Artificial Organs

WILEY

Artificial Organs

THOUGHTS & PROGRESS

Changes in Bone Turnover Markers Associated With a Single Dialysis Session

Alix Mackowiak[1] | Antoine Bouquegneau[2] | Bernard Dubois[2] | Elodie Grifnée[3] | Justine Demeuse[1] | Caroline Le Goff[1,3] | Mathias Loberg Haarhaus[4,5] | Per Magnusson[6,7] | Hanne Skou Jørgensen[8,9,10] | Pieter Evenepoel[8,11] | Pierre Delanaye[2] | Etienne Cavalier[1,3]

[1]Clinical Chemistry, Center for Interdisciplinary Research on Medicines (CIRM), University of Liège, Liège, Belgium | [2]Department of Nephrology, Dialysis and Transplantation, University Hospital of Liège, Liège, Belgium | [3]Department of Clinical Chemistry, University Hospital of Liège, Liège, Belgium | [4]Division of Renal Medicine, Department of Clinical Sciences, Intervention and Technology, Karolinska Institutet, University Hospital of Karolinska, Stockholm, Sweden | [5]Diaverum Sweden, Malmö, Sweden | [6]Department of Clinical Chemistry, Linköping University, Linköping, Sweden | [7]Department of Biomedical and Clinical Sciences, Linköping University, Linköping, Sweden | [8]Department of Microbiology, Immunology and Transplantation, Nephrology and Renal Transplantation Research Group, University of Leuven, Leuven, Belgium | [9]Department of Clinical Medicine, University of Aarhus, Aarhus, Denmark | [10]Department of Nephrology, University Hospital of Aalborg, Aalborg, Denmark | [11]Department of Nephrology and Renal Transplantation, University of Leuven, Leuven, Belgium

Correspondence: Alix Mackowiak (alix.mackowiak@uliege.be)

Received: 13 May 2025 | **Revised:** 16 June 2025 | **Accepted:** 20 June 2025

Funding: The authors received no specific funding for this work.

Keywords: bone turnover markers | dialysis membrane | hemodiafiltration | hemodialysis

ABSTRACT

Background: Chronic kidney disease (CKD) is associated with disturbances in mineral and bone metabolism, increasing fracture risk, particularly in advanced stages. Bone turnover markers (BTMs) such as bone alkaline phosphatase (BALP) and tartrate-resistant acid phosphatase 5b (TRACP5b), as well as collagen-derived markers including intact form of procollagen type I amino-terminal propeptide (iPINP) and β-cross-linked C-terminal telopeptide of type I collagen (β-CTX-I), are recommended to assess bone turnover. Although some BTMs are considered stable and independent of renal function, their behavior during dialysis sessions remains unclear.

Methods: To assess the impact of dialysis techniques and membrane types, serum samples were collected pre- and post-dialysis. BTM concentrations were analyzed using nonparametric statistics, and the clinical relevance of the observed changes was evaluated using the least significant change (LSC) thresholds.

Results: BALP and TRACP5b showed minor fluctuations post-dialysis, with changes remaining below their clinical significance thresholds. iPINP also showed limited variability. In contrast, β-CTX-I decreased significantly after hemodialysis, especially using high-permeability membranes, with changes exceeding the LSC threshold.

Conclusion: These findings suggest that BALP, TRACP5b, and PINP demonstrate robustness as BTMs in CKD patients undergoing dialysis. However, β-CTX-I is markedly affected by dialysis parameters and should be interpreted cautiously in this context.

1 | Background

Chronic kidney disease (CKD) is a major public health problem leading to systemic complications, including bone and mineral metabolism disturbances [1]. The management of chronic kidney disease-mineral and bone disorder (CKD-MBD) is critical due to its association with higher fracture and mortality risks, particularly in patients with advanced CKD and those undergoing hemodialysis

(HD) [2]. While bone histomorphometry remains the gold standard for assessing bone turnover, its invasiveness, cost, and need for specialized expertise limit its routine use. Consequently, Kidney Disease: Improving Global Outcomes (KDIGO) guidelines recommend bone alkaline phosphatase (BALP) to evaluate bone turnover in CKD patients [1]. As a promising bone biomarker, serum BALP is supposed to be stable and unaffected by kidney function [3]. Other bone markers, such as tartrate-resistant acid phosphate 5b (TRACP5b) [4] and intact form of procollagen type I amino-terminal propeptide (iPINP) [5], are also considered independent of renal function, unlike β-cross-linked C-terminal telopeptide of type I collagen (β-CTX-I) [6]. However, their behavior during hemodialysis has not been thoroughly evaluated, despite its importance for improving clinicians' confidence in their use for monitoring CKD patients. This study aims to evaluate the impact of dialysis on bone markers levels.

