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ORIGINAL ARTICLE

High-volume hemodiafiltration decreases the pre-dialysis concentrations of indoxyl sulfate and p-cresyl sulfate compared to hemodialysis: a post-hoc analysis from the HDFit randomized controlled trial

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Abstract

Background Although high-volume online hemodiafiltration has been associated with higher clearance and lower pre-dialysis concentration of middle molecular weight toxins compared to hemodialysis, its effect on protein-bound uremic toxins has shown inconclusive results. In this study, we investigated whether hemodiafiltration impacts pre-dialysis plasma levels of the toxins indoxyl sulfate, p-cresyl sulfate, and indole-3-acetic acid compared to high-flux hemodialysis.

Methods This is a post-hoc analysis of the multicenter, randomized controlled trial HDFit (ClinicalTrials.gov: NCT02787161). Uremic toxins were determined by high performance liquid chromatography at baseline, 3, and 6 months. Mean differences in monthly changes of pre-dialysis uremic toxin concentrations between hemodiafiltration and high-flux hemodialysis were analyzed using linear mixed-effect models.

Results One hundred ninety-three patients (mean age 53 years old, 71% males) were analyzed. There were no differences between groups regarding clinical and biochemical characteristics at baseline or duration of dialysis session and blood flows throughout the follow-up. Mean differences in rates of change (μM/month, [confidence interval CI]) in high-flux hemodialysis vs. hemodiafiltration were 2.4 [0.3 to 4.56], 3.94 [− 1.54 to 9.41] and 0.06 [− 0.6 to 0.5] for indoxyl sulfate, p-cresyl sulfate and indole-3-acetic acid, respectively. In the exploratory analysis, these differences in high-flux hemodialysis vs. hemodiafiltration subgroup with convective volume > 27.5 L were 2.86 [0.43 to 5.28], 7.43 [0.7 to 14.16] and − 0.19 [− 0.88 to 0.50].

Conclusion These exploratory findings suggest that hemodiafiltration is more effective in reducing indoxyl sulfate as compared to standard high-flux hemodialysis, and also that this effect was extended to p-cresyl sulfate in patients achieving higher convective volumes.

Jordana D. Lima, Murilo Guedes, Roberto Pecoits-Filho, Lia S. Nakao contributed equally.

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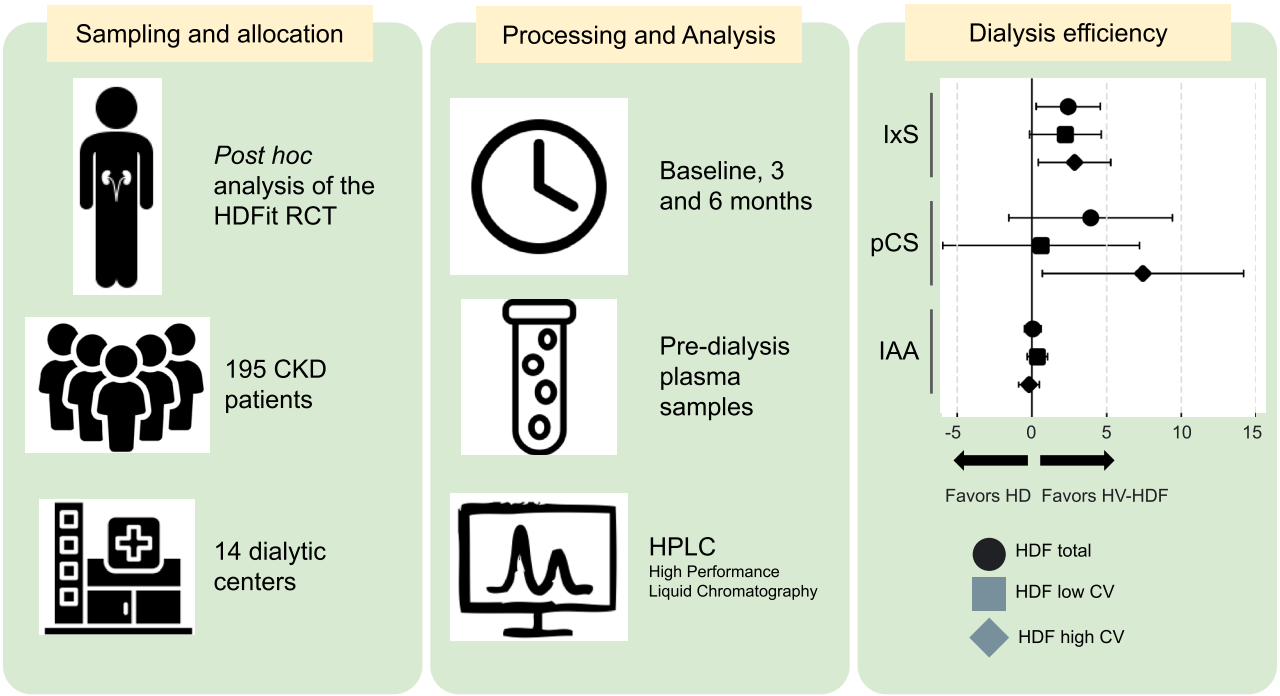
Graphical abstract



