $P=0.02$ ).

### **Associations of hemodialysis-related factors with NIRS- $r\text{SO}_2$ and frontal gray matter PET-CBF**

The mean UF volume, *i.e.* the fluid volume that was removed during hemodialysis, was  $1934\pm 781$  mL, and the mean UF rate, *i.e.* the rate of fluid removal, was  $6.7\pm 2.5$  mL/h/kg body weight. During hemodialysis, blood pH, tympanic temperature and hematocrit increased significantly, whereas the MAP did not change significantly (Table 3).

A significant interaction of pH with scan-order was present for the associations between pH and  $r\text{SO}_2$ , and between pH and CBF. A higher blood pH was associated with a

higher  $rSO_2$  at T3, as compared to T1, but with a lower CBF (Table 4; for detailed information on the interaction effects and confidence intervals, see Table S2). A higher hematocrit was significantly associated with a higher  $rSO_2$  at T2 and T3 as compared to T1, but not with CBF. MAP, tympanic temperature, and UF volume were not associated with  $rSO_2$ , whereas tympanic temperature and UF volume had a significant negative effect on CBF.

### **Associations of oxygenation-related factors, and markers of inflammation and endothelial activation with NIRS- $rSO_2$ and frontal gray matter PET-CBF**

$CaO_2$  and pentraxin-3 increased significantly during hemodialysis. Intradialytic  $pCO_2$ ,  $pO_2$ , CRP, and the endothelial activation markers did not change significantly (Table 3).

Of the oxygenation-related markers, a higher  $pCO_2$  was significantly associated with a lower  $rSO_2$  at T2, as compared to T1. Conversely, a higher  $pCO_2$  was significantly associated with a higher CBF (Table 4). Higher  $pO_2$  was significantly associated with higher  $rSO_2$ , whereas the association between  $pO_2$  and CBF yielded different effects over time (Table 4).  $CaO_2$  was positively associated with  $rSO_2$  whereas it was negatively associated with CBF. The inflammation markers were not associated with  $rSO_2$  or CBF. Of the endothelial activation markers, a higher angiopoietin-2 and AP 2:1 ratio was associated with a lower  $rSO_2$ , and a higher vWF was associated with a higher  $rSO_2$ . None of the endothelial activation makers had an association with CBF.

### **Adverse event**

One patient (identity 115) lost consciousness due to dialysis-induced hypotension shortly after the third NIRS/PET measurement. The mean decline in CBF of left and right frontal gray matter was 23% (both hemispheres -23%) and the mean frontal  $rSO_2$  decline was 27% (left -25%; right -28%). The patient made a full recovery without sequelae.

## **DISCUSSION**

In this study, we found a moderate correlation between frontal  $\Delta rSO_2$  as measured with NIRS and  $\Delta CBF$  of the frontal gray matter as measured with  $[^{15}O]H_2O$  PET during hemodialysis. The agreement analysis showed moderate agreement and a trend towards predominantly a fixed bias with underestimation of  $\Delta CBF$  by NIRS. Thus, NIRS could be a proxy for PET to capture intradialytic CBF changes, but some correction factor may be needed to correct for the underestimation of  $\Delta CBF$  by NIRS. Furthermore, considerable differences were noted with regard to associations of hemodialysis- and oxygenation-

related factors and markers of endothelial activation with  $rSO_2$  as compared to CBF. This underscores that  $rSO_2$  and CBF represent different physiological parameters of the brain.

Few studies have simultaneously performed cerebral oximetry and PET scanning. One study evaluated the change in cerebral blood volume ( $\Delta CBV$ ) as measured by NIRS, with  $\Delta CBV$  as measured by PET, during normoventilation and during  $pCO_2$  manipulation procedures.<sup>41</sup> They reported a moderate correlation ( $\rho = 0.56$ ) between  $\Delta CBV$ -NIRS and  $\Delta CBV$ -PET, and an underestimation of  $\Delta CBV$  by NIRS. Villringer *et al.* compared changes in  $rSO_2$  by NIRS with simultaneous changes in PET-CBF during rest and during cognitive activation tasks. They found a strong correlation ( $\rho = 0.88$ ) between  $\Delta \text{total-Hb}$  (*i.e.*, the sum of  $\Delta \text{oxy-Hb}$  and  $\Delta \text{deoxy-Hb}$ ) and  $\Delta CBF$ , if a penetration depth of near-infrared light of 0.9 cm into the brain cortex was assumed.<sup>42</sup> Another study from the same group showed strong correlations of  $\Delta \text{oxy-Hb}$  ( $\rho$  range: 0.74 to 0.75),  $\Delta \text{deoxy-Hb}$  ( $\rho$  range: -0.64 to -0.69), and  $\Delta \text{total-Hb}$  ( $\rho$  range: 0.88 to 0.93) with  $\Delta CBF$ , when assuming various penetration depths  $\leq 1.35$  cm of near-infrared light.<sup>43</sup> Several differences limit the comparison of their findings to ours. First, Villringer *et al.* compared NIRS to frontal CBF of a small semisphere, a so-called 'banana-shaped' region behind the NIRS optode. In contrary, we studied the correlation of NIRS with CBF of the total frontal gray matter. Second, Villringer *et al.* did not describe the correlation between  $\Delta rSO_2$  (*i.e.*,  $\Delta \text{oxy-Hb}/\Delta \text{deoxy-Hb}$ ) and  $\Delta CBF$ . Besides, neither the studies from this group nor other studies investigated patients with CKD or encompassed the hemodialysis process.

The hemodialysis process is a unique physiological stimulus involving many simultaneous hemodynamic and metabolic changes,<sup>44</sup> e.g. a change in pH due to bicarbonate infusion that is not necessarily accompanied by simultaneous changes in  $pCO_2$  or  $pO_2$ . Previous studies reported either no change in  $rSO_2$  during hemodialysis,<sup>45,46</sup> or an  $rSO_2$  decline only during the first 35 minutes of hemodialysis, with a subsequent increase in  $rSO_2$  yielding a net non-significant change at the end of hemodialysis.<sup>47</sup> Our study is new insofar that we simultaneously studied intradialytic changes in  $rSO_2$  by NIRS and changes in CBF by PET.

There is increasing interest in the utilization of NIRS to monitor adequacy of brain perfusion non-invasively. The underlying assumption is that changes in  $rSO_2$  reflect changes in CBF, which is theoretically correct if cerebral metabolism, CBV, and additionally  $CaO_2$ , blood transit time, and oxygen extraction fraction (OEF) remain constant.<sup>48</sup> However, to our knowledge, it is unknown whether this is true during the hemodialysis procedure. First, absolute systemic blood volume was reported to decline by 17% during hemodialysis,<sup>49</sup> but it is unknown whether CBV also declines. Second, during hemodialysis 10% of the patients of a hemodialysis cohort experienced prolonged hypoxia (arterial oxygen saturation  $< 90\%$  at least one third of the treatment time).<sup>50</sup> Third, apart from an

intradialytic effect, it was reported that cerebral oxygen metabolism,<sup>51</sup> blood transit time,<sup>52</sup> and oxygen extraction,<sup>51</sup> were altered in hemodialysis patients compared to controls. Nevertheless, since our primary study aim was to evaluate the correlation between  $\Delta rSO_2$  and  $\Delta CBF$ , we are not able to draw any conclusion on the other parameters such as CBV, OEF, or blood transit time.

The underestimation of CBF changes by NIRS seems to be related to predominantly a fixed bias, since with removal of one outlier no proportional bias was present anymore. The underestimation of  $\Delta CBF$  by NIRS could be the result of scattering effects of extracerebral tissue on the transmission of light. Computer modeling showed that in a typical tissue volume interrogated by NIRS, approximately 30% was brain and 70% was extracerebral tissue.<sup>53</sup>

Remarkably, we noted an absent correlation between  $\Delta rSO_2$  and  $\Delta CBF$ , defined as T1 versus T2 including the first 30 minutes of hemodialysis. Previous studies reported that  $PaO_2$  initially declined during the first 60 minutes of hemodialysis treatment.<sup>50, 54</sup> We speculate that early intradialytic changes in  $PaO_2$  influenced  $\Delta rSO_2$  rather than  $\Delta CBF$  between T1 and T2 thereby limiting the correlation. Of note, the divergent association of  $PaO_2$  with  $rSO_2$  as compared to CBF seems to underscore this hypothesis.

