We would like to thank the expert Reviewers for the comments and the effort in reviewing our manuscript. Each comment is addressed as follows.

Reviewer #3

The present study addresses the effectiveness of different forms of hemodialysis on the clearance of middle molecules, a question that has already been examined in much more comprehensive studies. The small cohort considered here does not yield any unexpected results for the reader. Currently, the manuscript exhibits significant limitations, starting with the poorly described methodology, which lacks clear information about the materials used. Without this information, the few presented results cannot be adequately interpreted. The major limitations listed below must be addressed:

Title and Abstract: The title of the manuscript does not correspond to the abstract, as the term 'hemodiafiltration' is not mentioned at all in the abstract. This discrepancy needs to be addressed to ensure consistency.

Additionally, the use of the abbreviation 'expanded HD' (xHD) is inconsistent throughout the abstract. A unified terminology should be maintained.

Introduction: The statement 'has made great advances' is overly exaggerated. Dialysis remains an inferior solution compared to transplantation. The statement should be moderated and rephrased accordingly.

The claim of a direct and singular causality between middle molecular weight toxins and conditions such as amyloidosis, chronic inflammation, cardiovascular disease, mineral-bone disease, and neuropathy is not scientifically sound. These toxins may contribute to these conditions, but presenting a monocasual relationship is inappropriate.

Methods: The methods section lacks essential details, specifically concerning the dialysis machines and filters used. Information about filters is critical as variations could explain the study's findings, given the known differences between various filter types.

There is a lack of clarity regarding the term 'expanded HD,' including the duration of the hemodialysis sessions and the specific filters used in each group. **Regarding study design, a group of patients transitioning from standard HD to online HDF would have provided more insightful results. The relevance of standard HD with low-flux filters is questionable, as it is considered outdated.** **Results: The results section lacks a baseline table including essential statistics (age, dialysis vintage, baseline renal disease, dialysis access). Providing such a table is a scientific standard, especially considering the small patient cohort.** **The labeling of groups is confusing: In Figure 1, 'baseline' is labeled as 'standard HD,' while in Figures 2B and 2C, 'before HD' and 'after HD' would be more accurately labeled as 'before xHD' and 'after xHD.'** **The statement regarding the comparison of Su-PAR, MCP-1, and activin-A clearance between the groups lacks a corresponding figure. Figure 4 only shows pre/post xHD comparisons. Please provide data for all groups, including percentage changes.** **Discussion: The statement that expanded HD could be a reliable and cost-effective alternative to on-line HDF requires clarification. Information on cost differences and practical feasibility, particularly in routine patient care, is necessary. In most dialysis centers, optimizing patient numbers is a priority, making expanded HD potentially less practical.** **The 2023 landmark study (CONVINCE study by Peter Blankestijn, DOI: 10.1056/NEJMoa2304820) demonstrated improved mortality with HDF compared to high-flux HD. The authors should address this study and incorporate its findings into the discussion.** **Conclusion: The final statement claiming that further randomized trials are necessary to evaluate the effects of MCO membranes on medium molecular weight substance clearance is misleading. The study lacks focus on MCO membranes, and more specific information regarding the methodology is required.** **General Comments: The study's relevance is questionable, given the established knowledge among nephrologists that HD with low-flux filters is outdated. This fundamental issue should be addressed.**		
1.	**Title and Abstract: The title of the manuscript does not correspond to**	We thank Reviewer 3 for the comment. In the abstract is clearly mentioned "Moreover, we investigated the clearance of Su-PAR, MCP-1 and Activin,	**Page 1**