Keywords Hemodiafiltration · Protein-bound uremic toxins · Post-hoc analysis · Convective volume

Introduction

High-volume online hemodiafiltration (HV-HDF) has demonstrated better clinical outcomes compared to hemodialysis (HD), such as improved tolerance to sessions [1], better blood pressure control [2], and reduction of mortality [3–5]. These improvements in clinical outcomes may be, at least in part, attributed to a decrease in uremic toxicity observed in patients treated with HV-HDF compared to standard HD.

HV-HDF enhances the reduction ratios of uremic solutes [6], particularly those with middle molecular mass (> 500 Da), such as β2-microglobulin [7], fibroblast growth factor 23 [8], and cystatin C [9], compared to conventional HD, possibly due to its higher achieved convective volume and the consequent sieving of larger molecules. However, as demonstrated for β2-microglobulin [7], increased reduction ratios do not necessarily lead to diminished pre-dialysis concentrations, which will ultimately reflect the cumulative exposure of tissues to uremic solutes, since pre-dialysis concentrations depend on both removal and production rates of the solutes.

Indoxyl sulfate (IxS), p-cresyl sulfate (pCS), and indole-3-acetic acid (IAA) are among the most often investigated protein-bound uremic toxins (PBUT), and their binding to albumin [10] limits their removal during standard HD sessions. These protein-bound uremic toxins have detrimental effects on bone, immunological, neurological, cardiovascular and renal systems, and may be the missing link between CKD progression and high incidence of cardiovascular complications [11]. Indeed, observational studies associated increased blood levels of total indoxyl sulfate [12], free p-cresyl sulfate [13], and total indole-3-acetic acid [14] with increased risk of overall and cardiovascular mortality of CKD patients. HV-HDF has been shown to increase the reduction ratios of several protein-bound uremic toxins compared to high-flux HD [15, 16]. However, well-designed studies evaluating the effect of HV-HDF vs. high-flux HD (current standard of care) on pre-dialysis concentrations of protein-bound uremic toxins are lacking, with a few heterogeneous randomized controlled trials (RCTs) showing conflicting results [16–20].

Therefore, we carried out a post-hoc analysis of the HDFit RCT [21, 22] following 193 patients to assess whether HV-HDF treatment results in lower pre-dialysis

levels of indoxyl sulfate, p-cresyl sulfate, and indole-3-acetic acid. We also explored whether higher convective volumes achieved during HV-HDF proportionally reduce pre-dialysis protein-bound uremic toxin levels over time compared to high-flux HD. To our knowledge, this is the largest post-hoc analysis of an RCT comparing the effect of HV-HDF to high-flux HD on the pre-dialysis levels of protein-bound uremic toxins in an adult population that consistently achieved high convective volumes during the trial period.