Another remarkable finding was the left-right asymmetry in the correlation and Bland-Altman analyses. Because removal of one outlier did not change this asymmetry, we consider this asymmetry a change finding.

We found that on average CVR did not change significantly during hemodialysis. This constant CVR could suggest that static autoregulation might have been disturbed. However, we cannot draw any definite conclusions, since this was not an autoregulation study and many factors change simultaneously during hemodialysis (e.g. pH, hematocrit), which might directly affect CVR.

Four patients experienced an  $rSO_2$  drop of >20% during hemodialysis. A 20%  $rSO_2$  decline has been proposed as predictor of cerebral ischemia in patients during carotid endarterectomy and cardiac surgery.<sup>55, 56</sup> A >15% drop in  $rSO_2$  during hemodialysis was shown to correlate with executive function decline at 1-year follow-up.<sup>17</sup> Therefore, although NIRS tended to underestimate PET-CBF with increasing CBF changes, in our opinion NIRS is still a promising technique to monitor declines in cerebral oxygenation during hemodialysis. Intradialytic changes in cerebral oxygenation might yield important information on intradialytic brain homeostasis, apart from intradialytic changes in cerebral perfusion. More studies are needed to explore whether large intradialytic  $rSO_2$  drops are associated with incident cerebral ischemic injury and decline of cognitive function during follow-up.

The second aim of this study was to explore associations of several clinical and

laboratory parameters with  $rSO_2$  as compared to CBF. No association of MAP with  $rSO_2$  was found, similar to previous studies.<sup>45,46</sup> Remarkably, pH was positively associated with  $rSO_2$  and negatively with CBF. The positive association of pH with  $rSO_2$  might be explained by a leftward shift of the oxygen-hemoglobin dissociation curve due to the increase in pH during dialysis, thereby theoretically increasing  $rSO_2$ . However, others have reported a negative association of pH with  $rSO_2$  in dialysis patients.<sup>57</sup> Further examination is required on the effects of pH on CBF and  $rSO_2$  during hemodialysis, especially because the intradialytic change in pH is a modifiable factor by lowering the bicarbonate concentration in the dialysate.

To our knowledge, potential associations of inflammation and endothelial activation markers with cerebral tissue oxygenation have not been reported previously. We found an association between endothelial activation markers and  $rSO_2$ , since a higher angiotensin-2 and angiotensin 2:1 ratio was associated with lower  $rSO_2$ . Angiotensin-1 stabilizes the endothelium, whereas angiotensin-2 functions as a vessel-destabilizing molecule.<sup>58</sup> A higher angiotensin 2:1 ratio seems to represent loss of endothelial barrier integrity,<sup>59</sup> and might be an early marker of endothelial activation and dysfunction.<sup>60,61</sup> A possible explanation for the association between angiotensin-2, and the angiotensin 2:1 ratio with  $rSO_2$  might be found in the lungs. Recently, angiotensin-2, which is stored in pulmonary epithelial cells, was suggested to have effects on gas exchange.<sup>62</sup> Nevertheless, the relation between angiotensin-2 and the angiotensin 2:1 ratio and  $rSO_2$  needs further examination, and is beyond the scope of this study.

There are a number of potential weaknesses to this study. The sample size of this study was small and one outlier might have had a relative large influence in the analyses. Furthermore, the findings on our second study aim, *i.e.*, associations of hemodialysis and oxygenation-related factors, and inflammation and endothelial activation markers with  $rSO_2$  and CBF, should be purely considered as hypothesis generating. Larger studies are needed to evaluate the effect size of various factors and markers dynamically by multivariate analysis, especially because various hemodynamic, metabolic, and laboratory characteristics change simultaneously during hemodialysis. Second, NIRS and CBF measurements were performed in a supine position, whereas in general patients are in a semi-upright sitting position during hemodialysis. A semi-upright sitting position might have changed the  $rSO_2$  and CBF values but should not alter the correlation between both. Third, for a future study the use of a NIRS device that provides oxyHb, deoxyHb and total Hb levels is advised, because oxyHb better relates to arterial inflow than  $rSO_2$ , which is a mix of arterial and venous circulation. Besides, such study might also provide more information on transit time (changes) during hemodialysis, which we were not able to take into account.



In conclusion, NIRS could be used as a proxy to PET to detect intradialytic CBF changes, but a correction factor may be needed to correct for the underestimation of CBF changes by NIRS. The different associations of hemodialysis- and oxygenation-related factors and markers of endothelial activation with  $rSO_2$  as compared to CBF underscore that NIRS and PET capture different physiological parameters of the brain.

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**Table 1** Patient characteristics (N=12)

Age (yr)	75.4 ± 5.2
Men (%)	7 (58%)
BMI (kg/m <sup>2</sup> )	26.6 ± 3.5
<i>Primary kidney disease: (%)</i>	
Glomerulonephritis	4 (33%)
Diabetes	1 (8%)
Vascular	3 (25%)
Other diagnosis	3 (25%)
Unknown	1 (8%)
Current smoker	4 (33%)
Diabetes	3 (25%)
Hypertension	11 (73%)
Myocardial infarction	2 (17%)
Heart failure	1 (8%)
Peripheral artery disease	1 (8%)
COPD	1 (8%)
<i>Medication:</i>	
CCB	4 (33%)
Nitrate	3 (25%)
ACE inhibitor	1 (8%)
Angiotensin receptor blocker	1 (8%)
Beta-blocker	9 (75%)

Data are presented as means ± SD or (range), or number and percentages (%).ACE: angiotensin-converting enzyme; BMI: body mass index; CBF: cerebral blood flow; CCB: calcium channel blocker.

**Table 2** Intradialytic changes in NIRS-rSO<sub>2</sub> and in PET-CBF

Region	Before start HD			After start HD			At the end of HD		Dialysis treatment effect	
	T1	T2	T3	T1 vs. T3	T2 vs. T3					
<i>Regional oxygen saturation -measured by NIRS:</i>										
Frontal left (%)	54.8 ± 5.7	51.6 ± 5.7	51.2 ± 7.3	-4.2 (-6.6; -1.8) **	-1.0 (-3.4; 1.4)					
Frontal right (%)	54.1 ± 5.2	51.6 ± 6.5	50.6 ± 7.5	-3.1 (-6.4; 0.3)	-0.6 (-3.5; 2.4)					
<i>Cerebral blood flow - measured by PET:</i>										
Frontal GM left (mL/100g/min)	44.1 ± 7.8	42.7 ± 6.2	38.3 ± 5.4	-5.9 (-10.4; -1.5) **	-4.8 (-8.7; -0.9) *					
Frontal GM right (mL/100g/min)	44.7 ± 7.7	43.5 ± 6.7	38.8 ± 5.1	-6.2 (-10.6; -1.7) **	-5.1 (-9.1; -1.1) *					

Data are presented as unadjusted means ± SD. Dialysis treatment effects are obtained from linear mixed effects models and are presented as estimated mean difference (95% CI), \*  $P < 0.05$ , \*\*  $P < 0.01$ .. Abbreviations: GM: gray matter; HD: hemodialysis.

