

# Soluble CD146 and BNP dissect overhydration in functional components of prognostic relevance in hemodialysis patients.

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## **Significance Statement** (120 words)

## **Abstract** (250 words)

Background:

Methods:

Results:

Conclusions:

## Introduction

Volume management is a critical element in the care of hemodialysis (HD) patients and requires accurate assessment of both the hydration state and the underlying pathophysiology. Overhydration, a state of generalized edema formation, is a frequent condition in HD patients and is associated with reduced survival.<sup>1-6</sup> In patients with end-stage renal disease (ESRD) overhydration is related either to renal salt and water retention and insufficient ultrafiltration during HD or concomitant congestive heart failure (CHF). Indeed, increased intra-cardiac and upstream vascular hydrostatic pressures (systemic congestion) may contribute to edema formation in patients with ESRD and CHF.<sup>7,8</sup> The dissection of overhydration in cardiac and non-cardiac components in the individual HD patient is challenging because ESRD and CHF often coexist and because rapid and reliable diagnostic tools are lacking. However, the differentiation of the underlying mechanisms is of significant clinical relevance since it might implicate different treatments.

Bioimpedance analysis has become a popular tool in the assessment of overhydration in HD patients.<sup>1</sup> Body composition measurement (BCM) by bioimpedance not only allows to quantify malnutrition and sarcopenia,<sup>9</sup> but also to provide a quantitative estimation of fluid compartments. This general estimate of the volume status supports the clinician in HD volume management but does not distinguish the underlying mechanisms of overhydration.

Cardiovascular biomarkers might provide complementary diagnostic information for this purpose. Natriuretic peptides - B-type natriuretic peptide (BNP) and the amino-terminal pro-BNP (NT-proBNP) - are released by cardiomyocytes in the presence of increased intra-cardiac pressures. Previous studies showed discordant results regarding the ability of natriuretic peptides to detect overhydration and guide volume management,<sup>10-16</sup> but consistently displayed excellent negative predictive values for CHF, even in HD patients.<sup>17,18</sup> Thus, natriuretic peptides are considered biomarker of cardiac dysfunction rather than overhydration in HD patients. Notably, elevated plasma concentrations of natriuretic peptides well correlated with mortality in HD patients.<sup>16,18-24</sup>

Our group recently described soluble CD146 (sCD146) as novel vascular biomarker, released by vessels in response to endothelial stretch.<sup>25-27</sup> The extra-cardiac origin of sCD146 provides complementary information to BNP, and its mode of release into circulation makes it a more promising direct marker of systemic congestion, regardless of cardiac function.

In this study, after validating sCD146 as a non-cardiac biomarker of systemic congestion in HD patients, by measuring BNP and sCD146 we dissected overhydration in its major functional components and prospectively tested the association between cardiac and non-cardiac components of overhydration with mortality in HD patients.

## **Methods**

### **Study population**

The study population consisted of 174 stable chronic HD patients. The first cohort of patients ("Zurich", n=30) underwent a mechanistic study to validate sCD146 as a biomarker of systemic congestion in HD. This part of the study was performed from October 1st to December 31<sup>st</sup>, 2016 at the University Hospital of Zurich, Switzerland. Inclusion criteria were age > 18 years and hemodialysis since > 1 month; exclusion criteria were acute illness or hospitalization in the last week before inclusion, implanted cardiac device, as it precludes reliable bioelectrical impedance measurement.

The second cohort of patients ("London", n=144) was used for external validation of the "Zurich" study and for the clinical outcome study, aimed at investigating the relationship between overhydration, cardiac dysfunction and outcome in stable hemodialysis patients. This part of the study was performed at the Royal Free Hospital of London, United Kingdom. Patients attending for their routine midweek outpatient dialysis session 5<sup>th</sup> and 6<sup>th</sup> May 2016 were included... and followed up until the end of May 2017....

Cardiac systolic dysfunction was defined as a reduced left ventricular ejection fraction...

### **Mechanistic "Zurich" study**

The mechanistic "Zurich" study consisted of 4 visits. The first visit was performed after a regular HD session, followed by a short inter-dialysis interval (duration: 2 days). The second visit was performed at the end of the short interval, before the HD session, and the third visit after the same HD session. The fourth visit was performed at the end of the subsequent prolonged inter-dialysis interval ("long interval", duration: 3 days). Briefly, the four visits allowed to study a short and a long inter-dialysis interval to test different levels of overhydration.