Methods

Trial design

HDFit is a prospective, multicenter, unblinded RCT investigating the impact of dialysis modality on objectively measured physical activity levels as a primary objective. The study included several exploratory goals, such as the establishment of a central repository of biological samples for the analysis of biomarkers (ClinicalTrials.gov: NCT02787161). Protein-bound uremic toxins were longitudinally determined as part of a pre-specified exploratory analysis to estimate the comparative efficacy of HV-HDF vs. high-flux HD on reducing mean levels of protein-bound uremic toxins over time. HDFit study design and methodology have been previously published [21, 22]. This study was conducted according to the principles of the Declaration of Helsinki and Good Clinical Practice guidelines.

Setting and participants

The Center for Epidemiology and Clinical Research (EPICENTER) managed the patient recruitment among 14 centers located in the South and Southeast regions of Brazil. All patients were clinically stable, and all of them provided informed consent before the start of the study activities. We excluded patients who were participating in another trial, had severe limitation in mobility/ambulation, were non-adherent with HD, had been previously treated with HDF, and/or had a life expectancy of < 3 months due to a non-renal comorbidity. Demographics, comorbidities, and other parameters were collected during the baseline period. During the trial, all subjects had a fistula/graft or permanent catheter with adequate flow as vascular access and had a previous Kt/V ≥ 1.2. The trial included 195 adult (age ≥ 18 years) end-stage kidney disease patients (dialysis vintage between 3–24 months). At baseline, 3-, and 6-month visits, pre-dialysis blood was collected and plasma was isolated and stored at -80 °C. Plasma samples from at least one-time point were available for 193 patients. More details on the study protocol can be found elsewhere [21, 22].

Interventions

Briefly, all patients were initially followed in run-in and screening periods totaling 4 weeks before randomization. During this period, patients who were on low-flux HD were switched to high-flux HD. After this phase, subjects were randomized 1:1 to continue high-flux HD or to start post-dilution HV-HDF. Standardized high-flux dialyzers were used for HDF (Fresenius Polysulfone HDF 100®) and HD (Fresenius FX Classix 100®). Dialysis technical parameters such as blood flow and convective volume were measured

Table 1 Characteristics at baseline of the included population

Variable	Overall	HD	HV-HDF	p-value
n	193	97	96	
Age [mean (SD)]	52.93 (15.07)	53.44 (14.36)	52.42 (15.82)	0.63
Male (%)	137 (71.00)	67 (69.10)	70 (72.90)	0.66
White (%)	114 (59.10)	54 (55.70)	60 (62.50)	0.41
Access (%)				0.93
Catheter	22 (11.40)	11 (11.30)	11 (11.50)	
Fistula	162 (83.90)	82 (84.50)	80 (83.30)	
Graft	9 (4.70)	4 (4.10)	5 (5.20)	
Diabetes (%)	67 (34.70)	40 (41.20)	27 (28.10)	0.08
Pre-dialysis SBP [mean (SD)]	153.26 (23.75)	151.97 (23.73)	154.58 (23.81)	0.44
Albumin [mean (SD)]	3.97 (0.36)	3.98 (0.38)	3.96 (0.34)	0.73
Hematocrit [mean (SD)]	33.66 (5.39)	33.52 (5.54)	33.80 (5.27)	0.72
KTV [mean (SD)]	1.54 (0.43)	1.50 (0.42)	1.58 (0.44)	0.22
Pre-dialysis weight [mean (SD)]	77.80 (16.02)	79.39 (16.79)	76.19 (15.12)	0.16
BMI [mean (SD)]	27.46 (5.05)	27.88 (5.51)	27.04 (4.55)	0.28

monthly. More details on the study protocol can be found elsewhere [21, 22].

Determination of IxS, pCS, and IAA

Plasma samples were processed as described [23]. Briefly, 100 µL of plasma was diluted with 260 µL water and heated (95 °C, 30 min). After 10 min on ice, samples were centrifuged (13,000 rpm in bench centrifuge, 4 °C, 20 min), and the supernatant was ultrafiltered with a 30 kDa-cutoff membrane (Amicon Ultra, Millipore). The ultrafiltrate (10 µL) was injected. Chromatographic determinations were performed as described [23]. During the run, fluorescence wavelengths varied: $\lambda_{exc} = 280$ nm and $\lambda_{em} = 383$ nm to indoxyl sulfate and indole-3-acetic acid and $\lambda_{exc} = 265$ nm and $\lambda_{em} = 290$ nm to p-cresyl sulfate [24]. Calibration curves were performed with authentic standards. Indoxyl sulfate and indole-3-acetic acid were purchased from Sigma, and p-cresyl sulfate was a gift from Dr. Griet Glorieux (University Hospital Ghent, Belgium).