2 | Methods

Remnant serum samples were collected from 43 patients (26 men and 17 women) undergoing hemodialysis at the Dialysis Department of the University Hospital of Liège. Among them, 18 were treated by conventional HD, and 25 underwent hemodiafiltration (HDF). Various dialysis membranes were employed during the sessions, including FX 10, FX CorDiax 80/100/800/1000, and VIE 21 A. Samples were collected before and after the dialysis session, centrifuged at 2630 *g* for 10 min, and stored at 4°C–8°C for a maximum of 24 h prior to routine analyses. Remnant samples were frozen at −80°C for up to 16 days.

Serum levels of BALP, TRACP5b, and iPINP were determined using the IDS iSYS platform (Immunodiagnostic Systems, Boldon, UK), whereas serum β-CTX-I levels were measured using the Elecsys β-CrossLaps assay (Roche Diagnostics, Basel, Switzerland). To account for hemoconcentration, all post-dialysis serum levels were adjusted using the factor $(1+(\Delta BW/0.2\times BW))$, where ΔBW represents the weight loss during dialysis and BW corresponds to the post-dialysis body weight [7].

To evaluate the difference in bone turnover markers (BTMs) levels after a dialysis session, nonparametric statistical tests (Wilcoxon and Mann–Whitney tests) were used due to the small sample size. Statistically significant changes in biomarker levels were then assessed against least significant change (LSC) thresholds, which quantifies the smallest change in a biomarker level that can be considered clinically significant. The LSC is calculated from analytical (based on daily quality controls) and biological [8] variation data for each analyzed marker. This approach is essential, as a statistically significant change is not necessarily clinically relevant and may not be directly attributable to the impact of dialysis.

3 | Results

Figure 1 illustrates the values of bone markers before and after dialysis, representing the overall trends in value changes following dialysis. The median values of all markers significantly decreased post-dialysis. BALP and TRACP5b showed slight reductions following both HD and HDF, whereas iPINP and β-CTX-I exhibited more pronounced declines.

Changes in bone markers values by dialysis and membrane type are summarized in Table 1. Results are presented as median percentage of variation with corresponding interquartile ranges, reflecting individual level changes post-dialysis. Decreases of BALP, TRACP5b, and iPINP were minor but statistically significant, whereas β-CTX-I decreases were more important. There was no difference between HD and HDF in changes observed for the bone markers, except for β-CTX-I. Membrane-specific analysis demonstrated that BALP and TRACP5b displayed limited variability. Collagen-derived biomarkers (iPINP and β-CTX-I) exhibited greater variability, with notable decreases using high-permeability membranes.

Median differences for BALP, TRACP5b, and iPINP remained below their LSC thresholds, indicating no clinically relevant changes. In contrast, β-CTX-I demonstrated a significant decrease that exceeded its LSC thresholds following HDF. These