Total body weight was measured at each visit, to calculate the net body weight increase during the short and the long intervals, respectively. Furthermore, overhydration was quantitatively assessed at each study visit

with portable whole-body bioimpedance spectroscopy device (Fresenius Medical Care GmbH, Bad Homburg, Germany). BCM was performed with the patient in a supine position according to the manufacturer's description. Based on a fluid assessment model using resistances to electrical currents of 50 discrete frequencies, the total body water (TBW), the extracellular water (ECW), the intracellular water (ICW) and the overhydration (OH) are calculated.<sup>28,29</sup> Relevant overhydration was previously defined as an OH/ECW ratio > 0.15<sup>1,2</sup>. Patient history and clinical evaluation were collected at each study visit. Comorbidities and HD specific information were retrieved from electronic medical charts.

### **Prospective clinical “London” outcome study**

The clinical “London” outcome study consisted of one visit, before a regular outpatient midweek hemodialysis session, followed by a prospective follow-up of 1 year for all-cause mortality. The quantitative assessment of overhydration was performed before the hemodialysis session using multifrequency bioimpedance InBody 720, Seoul, South Korea), in a standardized protocol [references 16. Fürstenberg A, Davenport A. Comparison of multifrequency bioelectrical impedance analysis and dual-energy X-ray absorptiometry assessments in outpatient haemodialysis patients. *Am J Kidney Dis.* 2011;57(1):123-9; Kumar S, Khosravi M, Massart A, Potluri M, Davenport A. Are serum to dialysate sodium gradient and segmental bioimpedance volumes associated with the fall in blood pressure with hemodialysis? *Int J Artif Organs.* 2014 Jan;37(1):21-8]. The total body water (TBW), the extracellular water (ECW) and the intracellular water (ICW) were calculated. Since the OH value was not determined in this cohort, relevant overhydration was defined in the presence of an ECW/TBW ratio > 0.4 according to the literature.<sup>1</sup> Clinical data, including information about cardiac systolic function and comorbidities, were retrieved from electronic medical charts.

### **Biomarker testing**

Venous blood samples for measurement of cardiovascular biomarkers were drawn at each visit, centrifuged within 6 hours and ethylene-diamine-tetraacetic acid (EDTA) plasma aliquots were stored at -80°C until analyzed at Lariboisière University Hospital, Paris, France. The concentration of sCD146 was determined by ELISA (CY-QUANT ELISA sCD146©, Biocytex, France) with a detection limit of sCD146 of 10 ng/mL and coefficients of variation for both repeatability and reproducibility < 20% in the measured range. Measurement of concentration of brain natriuretic peptide (BNP) was performed with the Architect i2000 platform (Abbott Diagnostics, Abbott Park, IL, USA).

## **Ethical considerations**

The study was conducted according to the standards of the Declaration of Helsinki and approved by the local ethics committee of Zurich and London (13/Lo/0912). All patients provided written informed consent (ClinicalTrials.gov NCT02962635).

## **Statistical analysis**

Continuous variables are expressed as median (interquartile range, IQR), categorical variables as number (percentage). Independent samples were compared using the Mann-Whitney U test or Chi-square test, as appropriate. Related samples were compared using the Wilcoxon signed rank test. Correlation analysis was performed using Spearman's correlation coefficient ( $\rho$ ). The diagnostic performance of the biomarkers was assessed by receiver operating characteristic (ROC) analysis and expressed as area under the curve (AUC). Survival was plotted with the Kaplan-Meier curve and differences between groups were assessed by the log-rank test. Univariate and covariate-adjusted Cox proportional hazards regression models were used to estimate the association between circulating biomarkers and risk of death. The covariates included in the regression models were a priori selected among baseline variables with known associations with study outcomes: age, sex, cardiac systolic dysfunction, relevant overhydration, anuria, log-transformed BNP, log-transformed sCD146. The null hypothesis was rejected with an adjusted two-sided p-value < 0.05. All analyses were performed with the use of IBM SPSS Statistics, Version 25.0. (IBM Corp, Armonk NY, USA).

# **Results**

## **Characterization of the study population**

The study population consisted of 174 hemodialysis patients, predominantly middle-aged men with ESRD (Table 1). Dialysis access was mostly an AV-fistula, and anuria was highly prevalent. Cardiovascular risk factors and vascular complications (coronary and peripheral artery disease) were highly prevalent.