Statistical analysis

Data distribution was assessed for normality. Continuous variables were summarized as means and standard deviations (SD) or medians and interquartile ranges (IQR). Categorical variables are reported as counts and proportions. When appropriate, Student's *t* tests or Mann–Whitney rank-sum-*U* tests were used. Intra-group comparisons were made by paired *t* tests over time periods.

For the primary analysis, we assessed the rate of change of protein-bound uremic toxins between intervention groups over time using a linear mixed effect model, allowing random intercept and random slopes for time and testing the time by intervention group interaction. For each protein-bound uremic toxin, the most appropriate model according to Akaike Information criterion was used. Missingness for protein-bound uremic toxins was assumed to be completely at random (MCAR).

A pre-planned secondary analysis aiming to evaluate the rate of change over time comparing high-flux HD to HV-HDF groups stratified by convective volumes was performed. The median convective volume of 27.5 L was used as a cut-off for defining the subgroups. All analyses were performed in R software 3.5.1.

Results

Out of 195 patients included in the trial, plasma samples from at least one-time point were available for 193 patients (high-flux HD: n = 97; HV-HDF: n = 96). There were no detectable differences at baseline between the two

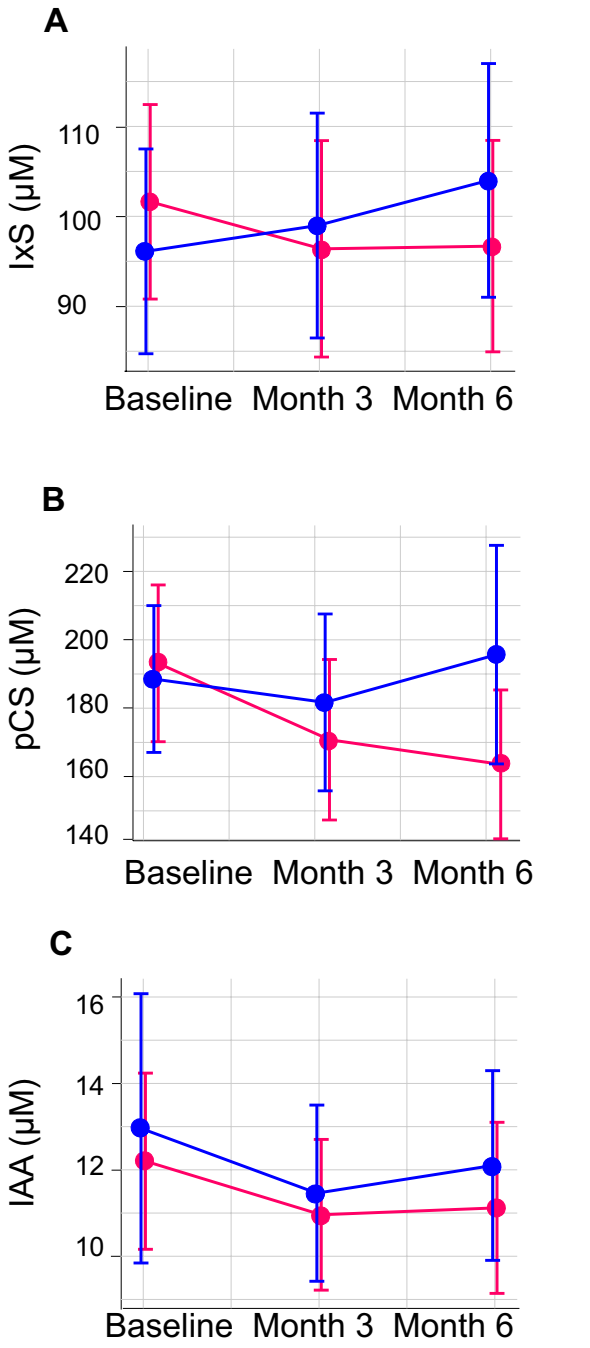


Fig. 1 Mean plasma concentrations of protein-bound uremic toxins over time. Pre-dialysis concentrations (mean ± SD) of indoxyl sulfate (IxS) (**A**), p-cresyl sulfate (pCS) (**B**) and indole-3-acetic acid (IAA) (**C**) at baseline, and at 3 and 6 months are shown. Note that IxS and pCS in HD patients increase over 6 months, while in HV-HDF patients they decrease. IAA, in contrast, is similar in both HD and HV-HDF patients over 6 months. Pink line: HV-HDF; Blue line: HD (colour figure online)