**Table 3** Intradialytic changes in Hemodialysis and Oxygenation-related factors, and in Inflammation and Endothelial activation markers

Region	Before start HD			After start HD			At the end of HD			Dialysis treatment effect <sup>a</sup>
	T1	T2	T3	T1	T2	T3	T1	T2	T3	
<i>Hemodialysis-related factors:</i>										
MAP (mmHg)	101 ± 11	105 ± 15	93 ± 17	-6 (-14; 1)						
Tympanic temperature (°C)	36.3 ± 0.5	36.2 ± 0.5	35.9 ± 0.6	-0.3 (-0.6; -0.01)*						
pH	7.38 ± 0.04	7.40 ± 0.03	7.48 ± 0.04	0.10 (0.08; 0.12)***						
Hematocrit (v/v)	0.33 ± 0.04	0.31 ± 0.04	0.34 ± 0.04	0.02 (0.006; 0.03)**						
<i>Oxygenation-related factors:</i>										
pO <sub>2</sub> (kPa)	12.2 ± 2.1	11.5 ± 1.8	12.5 ± 2.6	0.4 (-0.7; 1.5)						
pCO <sub>2</sub> (kPa)	5.0 ± 0.5	5.2 ± 0.5	5.1 ± 0.5	0.1 (-0.1; 0.3)						
CaO <sub>2</sub> (mL/dL)	14.0 ± 1.5	13.2 ± 1.7	14.9 ± 1.8	0.8 (0.4; 1.2)***						
<i>Inflammation markers:</i>										
C-reactive protein (mg/L)	7.9 ± 6.3	7.5 ± 6.1	8.8 ± 8.0	1.4 (-0.2; 3.1)						
Pentraxin-3 (ng/mL)	1.89 ± 0.83	2.08 ± 1.36	3.62 ± 1.72	1.65 (1.01; 2.30)***						
<i>Endothelial activation markers:</i>										
Angiopoiectin-1 (ng/mL)	14.1 ± 6.8	11.6 ± 5.0	15.6 ± 14.4	1.5 (-5.1; 8.2)						
Angiopoiectin-2 (ng/mL)	10.4 ± 3.5	9.7 ± 3.1	10.3 ± 3.2	-0.1 (-0.5; 0.3)						
Angiopoiectin 2:1 ratio	0.93 ± 0.51	1.02 ± 0.57	1.07 [0.44-1.82]	0.39 (-0.07; 0.85)						
VWF (%)	158 ± 43	141 ± 45	160 ± 49	2 (-12; 16)						

Data are presented as unadjusted means ± SD. <sup>a</sup> Dialysis treatment effects are obtained from linear mixed effects models including a random intercept, or a random intercept and slope (hematocrit, CRP, Pentraxin-3, and Angiopoiectin 2:1 ratio), and presented as estimated mean difference (95% CI), \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ . Abbreviations: CaO<sub>2</sub>, arterial oxygen content; MAP, mean arterial pressure; VWF, von Willebrand Factor.

**Table 4** Associations of Hemodialysis and Oxygenation-related factors, and of Inflammation and Endothelial activation markers with NIRS- $rSO_2$  (left panel), and frontal gray matter PET-CBF (right panel)

	Estimated effect on frontal $rSO_2$ (%)		Estimated effect on frontal gray matter CBF (mL/100g/min)	
	Effect at T1, T2, and T3 †	Interaction with time present ‡	Effect at T1, T2, and T3 †	Interaction with time present ‡
<i>Hemodialysis-related factors:</i>				
MAP (mmHg)	-	-	NA <sup>§</sup>	-
Tympanic temperature (°C)	-	-	-2.0*	-
pH (per 0.1)	5.0*	7.2*	-2.8***	-15.5**
Ht (per 0.1 mmol/L)	3.9*	1.2*	-	-
UF volume (L)	-	-	-4.8**	-
UF rate (mL/h/kg)	-0.5*	-	-1.2*	-
<i>Oxygenation-related factors:</i>				
$pO_2$ (kPa)	0.8*	-	-1.4**	0.4***
$pCO_2$ (kPa)	-5.1*	-	3.8**	-
$CaO_2$ (mL/dL)	-	0.3*	-1.4*	-
<i>Endothelial activation markers:</i>				
Angiopoietin 2 (ng/mL)	-	-1.7**	-	-
Angiopoietin 2:1 ratio	-5.1***	-	-	-
VWF (%)	0.06*	-	-	-

Associations were studied using linear mixed effects models including a random intercept, or a random intercept and slope, whether appropriate according to the likelihood-ratio test. The estimated effects (95% CI) of the individual characteristics on frontal  $rSO_2$  and frontal gray matter CBF that were significant are presented; \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ .

† For these characteristics, no interaction was present, meaning that the association of the characteristic with  $rSO_2$  or with CBF is similar for all time points. For example, a 1 point higher Angiopoietin 2:1 ratio is associated with a 5% lower  $rSO_2$ , independent of T1, T2 or T3.

‡ An interaction with time means that the association of the characteristic with  $rSO_2$  or CBF is different per time point, as compared to T1. For example, a 0.1 higher pH is associated with a 5% higher  $rSO_2$  at T2, and 7% higher  $rSO_2$  at T3, as compared to T1. For detailed information on confidence intervals and interaction effects, see Table S1 in the supplementary file.

§ The analysis of mean arterial pressure and CBF was inconclusive due to patient variation and missing values.<sup>14</sup> Markers of inflammation (pentraxin-3, and CRP) were not associated with  $rSO_2$  or CBF.

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Abbreviations: CaO<sub>2</sub>, arterial oxygen content; Ht, hematocrit; MAP, mean arterial pressure; NA, not available; n.s., not significant; rSO<sub>2</sub>, regional oxygen saturation; UF, ultrafiltration; vWF, von Willebrand Factor.