### **Mechanistic “Zurich” study on vascular sCD146 as a biomarker of systemic congestion**

The median value of circulating sCD146 in all studied patients at all time-points (120 measurements) was 530 ng/mL (range: 123 ng/mL to 1730 ng/mL). The median intra-individual variability was 43% (range: 6% to 354%, [Supplementary Figure 1](#)). Hemodialysis did not alter circulating sCD146, as shown by similar sCD146 levels before and after HD (543 ng/mL [384;625] vs. 492 ng/mL [393;646],  $p = 0.52$ , [Supplementary Figure 2](#)).

Moreover, we measured changes in body weight and sCD146 concentrations after a short (2 days) and a long (3 days) inter-dialysis interval. We observed an incremental increase in both body weight (+0.9 kg [+0.3;+1.8],  $p < 0.001$  and + 1.8 kg [+0.1;+2.7],  $p < 0.001$ ) and circulating sCD146 (+53 ng/mL [-16;+118],  $p = 0.006$  and +91 ng/mL [+20;+179],  $p < 0.001$ ) after the short and long intervals, respectively, [Figure 1](#).

Furthermore, we assessed the correlation between sCD146 and overhydration determined by BCM at the end of both short and long intervals ( $n = 60$ ), [Figure 2](#). Circulating sCD146 positively correlated with overhydration (Spearman’s rho 0.37,  $p = 0.004$ ). Circulating sCD146 showed good properties for diagnosing patients with relevant overhydration (area under the receiver operating characteristics curve, AUROC: 0.72 [0.58;0.86],  $p = 0.005$ ).

Finally, we evaluated the diagnostic properties of circulating sCD146 to detect dyspnea, as a clinical parameter of systemic congestion. At the end of both short and long intervals ( $n = 60$ ), 50% of patients reported dyspnea, defined as breathlessness at rest or during stress (New York Heart Association class II-IV). Dyspneic patients displayed higher plasma concentrations of sCD146 (633 ng/mL [541;770 ng/mL]) compared to patients without dyspnea (523 ng/mL [364;622 ng/mL],  $p = 0.006$ ), [Figure 3, left panel](#). Notably, BNP levels did not discriminate dyspneic and non-dyspneic patients (332 pg/mL [191;697] vs. 147 pg/mL [63;639],  $p = 0.08$ ). The AUROC of sCD146 for diagnosing dyspnea was 0.71 [0.58;0.84],  $p = 0.006$ ), [Figure 3, right panel](#). Plasma concentrations of sCD146 < 461 ng/mL had a sensitivity > 90% of not being associated with dyspnea.

### **Validation of the “Zurich” study in the “London” cohort**

The data obtained in the “Zurich” cohort were validated in the larger independent “London” cohort of 144 stable HD outpatients. We confirmed that subjects with relevant overhydration ( $n=84$ ) had higher levels of sCD146 (598 ng/mL [454;744] vs. 479 [416;597],  $p = 0.003$ ) compared to non-overhydrated subjects ( $n=60$ ). Circulating sCD146 levels positively correlated with overhydration (Spearman’s rho 0.28,  $p = 0.001$ ).



### **Cardiac and non-cardiac components of overhydration**

In a further analysis in the “London” cohort (n = 144), we found that the prevalence of relevant overhydration was much lower for patients with both low sCD146 and low BNP (29%) compared to patients with either an increase in one or both biomarkers (65-74%, Chi-square  $p < 0.001$ ), **Figure 4, left panel**. Notably, in the group of subjects with low BNP but high sCD146 (upper left quadrant) nearly 3 of 4 showed relevant overhydration, indicating that overhydration and systemic congestion correlated in most patients, but were not necessarily related to high BNP.

Furthermore, patients with cardiac systolic dysfunction (n = 48) had higher BNP levels (1507 pg/mL [504;4245] vs. 473 pg/mL [259;1368],  $p < 0.001$ ) compared to patients with preserved systolic function. However, sCD146 levels were similar in patients with or without cardiac systolic dysfunction (525 ng/mL [387;690] vs. 559 ng/mL [436;714],  $p = 0.47$ ). The prevalence of systolic dysfunction was ~2-3 fold among patients with elevated BNP (44-68%) compared to patients with low BNP, regardless to sCD146 (21-23%, Chi-square  $p < 0.001$ ), **Figure 4, right panel**. These findings indicate that overhydration and systemic congestion not always reflect CHF in HD patients and that circulating sCD146 is a biomarker of overhydration/systemic congestion independent of CHF.