	the abstract, as the term 'hemodiafiltration' is not mentioned at all in the abstract. This discrepancy needs to be addressed to ensure consistency.	comparing sHD, xHD and **on-line hemodiafiltration (HDF)**". We believe that it is clear that we compare the efficacy between standard HD, expanded HD and on-line hemodiafiltration.	
2.	**Additionally, the use of the abbreviation 'expanded HD' (xHD) is inconsistent throughout the abstract. A unified terminology should be maintained**	We thank the Reviewer 3 for the comment. We have now corrected the terminology.	**Pages 1-8**
3.	**Introduction: The statement 'has made great advances' is overly exaggerated. Dialysis remains an inferior solution compared to transplantation. The statement should be moderated and rephrased accordingly.**	We thank the Reviewer 3 for the comment. We have now moderated the statement accordingly "In recent decades, hemodialysis (HD) has been refined ~~made great advances~~ leading patients with end-stage kidney disease (ESKD) to better quality of life and longer survival compared to the past".	**Page 1**
4.	**The claim of a direct and singular causality between middle molecular weight toxins and conditions such as amyloidosis, chronic inflammation, cardiovascular disease, mineral-bone disease, and neuropathy is not scientifically sound. These toxins may contribute to these conditions, but presenting a monocasual**	We thank the Reviewer 3 for the comment. We have now modified the Introduction accordingly: "Nowadays, it is known that middle molecular weight uremic toxins are accumulated in ESKD and ~~are responsible for~~ can contribute to the appearance of many toxic effects such as amyloidosis, chronic inflammation, cardiovascular disease, mineral-bone disease, neuropathy, virtually covering all organ systems".	**Page 1**

	relationship is inappropriate.		
5.	**Methods: The methods section lacks essential details, specifically concerning the dialysis machines and filters used. Information about filters is critical as variations could explain the study's findings, given the known differences between various filter types.**	We thank the Reviewer 3 for the comment. We have now modified the Methods accordingly "All the participants were dialyzed with NIKKISO DBB EXA® machine. As for the dialyzers, we used FX10® in the standard HD group, FX100® in the online-HDF group and THERANOVA® in the xHD group. The flow rate was ranging between 300 and 400 ml/min and dialysate flow rate was 500 ml/min; calcium concentration was 1.75 mmol/l, magnesium concentration was 0.5 mmol/l, potassium concentration was 2 mmol/l and sodium concentration was 139mmol/l during the whole period of the study".	**Page 2**
6.	**There is a lack of clarity regarding the term 'expanded HD,' including the duration of the hemodialysis sessions and the specific filters used in each group.**	We thank Reviewer 3 for the comment. All the participants underwent three 4-hour dialysis sessions per week. Expanded hemodialysis, is a renal replacement therapy based on the use of medium cut-off membranes (MCO). In our study, we used the MCO membrane Theranova®. As for standard HD we used low flux dialyzers (FX10®), whereas high flux dialyzers (FX100®) were used in on-line hemodiafiltration. The dialyzer surface area was determined by the patient's body surface area. We have now modified the Method accordingly "All the participants were dialyzed with NIKKISO DBB EXA® machine. As for the dialyzers, we used FX10® in the standard HD group, FX100® in the online-HDF group and THERANOVA® in the xHD group. The flow rate was ranging between 300 and 400 ml/min and dialysate flow rate was 500 ml/min; calcium concentration was 1.75 mmol/l, magnesium concentration was 0.5 mmol/l, potassium concentration was 2 mmol/l and sodium concentration was 139mmol/l during the whole period of the study. Moreover, there was stable medical and nursing staff throughout the	**Page 2**

		duration of the study. All the participants underwent three 4-hour dialysis sessions per week".	
7.	**Regarding study design, a group of patients transitioning from standard HD to online HDF would have provided more insightful results. The relevance of standard HD with low-flux filters is questionable, as it is considered outdated**	We thank the Reviewer for the comment. We agree that a comparison between standard high-flux HD and on-line HDF could provide more insightful results. We decided to compare xHD and on-line HDF with low-flux HD, as in the Greek national health care system a mandatory quota is applied to the type of dialysis that patients receive regardless of possible actual medical needs and indications.	

