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 #1

1. **The first part of the study included only 7 patients, and the small sample size may have led to insufficient statistical power, affecting the stability and generalizability of the results. It might not have adequately captured individual differences, so the conclusions may be uncertain when applied to a wider population.**

2. **With only a 4-week intervention period, the study may not have been long enough to fully assess the long - term efficacy and safety of the dialysis methods. Long - term complications like chronic inflammation and cardiovascular disease require more prolonged observation to evaluate their development and the impact of dialysis.**

3. **The study used a non-randomised open - label design, which can introduce selection bias and confounding factors. A randomised controlled trial design is recommended for more objective results in future studies.**

4. **Data from a single centre may be influenced by factors specific to that environment, such as equipment and staff, which could affect the results and limit their generalisability.**

5. **Beyond basic patient characteristics, other factors that might affect dialysis outcomes, such as diet, medication use, and dialysis adherence, were not recorded or analysed in detail. These factors could interfere with the assessment of dialysis methods' effectiveness.**

6. **The study showed that xHD reduces certain middle - molecular - weight toxin levels, but it's unclear if these biochemical changes lead to actual clinical improvements like fewer cardiovascular events or lower mortality.**

7. **The study states that ethical approval and patient consent were obtained, but it doesn't go into detail about the consent process,**

patients' rights, and privacy protection.			
No	**Comment**	**Response**	**Page in Revised Manuscript**
1.	**The first part of the study included only 7 patients, and the small sample size may have led to insufficient statistical power, affecting the stability and generalizability of the results. It might not have adequately captured individual differences, so the conclusions may be uncertain when applied to a wider population**	We thank the Reviewer 1 for the comment. Indeed, the sample size, especially in the first part of the study, is small and it could lead to uncertain conclusions when applied to a wider population. This is clearly mentioned in the limitations of our study: "Moreover, the sample size was small, and this could have an impact on the robustness of our results". We have now modified the Discussion accordingly "Further randomized trials with larger sample size are necessary to clarify if the conclusions of our study could be applied to a wider population". However, as shown in figure 2, the difference in the concentration of β2-microglobulin after xHD both after the 1st session and at the end of follow up is quite substantial, thus, there is high probability that a larger sample would not alter our results.	**Page 8**
2.	**With only a 4 - week intervention period, the study may not have been long enough to fully assess the long - term efficacy and safety of the dialysis methods. Long - term complications like chronic inflammation and cardiovascular disease require more**	We thank the Reviewer 1 for the comment. We have now modified the Discussion: "Our study has strengths and limitations. The main limitation is that the duration of the intervention was 4 weeks which can be considered short, as long-term complications like cardiovascular disease and chronic inflammation require prolonged observation to draw safe conclusions; longer	**Page 8**

	prolonged observation to evaluate their development and the impact of dialysis.	interventions are necessary to further delineate the observed effects".	
3.	**The study used a non-randomised open - label design, which can introduce selection bias and confounding factors. A randomised controlled trial design is recommended for more objective results in future studies**	We thank the Reviewer 1 for the comment. We -indeed- used a non-randomised, open-label design in our study. However, the study population had very similar base line characteristics like age, dialysis vintage, type of vascular access and comorbidities (diabetes mellitus, hypertension, heart failure, coronary artery disease), so we believe that the bias is negligible. We have now modified the discussion to include this limitation: "Furthermore, in our study we did not follow a randomized design. A possible alternative could be performing a randomized cross-over design, but in that case, large wash-out periods would be needed to eliminate possible carry-over effects. In order to overcome this problem in study design, we carefully selected patients with very similar characteristics, like age, dialysis vintage, type of vascular access and comorbidities (diabetes mellitus, hypertension, heart failure, coronary artery disease), so we believe that the bias is negligible". Moreover, we have now modified the conclusion: "Further randomized controlled 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**
4.	**Data from a single centre may be influenced by factors specific to that environment, such**	We thank the Reviewer 1 for the comment. All the participants were dialyzed with the same haemodialysis machine (NIKKISO	**Page 2**