### **Prospective clinical “London” outcome study**

Based on the previous data, we investigated the prognostic impact of overhydration and its functional components on all-cause mortality in HD patients of the “London” cohort. During a median follow-up of 388 days, a total of 27 deaths occurred. In univariate analysis (**Table 2**) age, relevant overhydration, cardiac systolic dysfunction, and log-transformed BNP were shown to be associated with all-cause mortality, while sex, anuria, and log-transformed sCD146 were not. In multivariate analysis, systolic dysfunction and log-transformed BNP were still strongly associated with all-cause mortality, while age and relevant overhydration were not. BNP shows excellent properties for predicting all-cause mortality during follow-up (AUROC 0.81 [0.73;0.90],  $p < 0.001$ ), with specificity > 90% for values exceeding 3000 pg/mL.

**Figure 5** illustrates the marked difference in survival of hemodialysis patients according to the presence of relevant overhydration (log-rank  $p = 0.041$ ), systolic dysfunction (log-rank  $p < 0.001$ ), and high BNP (log-rank  $p = 0.001$ ) but not high sCD146 levels.

## Discussion

Optimal volume management in HD patients is of significant clinical relevance and relies on accurate assessment of overhydration and its functional components. Indeed, since CHF is highly prevalent in ESRD patients, overhydration may have both cardiac and non-cardiac components. Our study, after validating the novel vascular biomarker sCD146 as a non-cardiac biomarker of systemic congestion in HD patients, by combining two cardiovascular biomarkers (BNP and sCD146) dissected overhydration in its major functional components. Furthermore, we prospectively showed that cardiac systolic dysfunction and not systemic congestion *per se* is associated with high all-cause mortality in HD patients.

Vascular sCD146 is released from endothelial cells upon mechanical stress and is considered a biomarker of systemic congestion, independent from cardiac function.<sup>25-27,30,31</sup> In the mechanistic part of our study, we confirmed for the first time, in two independent cohorts of stable HD patients ("Zurich" and "London"), that sCD146 is an accurate non-cardiac biomarker of systemic congestion, as previously shown in CHF<sup>25-27</sup>. Indeed, circulating sCD146 concentrations well correlated with clinical markers such as weight gain, dyspnea and BCM-derived parameters of overhydration.

Using sCD146 (reflecting systemic congestion, independently from the cardiac function) and combining it with BNP and echocardiographic data (reflecting cardiac function) we dissected overhydration in its major functional components (cardiac vs. non-cardiac). Our data showed that overhydration (as determined by BCM), systemic congestion and cardiac dysfunction do not necessarily co-exist in HD patients. Our data showed that relevant overhydration might be associated with three biomarker phenotypes: high-BNP/low-sCD146 (indicating overhydration of cardiac origin), low-BNP/high-sCD146 (indicating overhydration of non-cardiac origin) and high-BNP/high-sCD146 (indicating overhydration of mixed origin).

The application of this novel biomarker-based dissection of functional components of overhydration led us to the second main finding of this study: the high all-cause mortality associated with overhydration in HD patients is strongly related to cardiac systolic dysfunction. In light of these data, the previously reported increased mortality related to overhydration is probably indicative of a higher prevalence of prognostically-relevant CHF among patients with BCM-determined overhydration, and not to systemic congestion *per se*.

## **Clinical significance**

Our findings need to be validated in prospective larger studies but might contribute to a change in the management of HD patients according to the biomarker profile. High-BNP patients have a high likelihood of CHF and display high mortality independently from sCD146 concentration. High-BNP/low-sCD146 might require extensive cardiovascular evaluation and specific CHF-therapy. High-BNP/high-sCD146 patients likely display overhydration of mixed origin and might benefit from a combination of specific CHF-therapy and intensive volume management (e.g., ultrafiltration). Low-BNP patients display good survival independently from sCD146 values. In these patients, sCD146 might help to guide volume management, in particular in the presence of symptoms such as dyspnea, but in most patients, a less restrictive volume management to avoid hypotension, cardiac stunning and anuria might be appropriate. Further studies correlating sCD146 levels with blood pressure and the incidence of cardiovascular events will provide additional data of interest for an optimal volume management. Furthermore, the application of a biomarker approach might lead to a more precise cardiovascular stratification of patients to be included in interventional studies aiming at the improvement of cardiovascular mortality on HD.