## REFERENCES

1. Bugnicourt JM, Godefroy O, Chillon JM, Choukroun G, Massy ZA: Cognitive disorders and dementia in CKD: The neglected kidney-brain axis. *J Am Soc Nephrol* 24: 353-363, 2013
2. Yao H, Takashima Y, Hashimoto M, Uchino A, Yuzuriha T: Subclinical cerebral abnormalities in chronic kidney disease. *Contrib Nephrol* 179: 24-34, 2013
3. Chai C, Wang Z, Fan L, Zhang M, Chu Z, Zuo C, Liu L, Mark Haacke E, Guo W, Shen W, Xia S: Increased number and distribution of cerebral microbleeds is a risk factor for cognitive dysfunction in hemodialysis patients: A longitudinal study. *Medicine (Baltimore)* 95: e2974, 2016
4. Zhang R, Liu K, Yang L, Zhou T, Qian S, Li B, Peng Z, Li M, Sang S, Jiang Q, Sun G: Reduced white matter integrity and cognitive deficits in maintenance hemodialysis ESRD patients: A diffusion-tensor study. *Eur Radiol* 25: 661-668, 2015
5. Hsieh TJ, Chang JM, Chuang HY, Ko CH, Hsieh ML, Liu GC, Hsu JS: End-stage renal disease: In vivo diffusion-tensor imaging of silent white matter damage. *Radiology* 252: 518-525, 2009
6. Kurella Tamura M, Vittinghoff E, Hsu CY, Tam K, Seliger SL, Sozio S, Fischer M, Chen J, Lustigova E, Strauss L, Deo R, Go AS, Yaffe K, CRIC Study Investigators: Loss of executive function after dialysis initiation in adults with chronic kidney disease. *Kidney Int* 91: 948-953, 2017
7. Murray AM, Seliger S, Lakshminarayan K, Herzog CA, Solid CA: Incidence of stroke before and after dialysis initiation in older patients. *J Am Soc Nephrol* 24: 1166-1173, 2013
8. McIntyre CW, & Goldsmith DJ: Ischemic brain injury in hemodialysis patients: Which is more dangerous, hypertension or intradialytic hypotension? *Kidney Int* 87: 1109-1115, 2015
9. Qian Q: Acid-base alterations in ESRD and effects of hemodialysis. *Semin Dial*; 31: 226-235, 2018
10. Burton JO, Jefferies HJ, Selby NM, McIntyre CW: Hemodialysis-induced repetitive myocardial injury results in global and segmental reduction in systolic cardiac function. *Clin J Am Soc Nephrol* 4: 1925-1931, 2009
11. Rysz J, Banach M, Cialkowska-Rysz A, Stolarek R, Barylski M, Drozd J, Okonski P: Blood serum levels of IL-2, IL-6, IL-8, TNF-alpha and IL-1beta in patients on maintenance hemodialysis. *Cell Mol Immunol* 3: 151-154, 2006
12. Jofre R, Rodriguez-Benitez P, Lopez-Gomez JM, Perez-Garcia R: Inflammatory syndrome in patients on hemodialysis. *J Am Soc Nephrol* 17: S274-80, 2006
13. Meyer C, Heiss C, Drexhage C, Kehmeier ES, Balzer J, Muhlfeld A, Merx MW, Lauer T, Kuhl H, Floege J, Kelm M, Rassaf T: Hemodialysis-induced release of hemoglobin limits nitric oxide bioavailability and impairs vascular function. *J Am Coll Cardiol* 55: 454-459, 2010
14. Polinder-Bos HA, Garcia DV, Kuipers J, Elting JWJ, Aries MJH, Krijnen WP, Groen H, Willemsen ATM, van Laar PJ, Strijkert F, Luurtsema G, Slart RHJA, Westerhuis R, Gansevoort RT, Gaillard CAJM, Franssen CFM: Hemodialysis induces an acute decline in cerebral blood flow in elderly patients. *J Am Soc Nephrol* 29: 1317-1325, 2018
15. MacEwen C, Watkinson P, Tarassenko L, Pugh C: Cerebral ischemia during hemodialysis-finding the signal in the noise. *Semin Dial*; 31: 199-203, 2018
16. Terborg C, Groschel K, Petrovitch A, Ringer T, Schnaudigel S, Witte OW, Kastrup A: Noninvasive assessment of cerebral perfusion and oxygenation in acute ischemic stroke by near-infrared spectroscopy. *Eur Neurol* 62: 338-343, 2009
17. MacEwen C, Sutherland S, Daly J, Pugh C, Tarassenko L: Relationship between hypotension and cerebral ischemia during hemodialysis. *J Am Soc Nephrol* 28: 2511-2520, 2017
18. Murkin JM, & Arango M: Near-infrared spectroscopy as an index of brain and tissue oxygenation. *Br J Anaesth* 103 Suppl 1: i3-13, 2009
19. Holzschuh M, Woertgen C, Metz C, Brawanski A: Comparison of changes in cerebral blood flow and cerebral oxygen saturation measured by near infrared spectroscopy (NIRS) after acetazolamide. *Acta Neurochir (Wien)* 139: 58-62, 1997
20. Metry G, Spittle M, Rahmati S, Giller C, Giller A, Kaufman A, Schneditz D, Manno E, Brenner Z, Boniece I, Ronco F, Ronco C, Levin NW: Online monitoring of cerebral hemodynamics during hemodialysis. *Am J Kidney Dis* 40: 996-1004, 2002
21. Stefanidis I, Bach R, Mertens PR, Liakopoulos V, Liapi G, Mann H, Heintz B: Influence of hemodialysis on the mean blood flow velocity in the middle cerebral artery. *Clin Nephrol* 64: 129-137, 2005
22. Hata R, Matsumoto M, Handa N, Terakawa H, Sugitani Y, Kamada T: Effects of hemodialysis on cerebral circulation evaluated by transcranial doppler ultrasonography. *Stroke* 25: 408-412, 1994
23. Regolisti G, Maggiore U, Cademartiri C, Cabassi A, Caiazza A, Tedeschi S, Antonucci E, Fiaccadori E: Cerebral blood flow decreases during intermittent hemodialysis in patients with acute kidney injury, but not in patients with end-stage renal disease. *Nephrol Dial Transplant* 28: 79-85, 2013

24. Skinner H, Mackaness C, Bedford N, Mahajan R: Cerebral haemodynamics in patients with chronic renal failure: Effects of haemodialysis. *Br J Anaesth* 94: 203-205, 2005
25. Postiglione A, Faccenda F, Gallotta G, Rubba P, Federici S: Changes in middle cerebral artery blood velocity in uremic patients after hemodialysis. *Stroke* 22: 1508-1511, 1991
26. Garrett ZK, Pearson J, Subudhi AW: Postural effects on cerebral blood flow and autoregulation. *Physiol Rep* 5: 10.14814/phy2.13150. Epub 2017 Feb 27, 2017
27. Mehagnoul-Schipper DJ, Vloet LC, Colier WN, Hoefnagels WH, Jansen RW: Cerebral oxygenation declines in healthy elderly subjects in response to assuming the upright position. *Stroke* 31: 1615-1620, 2000
28. Hammers A, Allom R, Koeppe MJ, Free SL, Myers R, Lemieux L, Mitchell TN, Brooks DJ, Duncan JS: Three-dimensional maximum probability atlas of the human brain, with particular reference to the temporal lobe. *Hum Brain Mapp* 19: 224-247, 2003
29. Meyer E: Simultaneous correction for tracer arrival delay and dispersion in CBF measurements by the H215O autoradiographic method and dynamic PET. *J Nucl Med* 30: 1069-1078, 1989
30. Roach RC, Koskolou MD, Calbet JA, Saltin B: Arterial O<sub>2</sub> content and tension in regulation of cardiac output and leg blood flow during exercise in humans. *Am J Physiol* 276: H438-45, 1999
31. Sjoberg B, Qureshi AR, Anderstam B, Alvestrand A, Barany P: Pentraxin 3, a sensitive early marker of hemodialysis-induced inflammation. *Blood Purif* 34: 290-297, 2012
32. Parikh SM: The angiotensin-Tie2 signaling axis in systemic inflammation. *J Am Soc Nephrol* 28: 1973-1982, 2017
33. Fang Y, Li C, Shao R, Yu H, Zhang Q, Zhao L: Prognostic significance of the angiotensin-2/angiotensin-1 and angiotensin-1/tie-2 ratios for early sepsis in an emergency department. *Crit Care* 19: 367-015-1075-6, 2015
34. Assa S, Hummel YM, Voors AA, Kuipers J, Westerhuis R, Groen H, Bakker SJ, Muller Kobold AC, van Oeveren W, Struck J, de Jong PE, Franssen CF: Hemodialysis-induced regional left ventricular systolic dysfunction and inflammation: A cross-sectional study. *Am J Kidney Dis* 64: 265-273, 2014
35. Krouwer JS: Why bland-altman plots should use X, not (Y+X)/2 when X is a reference method. *Stat Med* 27: 778-780, 2008
36. Bland JM, & Altman DG: Measuring agreement in method comparison studies. *Stat Methods Med Res* 8: 135-160, 1999
37. Yoon S, Zuccarello M, Rapoport RM: pCO<sub>2</sub> and pH regulation of cerebral blood flow. *Front Physiol* 3: 365, 2012
38. Eldehni MT, Odudu A, McIntyre CW: Randomized clinical trial of dialysate cooling and effects on brain white matter. *J Am Soc Nephrol* 26: 957-965, 2015
39. Vorstrup S, Lass P, Waldemar G, Brandt L, Schmidt JF, Johnsen A, Paulson OB: Increased cerebral blood flow in anemic patients on long-term hemodialytic treatment. *J Cereb Blood Flow Metab* 12: 745-749, 1992
40. Mathew RJ, Rabin P, Stone WJ, Wilson WH: Regional cerebral blood flow in dialysis encephalopathy and primary degenerative dementia. *Kidney Int* 28: 64-68, 1985
41. Rostrup E, Law I, Pott F, Ide K, Knudsen GM: Cerebral hemodynamics measured with simultaneous PET and near-infrared spectroscopy in humans. *Brain Res* 954: 183-193, 2002
42. Villringer K, Minoshima S, Hock C, Obrig H, Ziegler S, Dirnagl U, Schwaiger M, Villringer A: Assessment of local brain activation. A simultaneous PET and near-infrared spectroscopy study. *Adv Exp Med Biol* 413: 149-153, 1997
43. Hock C, Villringer K, Muller-Spahn F, Wenzel R, Heekeren H, Schuh-Hofer S, Hofmann M, Minoshima S, Schwaiger M, Dirnagl U, Villringer A: Decrease in parietal cerebral hemoglobin oxygenation during performance of a verbal fluency task in patients with alzheimer's disease monitored by means of near-infrared spectroscopy (NIRS)--correlation with simultaneous rCBF-PET measurements. *Brain Res* 755: 293-303, 1997
44. Kooman JP, Katzarski K, van der Sande FM, Leunissen KM, Kotanko P: Hemodialysis: A model for extreme physiology in a vulnerable patient population. *Semin Dial* 31: 500-506, 2018
45. Hoshino T, Ookawara S, Goto S, Miyazawa H, Ito K, Ueda Y, Kaku Y, Hirai K, Nabata A, Mori H, Yoshida I, Tabei K: Evaluation of cerebral oxygenation in patients undergoing long-term hemodialysis. *Nephron Clin Pract* 126: 57-61, 2014
46. Ookawara S, Ito K, Ueda Y, Miyazawa H, Hayasaka H, Kofuji M, Uchida T, Ishii H, Shindo M, Kitano T, Aomatsu A, Hirai K, Kaku Y, Hoshino T, Tabei K, Morishita Y: Differences in tissue oxygenation and changes in total hemoglobin signal strength in the brain, liver, and lower-limb muscle during hemodialysis. *J Artif Organs* 21: 86-93, 2018
47. Malik J, Kudlicka J, Lachmanova J, Valerianova A, Rocinova K, Bartkova M, Tesar V: Tissue ischemia worsens during hemodialysis in end-stage renal disease patients. *J Vasc Access* 18: 47-51, 2017