Table 2 Mean change from baseline within intervention groups

Variable	Intervention	Mean change from baseline (μM)	95% Lower CI	95% Upper CI	p-value
IxS	HD	7.5	−2.1	17.2	0.12
IxS	HV-HDF	−5.2	−13.2	2.9	0.20
pCS	HD	8.1	−16.1	32.2	0.51
pCS	HV-HDF	−29.5	−50.2	−8.7	0.005
IAA	HD	−0.8	−3.2	1.6	0.52
IAA	HV-HDF	−1	−2.9	0.8	0.27

CI confidence interval

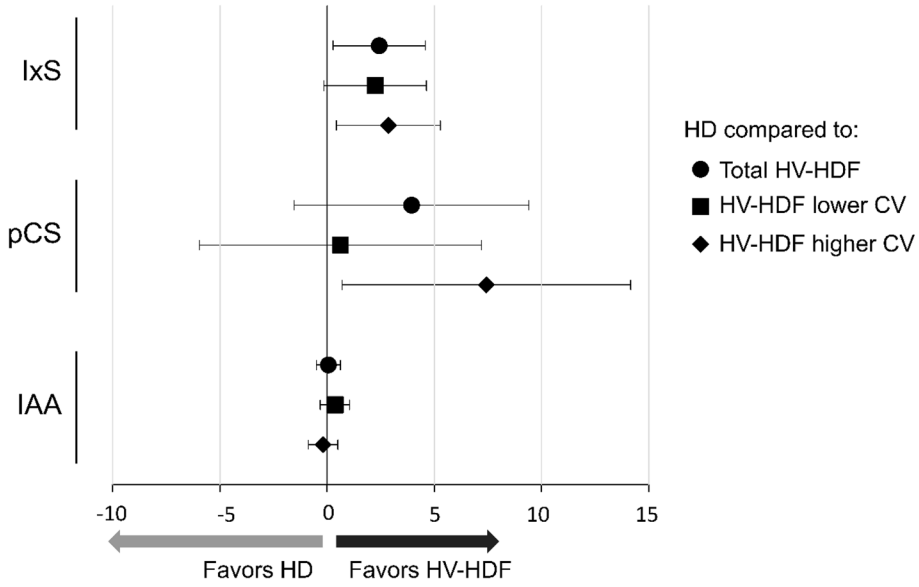


Fig. 2 Forest plot of the differences in monthly changes (μM/month) of pre-dialysis plasma protein-bound uremic toxin concentrations between HV-HDF and high-flux HD. The HDF group was stratified according to the achieved convective volume (CV), as lower (< 27.5 L) or higher convective volumes (> 27.5 L). Note that HV-HDF improved changes of indoxyl sulfate, when compared with HD. This effect was extended to p-cresyl sulfate in the subset of HV-HDF patients with higher convective volumes. Filled circles: total HV-HDF strata; Filled squares: lower convective volume subset; Filled diamonds: higher convective volume subset

intervention groups (Table 1). Overall, the median dialysis session time was 235 min (25th and 75th quartiles = 233 and 240), and mean blood flow was 362 ± 23 mL/min. In the HDF arm, the median achieved convective volume was 27.5 L, and most patients achieved convective volumes above the set target of 22 L (99%). Monthly mean convective volume (± SD) from baseline to 6 months was 27.6 ± 3.0, 27.4 ± 2.8, 27.1 ± 2.9, 27.2 ± 3.0, 27.3 ± 2.9 and 27.5 ± 2.9 L, respectively. The rates of dropout were 8% in the HDF and 11% in the HD arms.