## **Limitations**

This study has several limitations: (1) The validation of sCD146 in the mechanistic study was supported by consistent data from reference parameters, but for practical reasons, we did not include gold standard techniques (such as invasive venous pressure measurement). (2) A more precise characterization of the heart function might provide additional information of interest, e.g., the additional identification of patients with heart failure with preserved ejection fraction might be particularly informative, in consideration of the high prevalence of this condition in the HD population (ev. MEDIA-DHF?). (3) Including further parameters to assess overhydration and systemic congestion (e.g., by measuring the diameter of the inferior vena cava or by lung echocardiography) before and after dialysis might contribute to a more precise characterization of biomarker properties of sCD146 in this setting. Notably, the influence of other biological processes on circulating sCD146 (e.g., inflammation, angiogenesis) needs further investigation. (4) Absolute cut-off values of high and low BNP and sCD146 would be required for a direct clinical application of this approach, but larger study populations are required for this purpose. This further step would also help us to understand if patients with high BNP and low sCD146 represent a population of patients with CHF without systemic congestion, or if this group is an

artifact generated by the arbitrary definition of cut-offs. (5) The comparison between patients on HD and peritoneal dialysis might be particularly interesting to identify effects related to the procedure.

In summary, the combination of BNP and sCD146 dissects overhydration in functional components of prognostic value and is potentially relevant to optimize the management of HD patients.

## Author contributions

M.A., P.E.C. S.V.M. S.S. and A.M. designed the study. S.V.M., K.G., M.S., and A.D. carried out measurements. M.A. and P.E.C. analyzed the data and drafted the manuscript. All authors revised and approved the final version of the manuscript.

## Acknowledgements

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## Tables and Figure legends

**Table 1.** Patient characteristics of the study population

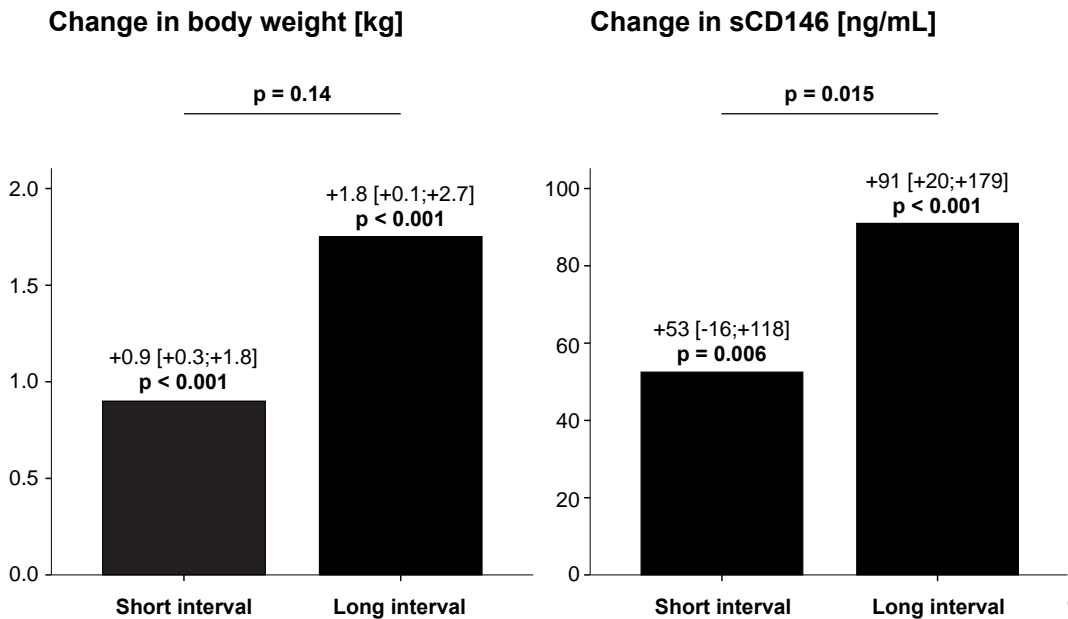
	Zurich cohort (n=30)	London cohort (n=144)	p-value
Age [years]	67 [56; 78]	73 [59; 81]	0.15
Male gender	25 (83%)	88 (61%)	0.021
Height [cm]	172 [162; 180]	163 [157; 171]	0.001
Weight [kg]	76 [62; 86]	71 [63; 79]	0.12
Etiology of kidney failure			xxx
Hypertensive / diabetic	10 (33%)	xxx	
Glomerulonephritis	8 (27%)		
Others	10 (33%)		
Unknown	2 (7%)		
Dialysis access			0.05
AV-fistula/graft	29 (97%)	119 (83%)	
Catheter	1 (3%)	25 (17%)	
Anuria (< 500 ml/day)	14 (47%)	106 (74%)	0.008
Arterial hypertension	30 (100%)	138 (96%)	0.59
Diabetes	11 (37%)	75 (52%)	0.16
History of coronary artery disease	12 (40%)	53 (37%)	0.84
History of chronic heart failure	4 (13%)	36 (25%)	0.23
History of peripheral artery disease	5 (17%)	23 (16%)	1.00
Plasma sCD146 [ng/mL] (before HD)	543 [390; 615]	542 [424;705]	0.55