48. Fantini S: Dynamic model for the tissue concentration and oxygen saturation of hemoglobin in relation to blood volume, flow velocity, and oxygen consumption: Implications for functional neuroimaging and coherent hemodynamics spectroscopy (CHS). *Neuroimage* 85 Pt 1: 202-221, 2014
49. Dasselaar JJ, Lub-de Hooge MN, Pruijm J, Nijhuis H, Wiersum A, de Jong PE, Huisman RM, Franssen CF: Relative blood volume changes underestimate total blood volume changes during hemodialysis. *Clin J Am Soc Nephrol* 2: 669-674, 2007
50. Meyring-Wosten A, Zhang H, Ye X, Fuerstinger DH, Chan L, Kappel F, Artemyev M, Ginsberg N, Wang Y, Thijssen S, Kotanko P: Intradialytic hypoxemia and clinical outcomes in patients on hemodialysis. *Clin J Am Soc Nephrol* 11: 616-625, 2016
51. Kanai H, Hirakata H, Nakane H, Fujii K, Hirakata E, Ibayashi S, Kuwabara Y: Depressed cerebral oxygen metabolism in patients with chronic renal failure: A positron emission tomography study. *Am J Kidney Dis* 38: S129-33, 2001
52. Pierro ML, Kainerstorfer JM, Civiletto A, Weiner DE, Sassaroli A, Hallacoglu B, Fantini S: Reduced speed of microvascular blood flow in hemodialysis patients versus healthy controls: A coherent hemodynamics spectroscopy study. *J Biomed Opt* 19: 026005, 2014
53. Hiraoka M, Firbank M, Essenpreis M, Cope M, Arridge SR, van der Zee P, Delpy DT: A monte carlo investigation of optical pathlength in inhomogeneous tissue and its application to near-infrared spectroscopy. *Phys Med Biol* 38: 1859-1876, 1993
54. Alfakir M, Moammar MQ, Ali MI, Alhatem E, Curran RD, Saoud RM, Chandran C, Khan MA, Debari VA: Pulmonary gas exchange during hemodialysis: A comparison of subjects with and without COPD on bicarbonate hemodialysis. *Ann Clin Lab Sci* 41: 315-320, 2011
55. Samra SK, Dy EA, Welch K, Dorje P, Zelenock GB, Stanley JC: Evaluation of a cerebral oximeter as a monitor of cerebral ischemia during carotid endarterectomy. *Anesthesiology* 93: 964-970, 2000
56. Murkin JM, Adams SJ, Novick RJ, Quantz M, Bainbridge D, Iglesias I, Cleland A, Schaefer B, Irwin B, Fox S: Monitoring brain oxygen saturation during coronary bypass surgery: A randomized, prospective study. *Anesth Analg* 104: 51-58, 2007
57. Ito K, Ookawara S, Ueda Y, Goto S, Miyazawa H, Yamada H, Kitano T, Shindo M, Kaku Y, Hirai K, Yoshida M, Hoshino T, Nabata A, Mori H, Yoshida I, Kakei M, Tabei K: Factors affecting cerebral oxygenation in hemodialysis patients: Cerebral oxygenation associates with pH, hemodialysis duration, serum albumin concentration, and diabetes mellitus. *PLoS One* 10: e0117474, 2015
58. Felcht M, Luck R, Schering A, Seidel P, Srivastava K, Hu J, Bartol A, Kienast Y, Vettel C, Loos EK, Kutschera S, Bartels S, Appak S, Besemfelder E, Terhardt D, Chavakis E, Wieland T, Klein C, Thomas M, Uemura A, Goerdts S, Augustin HG: Angiopoietin-2 differentially regulates angiogenesis through TIE2 and integrin signaling. *J Clin Invest* 122: 1991-2005, 2012
59. David S, Kumpers P, van Slyke P, Parikh SM: Mending leaky blood vessels: The angiopoietin-Tie2 pathway in sepsis. *J Pharmacol Exp Ther* 345: 2-6, 2013
60. Ong T, McClintock DE, Kallet RH, Ware LB, Matthay MA, Liu KD: Ratio of angiopoietin-2 to angiopoietin-1 as a predictor of mortality in acute lung injury patients. *Crit Care Med* 38: 1845-1851, 2010
61. Choi JS, Kwak KA, Park MJ, Kim YH, Gil HW, Song HY, Hong SY: Ratio of angiopoietin-2 to angiopoietin-1 predicts mortality in acute lung injury induced by paraquat. *Med Sci Monit* 19: 28-33, 2013
62. Wang H, Cade BE, Chen H, Gleason KJ, Saxena R, Feng T, Larkin EK, Vasani RS, Lin H, Patel SR, Tracy RP, Liu Y, Gottlieb DJ, Below JE, Hanis CL, Petty LE, Sunyaev SR, Frazier-Wood AC, Rotter JI, Post W, Lin X, Redline S, Zhu X: Variants in angiopoietin-2 (ANGPT2) contribute to variation in nocturnal oxyhaemoglobin saturation level. *Hum Mol Genet* 25: 5244-5253, 2016