Baseline concentrations of indoxyl sulfate, p-cresyl sulfate and indole-3-acetic acid were, respectively, 96.5 μM ± 54, 187.6 μM ± 102 and 12.9 μM ± 15 in the HD arm, and 101.7 μM ± 51, 193.1 μM ± 109 and 12.2 μM ± 9.7 in the HV-HDF arm. The results showed a more pronounced reduction of indoxyl sulfate and p-cresyl sulfate concentrations over time in the HV-HDF compared to the HD group (Fig. 1). The levels of p-cresyl sulfate decreased over time in the HV-HDF arm (− 29.5 μM) [95% CI − 50.2 to − 8.7, p = 0.005], and remained stable (8.1 μM) [95% CI − 16.1 to 32.2, p = 0.51] in the HD group. The concentrations of indoxyl sulfate tended to decrease in HV-HDF, but to increase in the HD group, although imprecision in the estimates of change from baseline was high (Table 2). The concentrations of indole-3-acetic acid remained similar in both groups (Fig. 1; Table 2).

In the primary analysis, the HV-HDF group showed a relative higher reduction rate of 2.4 μM/month [95% CI 0.30 to 4.56, p = 0.03] in indoxyl sulfate concentration over time than the high-flux HD group. For p-cresyl sulfate, HV-HDF promoted a reduction rate of 3.94 μM/month higher than high-flux HD [95% CI − 1.54 to 9.41, p = 0.16], while for indole-3-acetic acid, HDF did not lead to a different rate of high-flux HD (0.06 μM/month [95% CI − 0.6 to 0.5, p = 0.8] (Fig. 2).

In the secondary analysis, patients in the HDF group who achieved convective volumes higher than 27.5 L had, compared to the high-flux HD patients, a greater rate of concentration change for both indoxyl sulfate (2.86 μM/month) [95% CI 0.43 to 5.28, p = 0.04] and p-cresyl sulfate (7.43 μM/month) [95% CI 0.7 to 14.16, p = 0.03], while indole-3-acetic acid (− 0.19 μM/month) [95% CI − 0.88 to 0.50, p = 0.6] did not show changes over time between groups (Fig. 2).

Discussion

This post-hoc analysis of the HDFit trial suggests that HV-HDF increases the rate of reduction of total indoxyl sulfate in pre-dialysis plasma, leading to lower indoxyl sulfate levels over 6 months, compared to high-flux HD. Additionally, among patients who achieved higher convective volumes, p-cresyl sulfate levels were also reduced over time in the HV-HDF arm as compared to high-flux HD. To the best of our knowledge, this is the largest post-hoc analysis of a multicenter RCT comparing the efficacy of HV-HDF vs. high-flux HD in reducing the pre-dialysis concentration of protein-bound uremic toxins. Due to the protocol design, implementation, and trial size (which allowed for the stratification of achieved convective volume), these findings shed light on a debatable issue regarding the effectiveness of HV-HDF vs. high-flux HD in reducing protein-bound uremic toxin pre-dialysis levels and highlight the essential role of high convective volumes in such effect.

Indoxyl sulfate and p-cresyl sulfate are some of the most widely studied protein-bound uremic toxins [25]. Observational studies associated total indoxyl sulfate [12] and free p-cresyl sulfate [13] with increased cardiovascular and all-cause mortality rates. Particularly relevant to cardiovascular complications, indoxyl sulfate and p-cresyl sulfate have been demonstrated to cause vascular endothelial and smooth muscle cell dysfunctions, senescence, vascular calcification and inflammation, which increase the occurrence of atherosclerosis and cardiovascular events [26].