**Table 2.** Univariate and multivariate analysis for prediction of long-term all-cause mortality

	HR	CI	p-value		HR	CI	p-value
<b>Age</b>	1.03	0.99 - 1.06	0.052		1.03	0.99 - 1.07	0.09
<b>Sex</b>	1.08	0.50 - 2.36	0.84				
<b>Cardiac systolic dysfunction</b>	5.24	2.07 - 13.3	<0.001		3.43	1.23 - 9.09	0.018
<b>Relevant overhydration</b>	2.50	1.01 - 6.19	0.048		0.86	0.26 - 2.79	0.80
<b>Anuria</b>	0.80	0.35 - 1.82	0.59				
<b>log BNP</b>	7.29	3.44 - 15.5	<0.001		5.19	1.78 - 15.2	0.003
<b>log sCD146</b>	1.10	0.14 - 8.64	0.93				

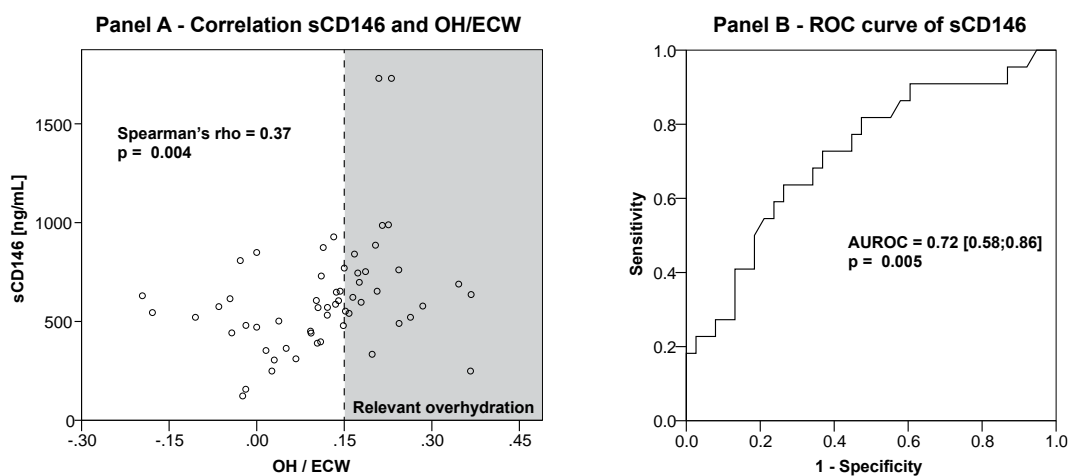


**Figure 1.** Median changes in body weight and sCD146 during the short and the long interval in the Zurich cohort.



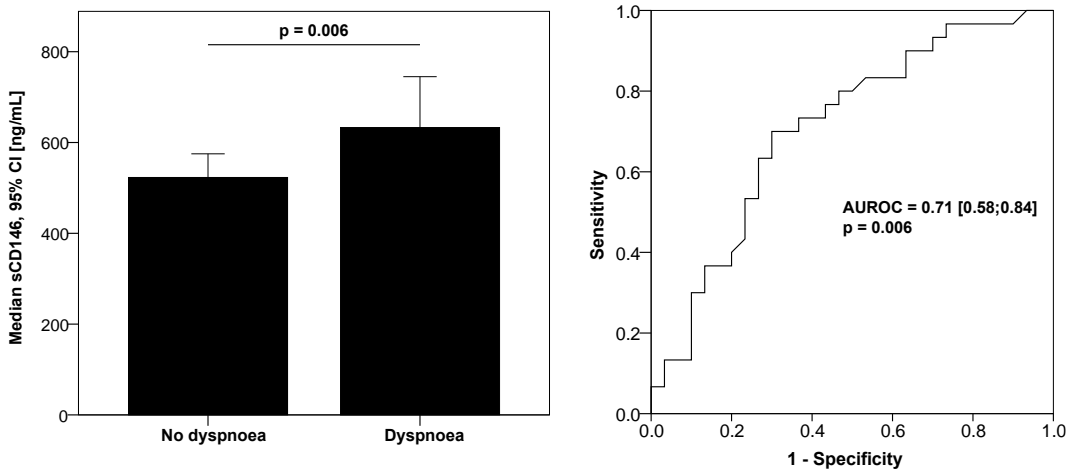
Legend: Median values and quartiles are reported. P-values refer to related samples Wilcoxon signed rank test. N=30