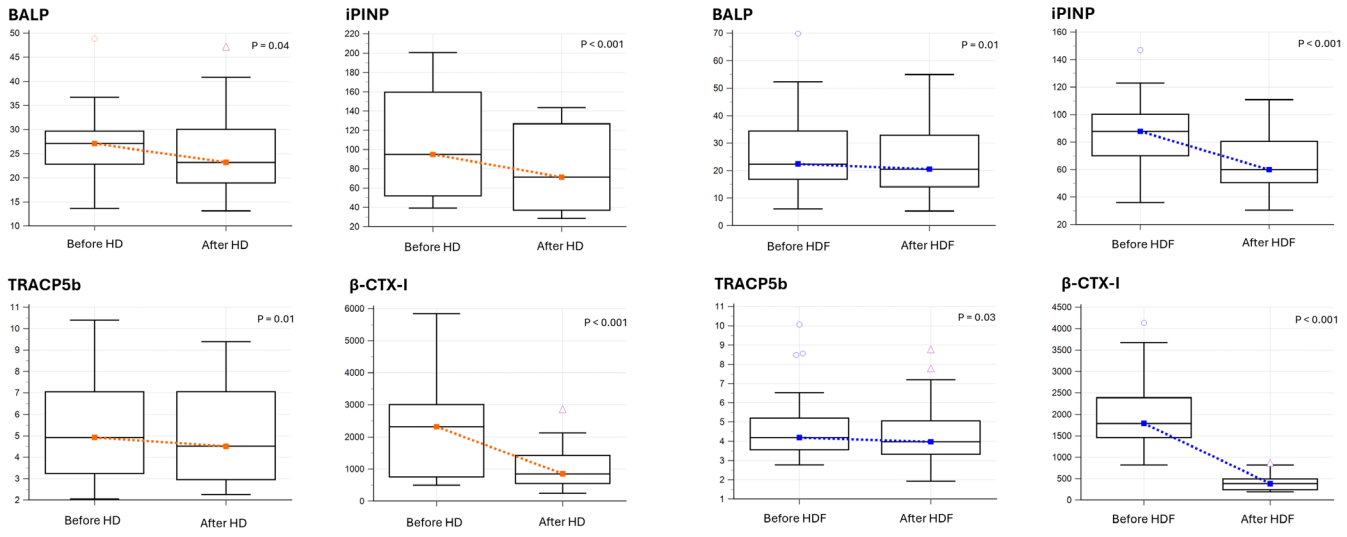

FIGURE 1 | Boxplots graphs of BALP, TRACP5b, iPINP, and β-CTX-I values before and after a single hemodialysis (HD) and hemodiafiltration (HDF) session. [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1 | Effect of the dialysis technique and dialysis membrane on bone biomarker values. Results are shown as median percentage of variation (IQR).

	Hemodialysis						Hemodiafiltration		
Membrane, *n*	**FX 10, *n* = 3**	**FX CorDiax 10, *n* = 4**	**FX CorDiax 80, *n* = 7**	**FX CorDiax 100, *n* = 2**	**VIE 21A, *n* = 2**	**Total, *n* = 18**	**FX CorDiax 800, *n* = 15**	**FX CorDiax 1000, *n* = 10**	**Total, *n* = 25**
BALP (115 kDa)	−3.45 (−11.17; 2.55)	−7.13 (−13.49; −2.68)	−14.55 (−16.56; −5.10)	8.83 (7.56; 10.11)	−7.43 (−9.82; −5.05)	−7.13[W] (−16.03; 0.43)	−6.48 (−14.38; 2.11)	−12.15 (−18.00; 0.16)	−6.57[W] (−15.59; 1.26)
TRACP5b (36 kDa)	−1.25 (−6.69; −2.19)	−14.06 (−20.52; −5.65)	−12.86 (−18.84; −4.64)	−1.25 (−6.32; 6.19)	−2.66 (−7.76; 2.44)	−10.16[W] (−15.54; 0.26)	−3.64 (−15.73; 5.3)	−5.81 (−24.74; −0.23)	−4.11[W] (−16.25; 2.57)
iPINP (35–70 kDa)	−18.44 (−18.76; −0.83)	−26.39 (−28.49; −19.87)	−26.38 (−30.84; −17.20)	−55.78 (−55.78; −55.78)	−15.22 (−16.42; −14.02)	−19.13[W] (−29.29; −16.99)	−20.94 (−27.90; −18.18)	−25.71 (−39.12; −21.84)	−24.40[W] (−30.90; −19.17)
β-CTX-I (3–8 kDa)	−6.45 (−7.49; 21.92)	−26.07 (−35.73; −13.41)	−66.42 (−69.89; −58.67)	−65.15 (−67.67; −62.62)	−50.45 (−66.10; −34.79)	−51.50[W, LSC] (−67.64; −17.61)	−82.94 (−84.01; −77.63)	−76.17 (−84.58; −67.45)	−81.41[W, LSC] (−84.23; −74.06)

variations can be attributed to differences in molecular weight, as β-CTX-I (3–8 kDa) is smaller than BALP (115 kDa), TRACP5b (36 kDa), and iPINP (35–70 kDa), which likely facilitates its diffusion across dialysis membranes.

4 | Conclusion

Bone markers, such as BALP, TRACP5b, iPINP, and β-CTX-I, are recommended to assess bone turnover in patients suffering from CKD. While some of these markers are considered stable and independent of renal function, their behavior during a dialysis session is less well understood. Investigating the post-dialysis fluctuations in their circulating levels was essential to enhance the reliability of clinical interpretations. Indeed, understanding these post-dialysis variations may facilitate the accurate interpretation of pre-dialysis levels during long-term patient monitoring and help to avoid masking an increase in BTM due to artificial decrease after dialysis.

Several limitations should be considered when interpreting the results. The small sample size limits the generalizability of the findings, and heterogeneity in dialysis parameters (e.g., duration and dialysate composition) may introduce variability. Patient-specific data, such as comorbidities and residual renal function, were not considered. Moreover, the risk of underestimation of hemoconcentration by the applied correction method, especially for the non-filtered parameters BALP and TRACP5b, must be accounted for [9].

