Suitability of $\alpha_1$-microglobulin reduction rate as a biomarker of removal efficiency of online hemodiafiltration: a retrospective cohort study

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Abstract

Background: Online hemodiafiltration (OL-HDF), whether in pre-dilution OL-HDF (pre-HDF) or post-dilution OL-HDF (post-HDF), is conducted to efficiently remove low molecular weight proteins from the blood of patients requiring dialysis. $\beta_2$-microglobulin ($\beta_2$-MG) and $\alpha_1$-microglobulin ($\alpha_1$-MG) are used as biomarkers to evaluate removal efficiency of OL-HDF.

We aimed to evaluate the relationship between $\beta_2$-MG and $\alpha_1$-MG reduction rates and the amount of albumin leakage. Furthermore, we statistically analyzed the relationship between the $\alpha_1$-MG reduction rate and $\alpha_1$-MG removal amount, and its suitability as a biomarker for evaluating the removal efficiency of OL-HDF.

Methods: We collected the results of regularly conducted routine evaluations to assess the efficiency of OL-HDF from cases of patients undergoing maintenance dialysis at our clinic from 2018 to 2019. Data on was collected on both pre-HDF and post-HDF sessions. $\beta_2$-MG and $\alpha_1$-MG reduction rates were analyzed. Regression analysis on reduction rates showed a significant correlation between the $\alpha_1$-MG reduction rate and the $\alpha_1$-MG removal amount.

Results: We conducted 435 tests on OL-HDF efficiency in 87 cases undergoing maintenance dialysis at our clinic in 2018 and 2019. There were 80.7 ± 4.5% for the $\beta_2$-MG reduction rate, 33.8 ± 9.4% for the $\alpha_1$-MG reduction rate, and 3.9 ± 1.8 g/s for the amount of albumin leakage. There was no correlation between the $\beta_2$-MG reduction rate and the $\alpha_1$-MG reduction rate, or between the amount of albumin leakage and $\beta_2$-MG reduction rate.

Conclusion: $\alpha_1$-MG reduction rate was found to correlate with its removal amount, demonstrating its suitability as a biomarker for evaluating the removal efficiency of OL-HDF.

Trial registration: Retrospectively registered.

Keywords: $\alpha_1$-microglobulin, Reduction rate of $\alpha_1$-microglobulin, Online hemodiafiltration, Biomarker for evaluation of removal efficiency

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Background

Online hemodiafiltration (OL-HDF), whether in pre-dilution OL-HDF (pre-HDF) or post-dilution OL-HDF (post-HDF), is conducted to efficiently remove low molecular weight proteins (LMWP) (molecular weight [MW]: 10,000–55,000), particularly larger LMWPs (MW > 30,000), from blood of dialysis patients. This is accomplished by increasing the convection volume from high-volume replacement fluid. Biomarkers are important for evaluating the efficiency of OL-HDF. The small molecular weight replacement fluid. Biomarkers are important for evaluating the efficiency of OL-HDF. The small molecule size of β₂-microglobulin (β₂-MG) (MW: 11,800, Stokes radius: 16Å) allows for removal dependent on both diffusion and convection. Therefore, β₂-MG cannot be used as a biomarker to accurately assess OL-HDF removal efficiency.

α₁-Microglobulin (α₁-MG) (MW: 33,000, Stokes radius: 28.4Å) is mainly removed by convection in dialysis therapy with a more suitable molecular size; hence, it has often been used as a biomarker to assess the efficiency of removal of middle- and large-MW solutes in Japan for over 30 years. Similarly, we reported a strong correlation between changes in the clinical symptoms of dialysis patients and changes in the α₁-MG reduction rate [1, 2]. We also reported that OL-HDF should aim for a β₂-MG reduction rate of 80% and an α₁-MG reduction rate of over 35% when performed to treat complications in long-term dialysis patients [3, 4].

There is a twofold difference in molecular weight between albumin (alb) (MW: 66,000, Stokes radius: 35.5Å) and α₁-MG, with a difference in Stokes radius of 20% [5]. Thus, it is impossible to separate α₁-MG and alb based on differences in molecular size alone and remove them using current dialysis membranes. Some degree of alb leakage is inevitable when removing α₁-MG at high efficiency. There is no report examining the appropriateness of the reduction rate of the α₁-MG reduction rate as a biomarker using statistical analysis. In this study, we evaluated the relationship between β₂-MG and α₁-MG reduction rates and the amount of alb leakage, as well as the relationship between the reduction rates of β₂-MG and α₁-MG based on the results of OL-HDF removal efficiency tests regularly conducted as routine examinations in 2018 and 2019. Furthermore, we statistically analyzed the relationship between the α₁-MG reduction rate and the α₁-MG removal amount, and we investigated whether this was a suitable biomarker for evaluating the removal efficiency of OL-HDF.