Previous studies investigated the impact of HV-HDF on pre-dialysis levels of the most relevant and studied protein-bound uremic toxins [16, 18–20, 27, 28]. An observational study showed that HV-HDF decreased the levels of protein-bound uremic toxins over 9 weeks compared to baseline, specifically total p-cresyl sulfate and free indole-3-acetic acid, but not total or free indoxyl sulfate [18]. Some compared HDF to low-flux HD [16, 19, 27, 28], which is not currently considered standard of care in hemodialysis. Most developed countries adopted high-flux as the preferred therapy, given the potential clinical benefits of high-flux over low-flux HD [29]. A trial with 37 non-randomized patients in the HV-HDF arm for 12 months did not highlight the potential of HV-HDF in reducing the levels of protein-bound uremic toxins [19]. However, this population consisted of pediatric patients, hampering comparisons with adult patients. Only two randomized studies in an adult population compared HDF to high-flux HD by analyzing pre-dialysis concentrations of protein-bound uremic toxins. The first reported the superiority of convection over conventional high-flux HD on protein-bound uremic toxin removal, analyzing the pre-dialysis concentration of total p-cresol, which represents the sum of p-cresyl sulfate and p-cresylglucuronide, after 2 weeks, in 14 patients [20]. The other is a prospective randomized crossover study, that analyzed 14 patients and did not confirm the superiority of HV-HDF in decreasing pre-dialysis levels of total indoxyl sulfate and p-cresyl sulfate, in spite of the higher reduction ratios determined for total indoxyl sulfate and p-cresyl sulfate [16]. Although our findings come from a post-hoc analysis, some points could be tentatively compared among these studies and ours: (i) Bammens et al. observed a reduction in pre-dialysis concentrations of total p-cresol in pre-dilution HDF, compared to high-flux HD. When comparing post-dilution HDF with high flux HD, no reduction was detected [20]. Both Krieter et al. and we compared post-dilution HDF to high-flux HD; (ii) the duration of treatments were 2 weeks [20], 6 weeks [16] and 6 months (ours). Krieter et al. [16] demonstrated a reduction in pre-dialysis levels of total indoxyl sulfate in HDF, compared to high-flux HD, only at week 3. No difference was observed for free indoxyl sulfate, free and total p-cresyl sulfate in week 3 or 6; (iii) both RCTs were crossover studies, with consecutive short periods (2–6 weeks) in each dialysis modality. Altogether, these data suggest that post-dilution HDF may improve the pre-dialysis concentrations of protein-bound uremic toxins, particularly indoxyl sulfate and p-cresyl sulfate, only at longer times. Indeed, we can estimate that at week 6, we could not find any difference between high-flux HD and HV-HDF (Fig. 1).

In addition, protein-bound uremic toxin concentrations could be importantly affected by the convective volume in HV-HDF. In agreement, higher convective volumes correlated with increased reduction ratios of indoxyl sulfate and p-cresyl sulfate in patients treated with HV-HDF [17], and a study with a low achieved convective volume reported no association with the percentage of change in pre-dialysis protein-bound uremic toxins over time [28]. Remarkably, the achieved convective volume in that study (17.3 ± 4.3 L) was lower than the current recommended target of 22 L. One may postulate that convective volumes below this threshold may yield solute removal comparable to predominantly diffusive modalities. Among the studies that analyzed HV-HDF and HD (either high or low-flux) in adult populations [16, 18, 27, 28], ours achieved the highest convective volume. According to our data, high convective volumes decreased the levels of indoxyl sulfate and p-cresyl sulfate. The canonical view of protein-bound uremic toxin removal during dialysis lies on the removal of the free fraction; the increased convection might increase the removal of the free fraction and induce the displacement of the bound toxin to its free state. However, our data also indicated that indole-3-acetic acid levels were similar in both the HD and HV-HDF groups. The lack of effect observed for this metabolite supports the view that toxins whose percentage of protein binding is relatively low may not benefit from HV-HDF, when compared to high flux HD. Protein binding is $> 90\%$ for indoxyl sulfate and

p-cresyl sulfate and < 70% for indole-3-acetic acid [10, 18]. Therefore, we suggest that HV-HDF, by incrementing convection in comparison to high-flux HD, allows the removal of the bound fraction of indoxyl sulfate and p-cresyl sulfate, but produces no additional effect on indole-3-acetic acid removal, for which diffusion plays a major role. However, we did not measure protein loss to confirm this hypothesis.