<table>
<tr>
<td>8.</td>
<td>Results: The results section lacks a baseline table including essential statistics (age, dialysis vintage, baseline renal disease, dialysis access). Providing such a table is a scientific standard, especially considering the small patient cohort.</td>
<td>We thank the reviewer for the comment. We have now modified our Results and added a table with baseline characteristics.

“The baseline demographic and clinical characteristics of the study population are presented in Table 1”.

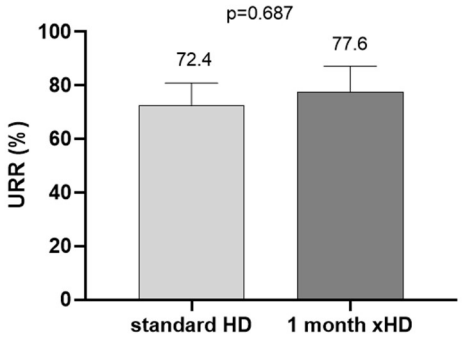
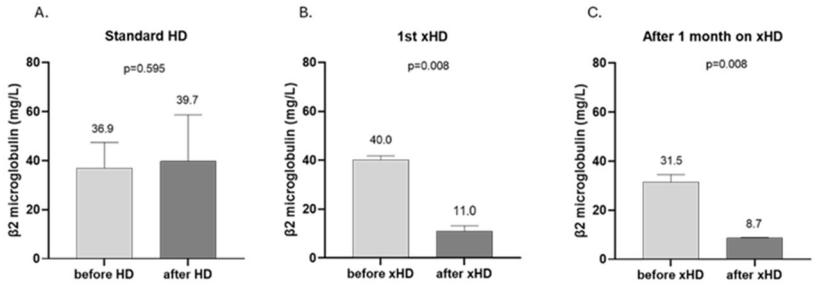
Table 1A
<table>
<tr><td>n</td><td>7</td></tr>
<tr><td>Male (n,%)</td><td>4,57%</td></tr>
<tr><td>Age (y)</td><td>64.2±10.8</td></tr>
<tr><td>Hemodialysis vintage (y)</td><td>10.8±4.6</td></tr>
<tr><td>Baseline Renal Disease</td><td></td></tr>
<tr><td>Diabetic Nephropathy (n,%)</td><td>2, 28.6%</td></tr>
<tr><td>Hypertensive Nephrosclerosis (n,%)</td><td>3, 43%</td></tr>
<tr><td>Glomerulonephritis (n,%)</td><td>2, 28.6%</td></tr>
<tr><td>Vascular access</td><td></td></tr>
<tr><td>AVF (n,%)</td><td>4, 57%</td></tr>
<tr><td>AVG (n,%)</td><td>1, 14.3%</td></tr>
<tr><td>Central catheter (n,%)</td><td>2, 28.6%</td></tr>
<tr><td>Comorbidities</td><td></td></tr>
</table>
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<td>Page 4</td>
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Diabetes mellitus (n,%)	2, 28.6%
Hypertension (n,%)	7, 100%
Coronary artery disease (n,%)	2, 28.6%
Heart failure (n,%)	1, 14.3%

Table 1B

	Standard HD	On-line HDF	xHD
n	10	10	10
Male (n,%)	7, 70%	7, 70%	7,70%
Age (y)	61.7±5.3	61.7±4.9	61.9±6.0
Hemodialysis vintage (y)	6.1±1.4	5.8±1.3	5.8±1.3
Baseline Renal Disease			
Diabetic nephropathy (n,%)	3, 30%	4, 40%	3, 30%
Hypertensive Nephrosclerosis (n,%)	4, 40%	3, 30%	30, 30%
Glomerulonephritis (n,%)	3, 30%	3, 30%	4, 40%