**Figure 2.** Correlation between overhydration and circulating sCD146 after the short and long interval (Panel A) and receiver operating characteristic curve of sCD146 (Panel B) for diagnosing relevant overhydration (OH/ECV > 0.15) in the Zurich cohort.



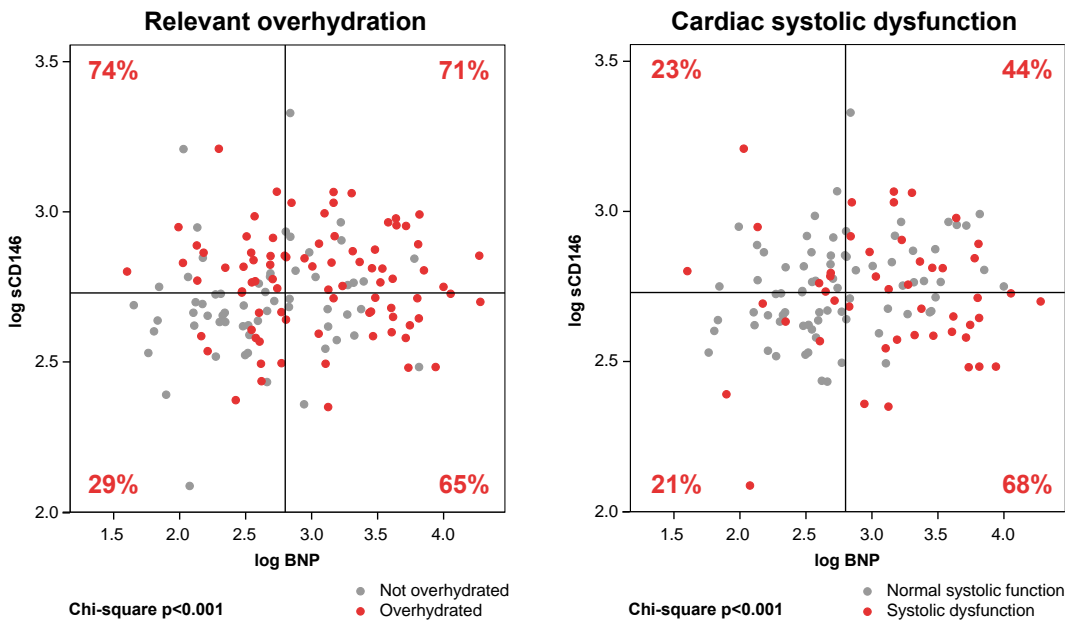
Legend: AUROC area under the receiver operating characteristic curve. Dotted lines refer to the definition of relevant overhydration (OH/ECW > 0.15). N=60

**Figure 3.** Circulating sCD146 according to symptoms of dyspnea and receiver operating characteristic curve of sCD146 for diagnosing dyspnea in the Zurich cohort.