As a main limitation, our study may not be generalized to the overall in-center hemodialysis population, given this is a post-hoc analysis of an RCT including stable patients on chronic in-center HD. Additionally, we did not have data on residual kidney function, which may modify the effect of HV-HDF vs. high-flux HD on the pre-dialysis concentrations of protein-bound uremic toxins. Moreover, the proportion of patients with diabetes at baseline was slightly lower in the HDF arm, which could have influenced the results. Also, we neither report nor adjusted for measures of inflammation, such as C-reactive protein, which could be important confounders. Importantly, the results we report for the between-group differences in the rate of removal for p-cresyl sulfate and indoxyl sulfate should be interpreted with caution. A higher rate of removal may not imply less cumulative exposure of pre-dialysis protein-bound uremic toxins at sufficient levels to impact clinical outcomes through the 6-month follow-up period. Finally, although the analyses of protein-bound uremic toxins were pre-specified in the study protocol, the results are exploratory, and thus hypothesis-generating only, since no power calculation was performed. However, our study has several strengths. First, as a post-hoc analysis of an RCT, our estimates may have a low risk of confounding, although we cannot rule out residual confounding. Second, we ensured that most patients had their pre-dialysis protein-bound uremic toxins measured during the follow-up, limiting potential post-randomization biases. Third, HDFit comprises fourteen Brazilian dialysis centers, and our sample sizes are larger compared to former studies evaluating protein-bound uremic toxins, which could minimize issues regarding power to detect clinically meaningful differences in pre-dialysis concentrations [16, 27, 28]. Additionally, our follow-up was relatively longer compared to the aforementioned studies, and our dropout rates were remarkably low. Moreover, we compared HV-HDF to high-flux HD, which increases the external validity of our findings considering the current real-world dialysis practices and standard of care. Finally, we determined pre-dialysis concentrations of protein-bound uremic toxins, which reflect the actual uremic environment to which cells and tissues are exposed between two dialytic treatments. Indeed, the achievement of higher reduction ratios for protein-bound uremic toxins during dialysis may not impact on the pre-dialysis levels of protein-bound uremic toxins [16], as observed also for β2-microglobulin [7].

In summary, in this post-hoc analysis of the largest published RCT comparing HV-HDF to high-flux HD with protein-bound uremic toxins as a predefined exploratory outcome, we present robust and consistent data on reduction rates in pre-dialysis concentrations of indoxyl sulfate, and of p-cresyl sulfate in the subgroup of patients achieving higher convective volumes, favoring HV-HDF. These exploratory findings suggest, that HV-HDF is more effective in reducing protein-bound uremic toxins as compared to standard high-flux HD. Further studies, namely RCTs are required to confirm these results.

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Author contributions Conception or design: ANMA, ABR, MEC, ACN, CEPF, RPF, LSN; Analysis and interpretation of data: JDL, MHG, SDR, RPF, LSN; Drafting the article or revising it: JDL, MHG, RPF, LSN; Providing intellectual content of critical importance to the work described: all authors; Final approval of the version to be published: all authors.

Declarations

Conflict of interest RPF receives research grants, consulting fees, and honoraria from Baxter Healthcare and Fresenius Medical Care. CEPF and ACN receive consulting fees and speaker honorarium from Fresenius Medical Care. ABB is an employee of Fresenius Medical Care Brazil. MEC receives research grants, consulting fees, and honoraria from Baxter Healthcare and Fresenius Medical Care. All other authors have no relevant financial or non-financial interests to disclose. The funding agencies had no role in the study design, sampling collection, analyses or the interpretation of the data.

Ethics approval In this post-hoc analysis, the use of all samples was previously allowed by the participants through a written consent form in the original HDFit study [22]. All the authors attest to the veracity of the data and affirm that these were not published elsewhere.

Consent to participate and for publication All participants from the original study HDFit [21, 22] underwent the appropriate consent policies.

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