	as equipment and staff, which could affect the results and limit their generalisability	DBB EXA®). Moreover, in each separate group (hemodialysis, expanded hemodialysis and on-line hemodiafiltration), we used dialyzers with same characteristics (K_{UF} and K_oA), whereas the surface area was determined according to the patient's body surface area. In more details, we used FX10®, FX100® and THERANOVA® in the standard HD, on-line HDF and xHD, respectively. 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. Finally, there was stable medical and nursing staff throughout the duration of the study. We have now added this information in the Methods:"All the participants were dialyzed with NIKKISO DBB EXA® machines. 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 duration of the study".	
5.	**Beyond basic patient characteristics, other factors that might affect dialysis outcomes,**	We thank the Reviewer 1 for the comment. All the participants underwent three 4-hour dialysis sessions per week. As for diet, all	**Page 2**

	such as diet, medication use, and dialysis adherence, were not recorded or analyzed in detail. These factors could interfere with the assessment of dialysis methods' effectiveness.	the participants were strongly advised to have a protein intake of 1-1.2g/kg/day and a daily energy intake of 30-35 Kcal/Kg (ideal body weight); they were also advised to strictly reduce salt consumption <2gr/day. As for medication, no changes were allowed during the study period, and all the participants were strongly advised to receive their medication with no deviations. We have now added this information in Methods: "All the participants underwent three 4-hour dialysis sessions per week. As for diet, all the participants were strongly advised to have a protein intake of 1-1.2g/kg/day and a daily energy intake of 30-35 Kcal/Kg; they were also advised to strictly reduce salt consumption <2gr/day. As for medication, no changes were allowed during the study period, and all the participants were strongly advised to receive their medication with no deviations".	
6.	**The study showed that xHD reduces certain middle - molecular - weight toxin levels, but it's unclear if these biochemical changes lead to actual clinical improvements like fewer cardiovascular events or lower mortality.**	We thank the Reviewer 1 for the comment. CKD is associated with both atheromatous cardiovascular disease (CVD) and non-atheromatous CVD. The increased risk of CVD is due to a combination of traditional cardiovascular risk factors (diabetes mellitus, hypertension, dyslipidemia, older age etc.) and non-traditional risk factors like uremic toxins accumulation. These uremic toxins originate from endogenous metabolism, exogenous intake or microbial metabolism. Several of these toxins, like β2-microglobulin, are associated with greater risk for CVD and mortality, especially in patients undergoing hemodialysis for prolonged time (Cheung, Alfred K et al, J Am Soc Nephrol 2006 Feb;17(2):546-55)	**Page 8**

		The superiority of on-line HDF is based on better removal of uremic toxins which improve the cardiovascular outcomes. In a recent multicenter randomized control trial xHD was compared to on-line HDF as for cardiovascular parameters. According to the results, the estimated parameters as well as cardiovascular and all-cause mortality were similar in both groups (Y. Lee et al, Sci Rep 11, 10807 (2021). As far as it concerns our study, a longer observation time is needed to clarify if this reduction of middle-molecular weight toxins could lead to a reduction in cardiovascular events and mortality. We have now modified the Conclusions accordingly: "In conclusion, this prospective non-randomized open label study showed that ~~expanded~~ xHD is very effective in the clearance of medium molecular weight uremic toxins which could potentially confer a survival benefit if associated with the reduction ~~with~~ of molecules such as MCP-1 and Su-PAR; however, a longer observation period is needed to clarify if this reduction of middle-molecular weight toxins could lead to a reduction in cardiovascular events and mortality"	
7.	**The study states that ethical approval and patient consent were obtained, but it doesn't go into detail about the consent process, patients' rights, and privacy protection.**	We than the Reviewer 1 for the comment. We have now modified the Methods accordingly: "All the participants provided signed consent for their participation in the study, after being thoroughly informed about the study procedures, potential risks and benefits".	**Page 2**