## **SUPPLEMENTAL MATERIAL**

### **Supplementary METHODS**

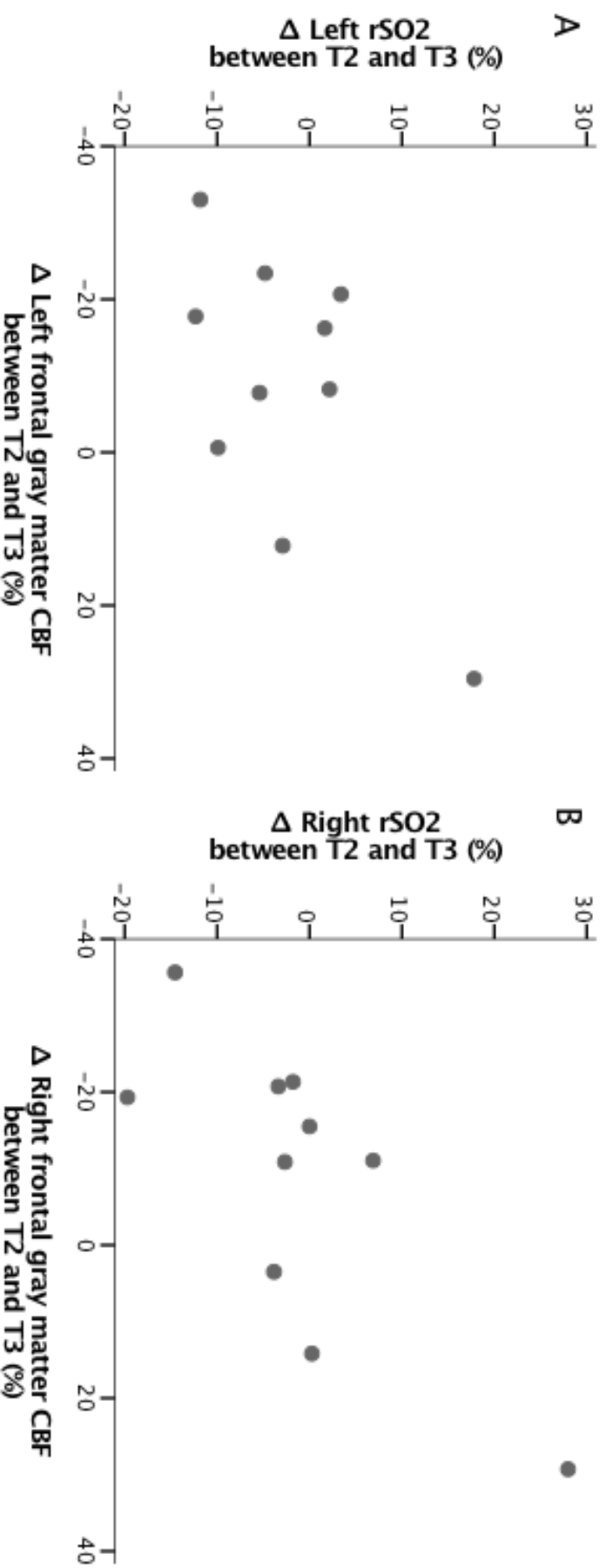
#### **Additional information on Dialysis Settings**

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).

#### **Additional information on Image Reconstruction and Preprocessing**

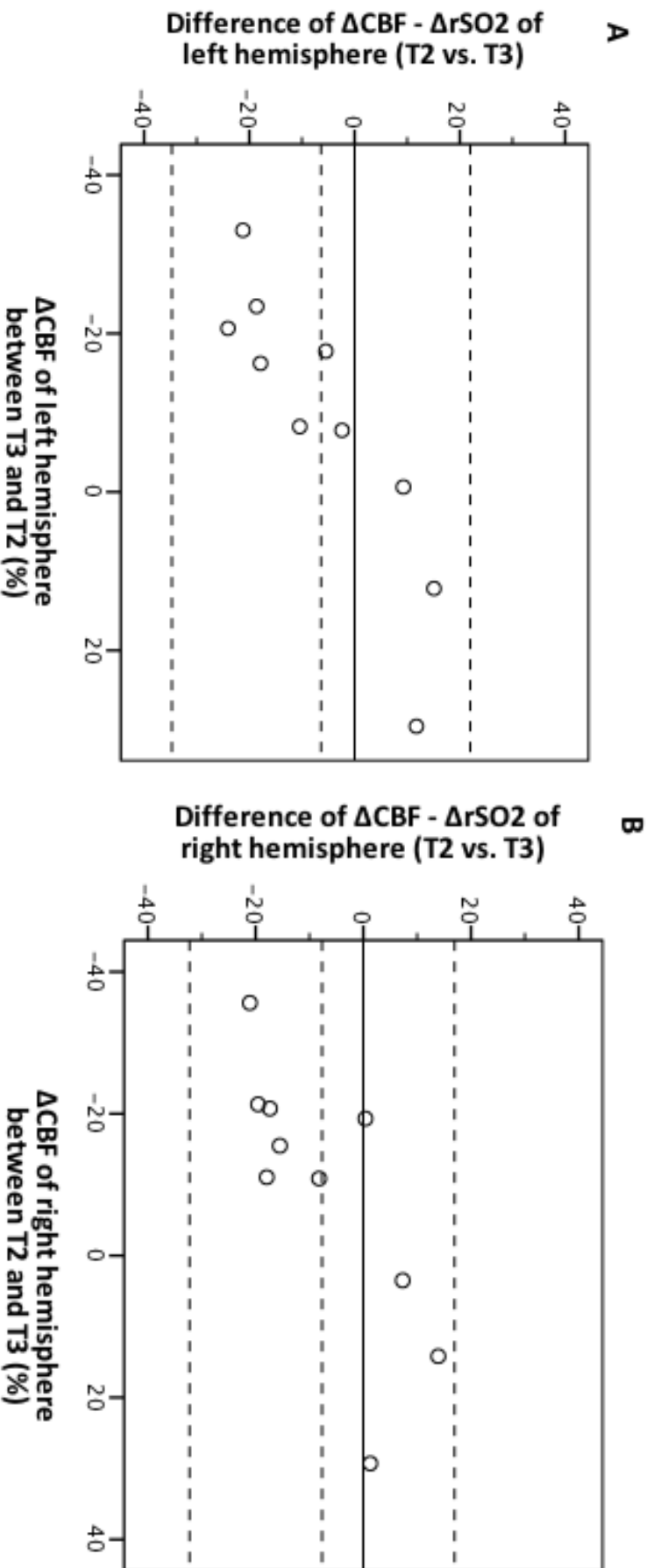
We used the 3D T2-FLAIR images for the registration process, because the 3D acquisition of the T1-weighted sequence was not available. Furthermore, several patients had marked brain atrophy and white matter lesions. Therefore, we used the population-based gray matter/white matter (GM/WM) maps to segment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

**Supplementary Figure S1** Correlation of  $\Delta$  rSO<sub>2</sub> and  $\Delta$  Frontal gray matter Cerebral Blood Flow between T2 and T3



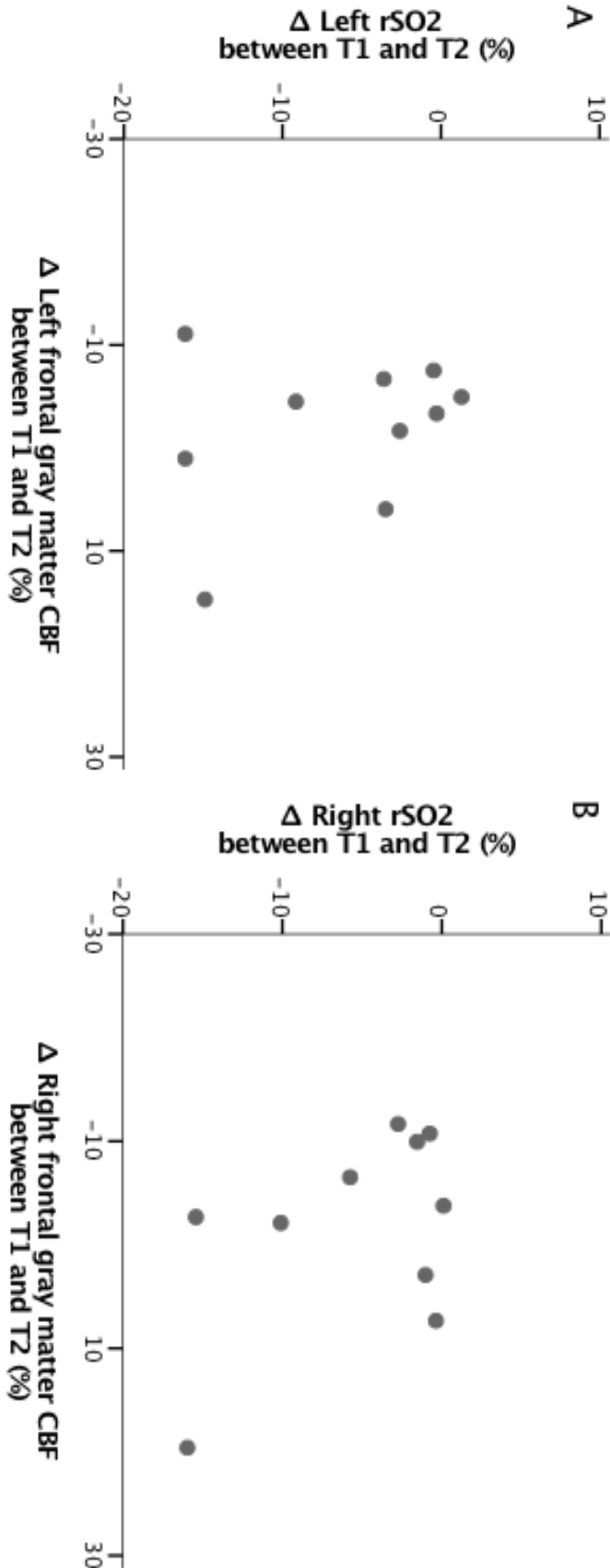
**Fig S1.** Scatter plots of  $\Delta$  Cerebral Blood Flow (X-axis) and  $\Delta$  Regional Oxygen Saturation (Y-axis) calculated between T3 and T2, displayed per left (S1A) and right (S1B) hemisphere. Correlation coefficient for the left hemisphere:  $p = 0.64$  ( $P = 0.048$ ), and the right hemisphere:  $p = 0.76$  ( $P = 0.01$ ).