These findings suggest that BALP, TRACP5b, and iPINP exhibit low variability across various dialysis conditions, with changes remaining below the LSC, which indicates negligible influences of dialysis. In contrast, β-CTX-I levels decreased significantly following HD and HDF, likely due to its smaller molecular size, which suggests a high influence of dialysis. These results are relevant for the clinical interpretation of BTMs, which play a central role in the clinical management of CKD-associated osteoporosis [10]. Accordingly, these results support the hypothesis that BALP, TRACP5b, and iPINP are robust bone markers in CKD-MBD patients, while the variability of β-CTX-I limits its reliability in this population.

Author Contributions

Etienne Cavalier: conceptualization, methodology, review and editing, supervision. **Alix Mackowiak:** methodology, data analysis/interpretation, writing. **Antoine Bouquegneau:** review and editing. **Bernard Dubois:** review and editing. **Elodie Grifnée:** review and editing. **Justine Demeuse:** review and editing. **Caroline Le Goff:** review and editing. **Mathias Loberg Haarhaus:** review and editing. **Per Magnusson:** review and editing. **Hanne Skou Jørgensen:** review and editing. **Pieter Evenepoel:** review and editing. **Pierre Delanaye:** review and editing.

Conflicts of Interest

The authors declare no conflicts of interest.

References

1. Kidney Disease: Improving Global Outcomes (KDIGO) CKD-MBD Work Group, "KDIGO Clinical Practice Guideline for the Diagnosis,

Evaluation, Prevention, and Treatment of Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD)," *Kidney International. Supplement* 76, no. 113 (2009): S1–S130, https://doi.org/10.1038/ki.2009.188.

2. D. Smout, H. S. Jørgensen, E. Cavalier, and P. Evenepoel, "Clinical Utility of Bone Turnover Markers in Patients With Chronic Kidney Disease," *Current Opinion in Nephrology and Hypertension* 31, no. 4 (2022): 332–338, https://doi.org/10.1097/MNH.0000000000000798.

3. A. Nizet, E. Cavalier, P. Stenvinkel, M. Haarhaus, and P. Magnusson, "Bone Alkaline Phosphatase: An Important Biomarker in Chronic Kidney Disease – Mineral and Bone Disorder," *Clinica Chimica Acta* 501 (2020): 198–206, https://doi.org/10.1016/j.cca.2019.11.012.

4. K. Shidara, M. Inaba, S. Okuno, et al., "Serum Levels of TRAP5b, a New Bone Resorption Marker Unaffected by Renal Dysfunction, as a Useful Marker of Cortical Bone Loss in Hemodialysis Patients," *Calcified Tissue International* 82, no. 4 (2008): 278–287, https://doi.org/10.1007/s00223-008-9127-4.

5. P. Delanaye, J. C. Souberbielle, M. H. Lafage-Proust, G. Jean, and E. Cavalier, "Can We Use Circulating Biomarkers to Monitor Bone Turnover in CKD Haemodialysis Patients? Hypotheses and Facts," *Nephrology, Dialysis, Transplantation* 29, no. 5 (2014): 997–1004, https://doi.org/10.1093/ndt/gft275.

6. M. G. Vervloet and V. M. Brandenburg, "Circulating Markers of Bone Turnover," *Journal of Nephrology* 30, no. 5 (2017): 663–670, https://doi.org/10.1007/s40620-017-0408-8.

7. E. Cavalier, P. U. Torres, B. E. Dubois, et al., "Impact of the Type of Dialysis Membranes on the Circulating Concentration of Markers of Vitamin D Metabolism," *International Journal of Artificial Organs* 40, no. 2 (2017): 43–47, https://doi.org/10.5301/ijao.5000565.

8. EFLM Biological Variation, accessed February 4, 2025, https://biologicalvariation.eu/.

9. M. Gomez, M. Arias-Guillén, and F. Maduell, "An Extended Kinetic Model-Based Correction Factor Equation to Account Hemodialysis Post-Treatment Hemoconcentration," *International Journal of Artificial Organs* 46, no. 5 (2023): 314–317, https://doi.org/10.1177/03913988231160803.

10. M. Ketteler, G. A. Block, P. Evenepoel, et al., "Executive Summary of the 2017 KDIGO Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD) Guideline Update: What's Changed and Why It Matters," *Kidney International* 92, no. 1 (2017): 26–36, https://doi.org/10.1016/j.kint.2017.04.006.