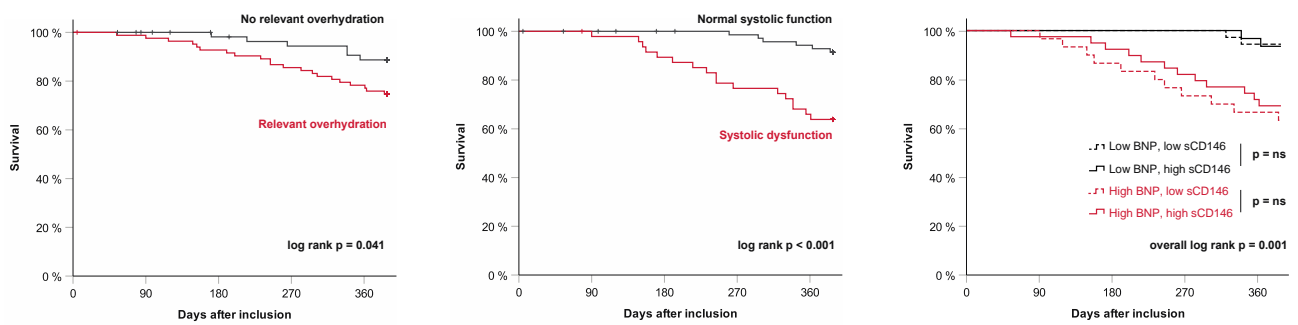
Legend: AUROC area under the receiver operating characteristic curve. N=60

**Figure 4.** Distribution of relevant overhydration and cardiac systolic dysfunction according to circulating concentrations of sCD146 and BNP (n=144) in the London cohort



Legend: Values of BNP and sCD146 were log-transformed. Groups were divided according to median values of BNP and sCD146 (represented by lines). N=144

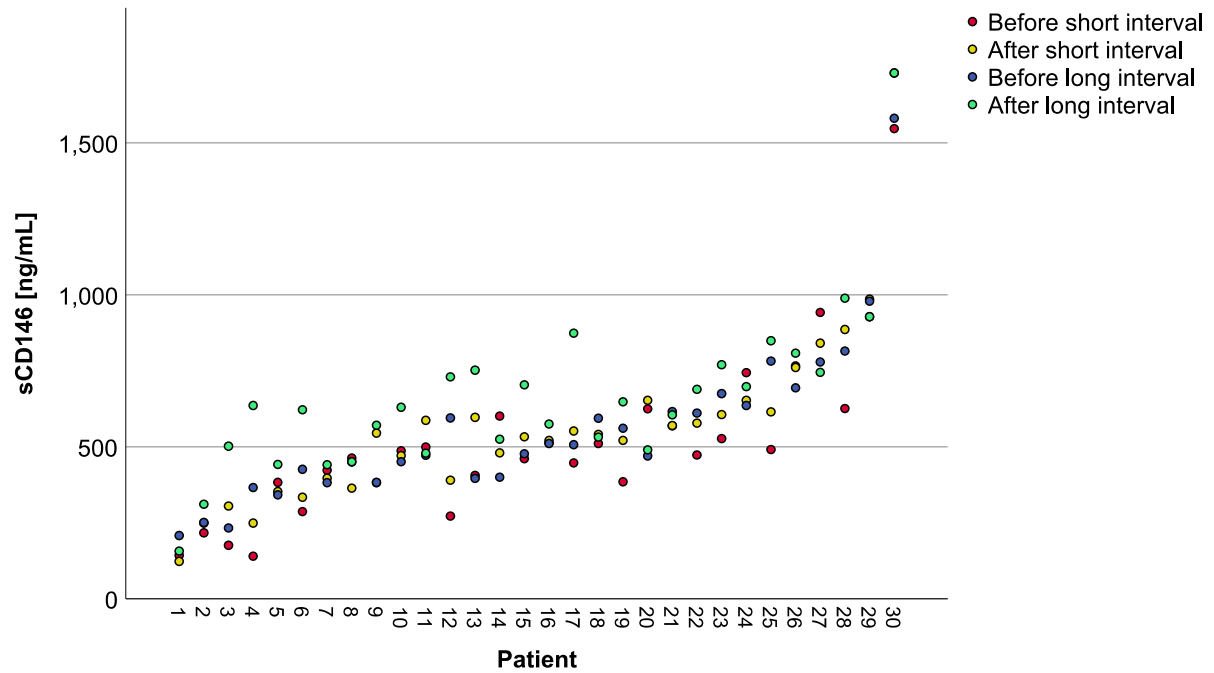
**Figure 5. Survival of hemodialysis patients according to the presence of relevant overhydration, cardiac systolic dysfunction and biomarker concentrations in the London cohort.**



Legend: Relevant overhydration was defined as  $ECW / TBW > 0.4$ . Low/high BNP defined according to the median (631 pg/mL), low/high sCD146 defined according to the median (542 ng/mL).  $N=144$

# Supplemental material

**Supplementary Figure 1 – Concentrations of sCD146 in each patient according to time-points**



**Supplementary Figure 2 – Plasma concentrations of sCD146 before and after hemodialysis**