**Supplemental Figure S2** Bland-Altman plot of NIRS-estimated versus PET-based CBF change between T2 and T3



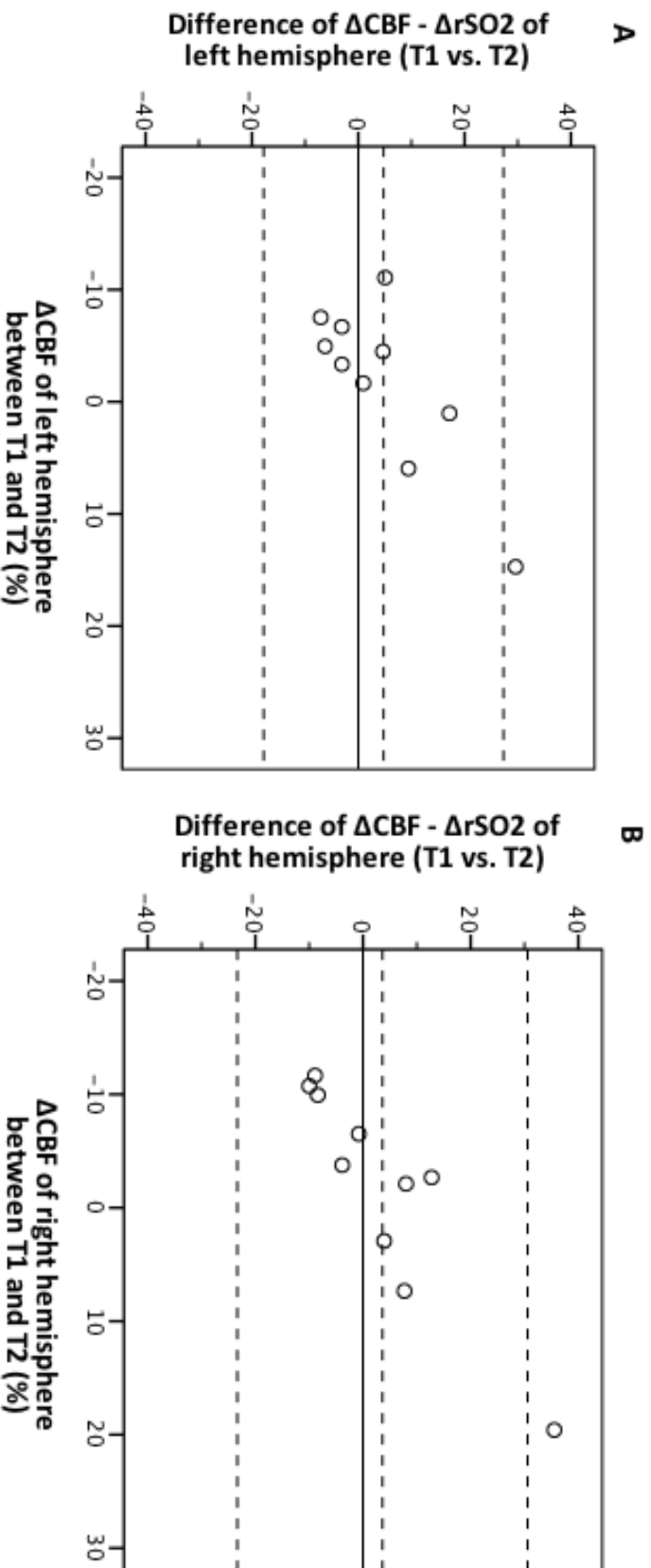
**Fig S2.** Bland-Altman plot of changes in  $r\text{SO}_2$  ( $\Delta r\text{SO}_2$ ) and in CBF ( $\Delta\text{CBF}$ ) between T2 (early after start of hemodialysis) and T3 (at the end of hemodialysis), displayed for the left (S2A) and right (S2B) hemisphere. The X-axis represents  $\Delta\text{CBF}$  (%), while the Y-axis represents the difference between  $\Delta\text{CBF}$  and  $\Delta r\text{SO}_2$ . The central solid line represents zero bias. The upper and lower dashed lines indicate overall bias, which was not significant (left hemisphere: -6%  $P=0.2$ ; right hemisphere: -8%,  $P=0.2$ ). The upper and lower dashed lines represent limits of agreement: -35% and 22% for the left, and -33% and 17% for the right hemisphere, respectively. Linear regression modeling suggested the presence of proportional bias (left hemisphere:  $P=0.001$ , regression equation:  $Y = -0.5 + 0.7x$ ; right hemisphere:  $P=0.01$ , regression equation:  $Y = -3.3 + 0.5x$ ). This suggests that NIRS increasingly underestimated  $\Delta\text{CBF}$  with larger( $r$ ) changes in CBF.

**Supplemental Figure S3** Correlation of  $\Delta rSO_2$  and  $\Delta$  Frontal gray matter Cerebral Blood Flow between T1 and T2