		Vascular access					
		AVF (n,%)	6, 60%	7, 70%	6,60%		
		AVG (n,%)	2, 20%	2, 20%	2, 20%		
		Central catheter (n,%)	2, 20%	1, 10%	2, 20%		
		Comorbidities					
		Diabetes mellitus (n,%)	4, 40%	4, 40%	4, 40%		
		Hypertension (n,%)	10, 100%	9, 90%	10, 100%		
		Coronary artery disease (n,%)	2, 20%	2, 20%	1, 10%		
		Heart failure (n,%)	1, 10%	0, 0%	1, 10%		

9.	**The labeling of groups is confusing: In Figure 1, 'baseline' is labeled as 'standard HD,' while in Figures 2B and 2C, 'before HD' and 'after HD' would be more accurately labeled as 'before xHD' and 'after xHD.'**	We thank the reviewer for the comment. We have now changed the labeling in Figure 1 and the legend of the figure:  **Figure 1.** URR (%) at ~~baseline~~ standard HD and after 4 weeks on ~~expanded~~ xHD ~~(xHD, expanded HD)~~. We have also modified the labeling in Figures 2B and 2C  **Figure 2.** β2-microglobulin concentrations before and after HD (A), first xHD session (B) and one month after xHD implementation (C)~~(xHD; expanded HD)~~. (A presents mean±SD; B and C present	**Page 5**

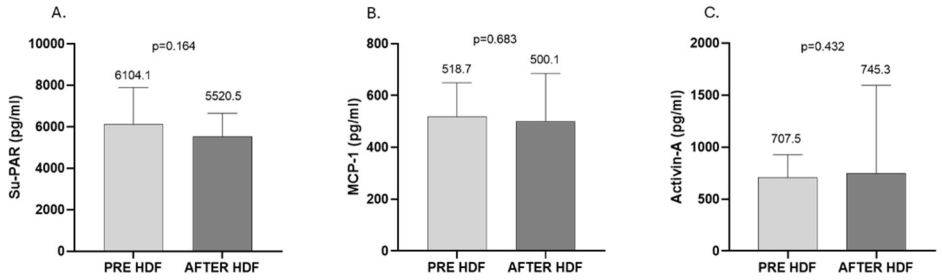
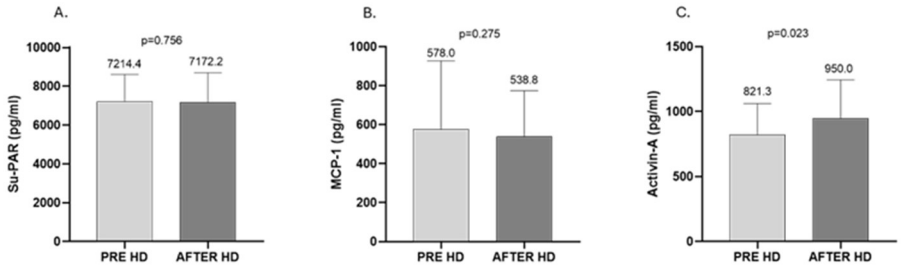
		median, IQR).	
10.	**The statement regarding the comparison of Su-PAR, MCP-1, and activin-A clearance between the groups lacks a corresponding figure. Figure 4 only shows pre/post xHD comparisons. Please provide data for all groups, including percentage changes.**	We thank the Reviewer 3 for the comment. We have now added Figure 5 and Figure 6  **Figure 5.** Effect of on-line HDF in the clearance of Su-PAR (A), MCP-1 (B) and Activin (C). (A and B present mean±SD; C presents median, IQR).  	**Pages 6,7**