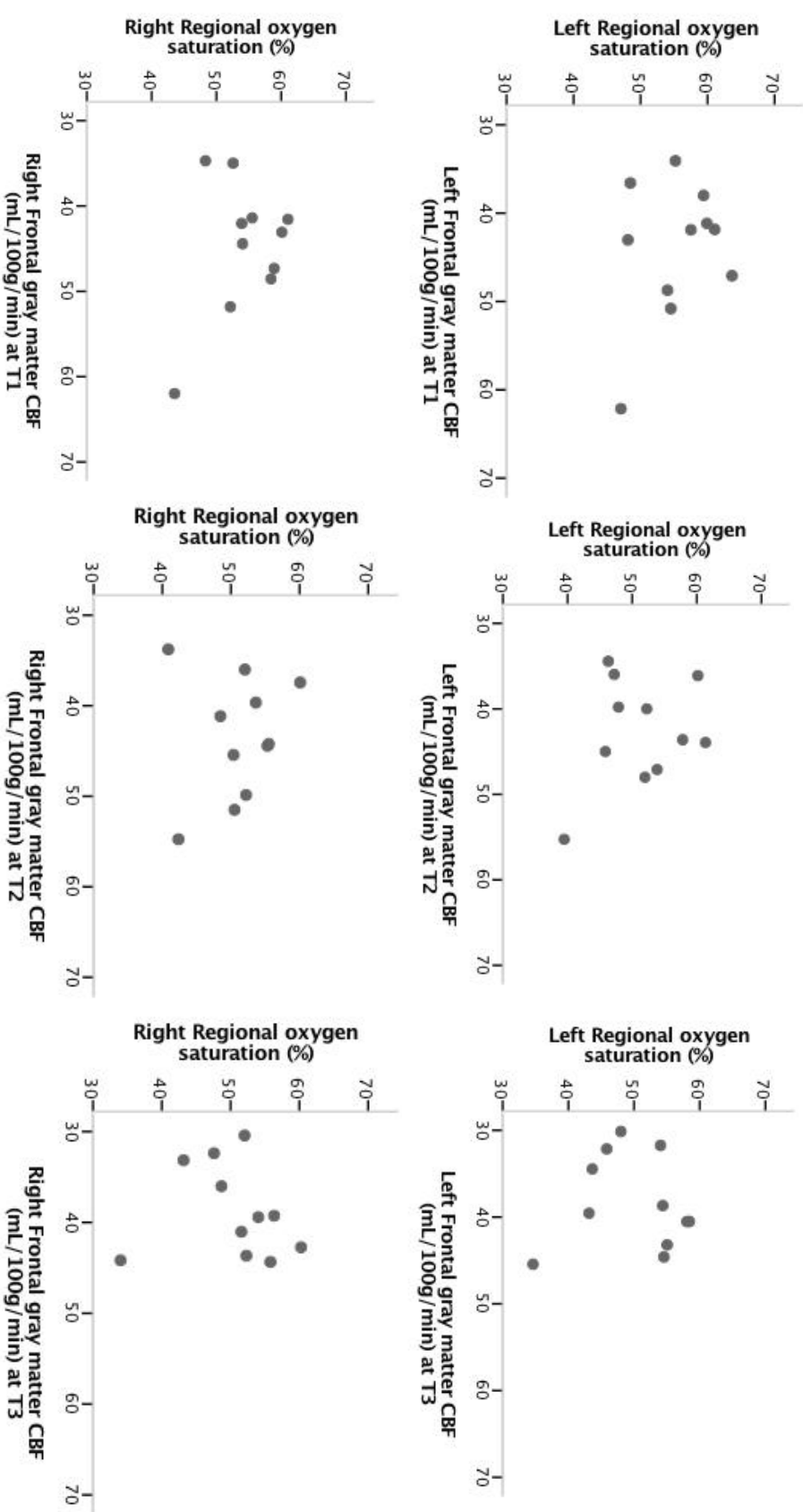
**Fig S3.** Scatter plots of  $\Delta$  Cerebral Blood Flow (X-axis) and  $\Delta$  Regional Oxygen Saturation (Y-axis) calculated between T2 and T1, displayed per left (S3A) and right (S3B) hemisphere. Correlation for the left hemisphere:  $p=0.09$  ( $P=0.4$ ), and the right hemisphere:  $p=0.21$  ( $P=0.6$ ).

**Supplemental Figure S4** Bland-Altman plot of NIRS-estimated versus PET-based CBF change between T1 and T2



**Fig S4.** Bland-Altman plot of changes in rSO<sub>2</sub> ( $\Delta$ rSO<sub>2</sub>) and in CBF ( $\Delta$ CBF) between T1 (before start of hemodialysis) and T2 (early after start of hemodialysis), displayed for the left (S4A) and right (S4B) hemisphere. The X-axis represents  $\Delta$ CBF (%), while the Y-axis represents the difference between  $\Delta$ CBF and  $\Delta$ rSO<sub>2</sub>. The central solid line represents zero bias. The upper and lower dashed lines indicate overall bias, which was not significant (left hemisphere: 5%  $P=0.2$ ; right hemisphere: 4%,  $P=0.4$ ). The upper and lower dashed lines indicate limits of agreement: -18% and 27% for the left, and -23% and 31% for the right hemisphere, respectively. Linear regression suggested the presence of proportional bias (left hemisphere:  $P=0.004$ , regression equation:  $Y = 7.0 + 1.3x$ , right hemisphere:  $P<0.001$ , regression equation:  $Y = 5.9 + 1.3x$ ). This suggests that NIRS increasingly underestimated  $\Delta$ CBF with larger( $r$ ) changes in CBF.

**Supplemental Figure S5** Correlation between absolute values of  $rSO_2$  and Frontal gray matter Cerebral Blood Flow



**Fig S5.** Scatter plots of frontal gray matter CBF (X-axis) and regional oxygen saturation (Y-axis) at T1, T2, and T3, and displayed per left and right hemisphere. No significant correlations were found.

**Supplemental Table S1** Associations of Hemodialysis and Oxygenation-related factors, and of Inflammation and Endothelial activation markers with NIRS-rSO<sub>2</sub> (left panel), and frontal gray matter PET-CBF (right panel)

	Estimated effect on frontal rSO <sub>2</sub> (%)			Estimated effect on frontal gray matter CBF (mL/100g/min)		
	Overall effect	Characteristic * T2	Characteristic * T3	Overall effect	Characteristic * T2	Characteristic * T3
Interaction effect with time						
<i>Hemodialysis-related factors:</i>						
Mean arterial pressure (mmHg)	None			NA <sup>§</sup>		
Tympanic temperature	None					
pH (per 0.1 change)	-1.2 (-6.4; 3.9)	6.2 (1.2; 11.1)*	8.4 (0.7; 16.1)*	-2.0 (-3.7; -0.3)*	-10.2 (-13.5; -6.8)***	-22.9 (-36.0; -9.8)**
Hematocrit (per 0.1 mmol/L)	8.6 (1.4; 15.7)*	-4.7 (-9.3; -0.1)*	-7.4 (-13.8; -1.0)*	None		
Ultrafiltration volume (per L)	None			-4.8 (-7.6; -2.0)**		
Ultrafiltration rate (mL/h/kg)	0.3 (-1.0; 1.5)	-0.8 (-1.4; -0.1)*	-0.9 (-2.2; 0.3)	-1.2 (-2.2; -1.0)*		
<i>Oxygenation-related factors:</i>						
pO <sub>2</sub> (kPa)	0.8 (0.1; 1.5)*			-2.3 (-2.9; -1.8)***	0.9 (0.4; 1.4)**	2.7 (1.6; 3.9)***
pCO <sub>2</sub> (kPa)	-0.4 (-4.5; 3.7)	-4.7 (-8.5; -1.0)*	-5.7 (-12.2; 0.8)	3.8 (1.1; 6.4)**		
CaO <sub>2</sub> (mL/dL)	2.0 (0.3; 3.7)*	-1.1 (-2.2; -0.002)	-1.7 (-3.3; -0.01)*	-2.7 (-5.0; -0.4)*	1.3 (0.1; 2.4)*	1.7 (-1.0; 4.4)
<i>Endothelial activation markers:</i>						
Angiopoiectin 2 (ng/mL)	-0.9 (-1.7; -0.1)*	-0.2 (-0.8; 0.4)	-0.8 (-1.4; -0.3)**	None		
Angiopoiectin 2:1 ratio	-5.1 (-7.4; -2.8)***			None		
von Willebrand Factor (%)	0.06 (0.02; 0.1)*			None		

Associations were studied using linear mixed effects models including a random intercept, or a random intercept and slope, whether appropriate according the likelihood-ratio test. The estimated effect (95%CI) of the individual characteristics on frontal rSO<sub>2</sub> and frontal gray matter is presented. \*P<0.05, \*\*P<0.01, \*\*\* P<0.001. The models with an interaction could be interpreted by adding the overall effect to the interaction effect, e.g. a 0.1 pH increase is associated with a (-1.2 + 6.2=) 5% rSO<sub>2</sub> increase at T2, and a (-1.2 + 8.4=) 7.2% rSO<sub>2</sub> increase at T3, as compared with T1. Markers of inflammation (pentraxin-3, and CRP) were not associated with rSO<sub>2</sub> or CBF. <sup>§</sup>The analysis of mean arterial pressure and CBF was inconclusive due to patient variation and missing values. <sup>20</sup> CaO<sub>2</sub>, arterial oxygen content; NA, not available; rSO<sub>2</sub>, regional oxygen saturation.