		Figure 6. Effect of standard HD in the clearance of Su-PAR (A), MCP-1 (B) and Activin (C). (A and C present mean±SD; B presents median, IQR).	
11.	**Discussion: The statement that expanded HD could be a reliable and cost-effective alternative to on-line HDF requires clarification. Information on cost differences and practical feasibility, particularly in routine patient care, is necessary. In most dialysis centers, optimizing patient numbers is a priority, making expanded HD potentially less practical.**	We thank the Reviewer 3 for the comment. It seems that xHD provides the possibility of performing Hemodialysis of similar efficacy to on-line HDF without increasing the cost and technical complexity by requiring specific equipment and large volumes of ultrapure dialysis replacement fluid (Ana García-Prieto et al, Clinical Kidney Journal, 2023, vol. 16, no. 7, 1071–1080). A cost-utility analysis based on the Convective Transport Study (CONTRAST), compared on-line HDF with low-flux HD. According to the results on-line HDF could not be considered as a cost-effective treatment for patients with ESKD (Nephrol Dial Transplant (2013) 28: 1865–1873). Michael J. Blackowicz et al, performed economic evaluation of xHD. In this 24-week clinical trial participants were randomized to receive hemodialysis with either THERNOVA® dialyzer or with a similar size high-flux dialyzer. All cause hospitalization rate was 45% lower with THERANOVA® compared to high-flux HD. Moreover, average annual estimated cost of hospitalization was $6098 lower with THERANOVA® compared to high-flux HD. Compared to high-flux HD, average annual estimated cost associated with THERANOVA use was $4772 lower per patient (Michael J. Blackowicz et al, Hemodialysis International. 2022;26:449–455). Ariza et al, found that the doses per patient of ESA, iron, insulin and hypertension medications, and hospitalization length measured by hospital day per patient-year were significantly lower for HDx patients compared with HF-HD patients (Ther Apher Dial2021;25:621–7). According to these studies, it seems that xHD is cost-effective compared to on-line HDF, however this is based on observational studies and the results should be confirmed in cost-effectiveness studies.	

		We have now modified the Discussion accordingly: "As for on-line HDF, it seems that ~~expanded~~ xHD could be a reliable alternative method, with adequate results and less cost, as xHD does not require specific equipment and large volume of ultrapure dialysis replacement fluid; however, this should be confirmed with cost-effectiveness studies"	
12.	**The 2023 landmark study (CONVINCE study by Peter Blankestijn, DOI: 10.1056/NEJMoa2304820) demonstrated improved mortality with HDF compared to high-flux HD. The authors should address this study and incorporate its findings into the discussion**	We thank the Reviewer 3 for the comment. We have now added the results of this study in the Discussion: "Peter J. Blankestijn et al compared the effect of hemodialiltration or hemodialysis on mortality. In this multinational, randomized, controlled trial, 683 patients were randomized to receive high dose hemodiafiltration and 677 to receive high flux hemodialysis. According to the results, the use of high dose hemodiafiltration resulted in a lower risk of death from any cause than conventional high-flux hemodialysis (hazard ratio, 0.77; 95% confidence interval, 0.65 to 0.93)"	**Page 7**
13.	**Conclusion: The final statement claiming that further randomized trials are necessary to evaluate the effects of MCO membranes on medium molecular weight substance clearance is misleading. The study lacks focus on MCO membranes, and more specific information regarding the methodology is required**	We thank the Reviewer 3 for the comment. We would like to clarify that expanded Hemodialysis is an emerging renal replacement therapy based on the use of medium cut-off (MCO) membranes. Thus, when we mention MCO membranes we mean expanded Hemodialysis. In order to be sure that this is clear, we have now modified the conclusion accordingly: "Further randomized trials are warranted to fully elucidate the effects of xHD on the clearance of medium molecular weight substances and whether they could have an impact on morbidity and mortality associated with HD".	**Page 8**

14.	**General Comments: The study's relevance is questionable, given the established knowledge among nephrologists that HD with low-flux filters is outdated. This fundamental issue should be addressed.**	We thank the Reviewer 3 for the comment. We decided to compare xHD and on-line HDF with low-flux HD, as in the Greek national health system a mandatory quota is applied to the type of dialysis that patients receive regardless of possible actual medical needs and indications.	