Methods

Four hundred thirty-five tests for evaluating OL-HDF efficiency in 87 patients undergoing maintenance dialysis at our clinic between January 2018 and December 2019 (male/female: 60/27, age: 60.5 ± 12.0 years, dry weight: 60.5 ± 13.0 kg, dialysis vintage: 146.0 ± 130.9 months).

Pre- and post-HDF comprised 304 and 131 sessions, respectively. Blood flow rate (Qb) was 261.5 ± 28.5 mL/min, total dialysate flow rate (Qd total) was 400 mL/min for 22 cases and 500 mL/min for 413 cases, and replacement fluid volume was 48.7 ± 8.2 L/session(s) for pre-HDF and 14.2 ± 3.1 L/s for post-HDF. Treatment time was 4.1 ± 0.2 h (Table 1). Different types of hemodialyzers commercially available in Japan were used. The obtained test values were used to investigate the relationship between β₂-MG and α₁-MG reduction rates and the amount of alb leakage, as well as the relationship between the β₂-MG reduction rate and α₁-MG reduction rate.

Thereafter, the relationship between the α₁-MG reduction rate and its removal amount was analyzed, and whether the reduction rate was suitable for evaluating the removal efficiency was investigated. This was done by setting a 95% prediction interval of the regression curve created from the α₁-MG removal amount (horizontal axis, mg) and reduction rate (vertical axis, %), and extracting test values that were outliers from this interval from the regression analysis. Values with reduction rates that were lower than the lower limit or higher than the upper limit of the calculated 95% prediction interval were defined as outlier test values. A histogram and Q-Q plot was used to assess if a set of data have a normal distribution. Continuous variables were expressed as the mean ± standard deviation (SD) and categorical variables as frequencies. A P value of < 0.05 was considered statistically significant, and all P values were two-sided. All statistical analyses were performed using SPSS statistics ver. 23.0 (IBM Japan, Ltd., Tokyo, Japan) software.

Post-dialysis values used to calculate the β₂-MG and α₁-MG reduction rates were values that were corrected by the hematocrit level to exclude concentration effects. The waste dialysate was partially stored throughout the

| Table 1 | Patients background and treatment mode |
|----------|--------------------------------------|
| Total patient count | 87 |
| Age (years) | 60.5 ± 12.0 |
| Dialysis vintage (months) | 146 ± 130.9 |
| Sex, M/F | 60/27 |
| Body weight (kg) | 58.9 ± 13.0 |
| Total number of evaluations | 435 |
| Qb (mL/min) | 261.5 ± 28.5 |
| Qd total (mL/min) | 400: 22 cases 500: 413 cases |
| Treatment time (h) | 4.1 ± 0.2 |
| Replacement fluid (L/session) | Pre: 48.7 ± 8.2  Post: 14.2 ± 3.1 |
| Membrane area (m²) | 2.1 ± 0.1 |

Hemodialyzers Various kinds, 10 types
dialysis session at a rate of 2 L/h, after which the total amount stored was well-mixed, and part of this was used to measure the β2-MG and α1-MG amounts removed and the amount of alb leakage.

This study has been approved by our institutional committee on human and/or animal research. All patients provided informed written consent.

Results

We conducted 435 tests on OL-HDF efficiency in 87 cases undergoing maintenance dialysis at our clinic in 2018 and 2019 (male/female: 60/27, age: 60.5 ± 12.0 years, dry weight: 60.5 ± 13.0 kg, dialysis vintage: 146.0 ± 130.9 months). Pre-HDF comprised 304 sessions, and post-HDF comprised 131 sessions. Qb was 261.5 ± 28.5 mL/min, Qd total was 400 mL/min for 22 cases and 500 mL/min for 413 cases, and replacement fluid volume was 48.7 ± 8.2 L/session(s) for pre-HDF and 14.2 ± 3.1 L/s for post-HDF. Treatment time was 4.1 ± 0.2 h/s (Table 1). Ten types of hemodiafilters commercially available in Japan were used (Table 2). The membrane surface area was 2.1 ± 0.1 m².

The results of all 435 tests (mean ± SD) were 80.7 ± 4.5% for the β2-MG reduction rate, 33.8 ± 9.4% for the α1-MG reduction rate, and 3.9 ± 1.8 g/s for the amount of alb leakage. There were six cases in ten sessions

| Membrane material | Manufacturer       | SC  | β2-MG | Albumin |
|-------------------|-------------------|-----|-------|---------|
| GDF               | PEPA Innkiso      | 0.87| 0.03  |
| GDF-M             | PEPA Innkiso      | 0.88| 0.01  |
| MFX-U eco         | PES Nipro         | 0.91| 0.01  |
| FIX-S eco         | ATA               | 0.93| 0.01  |
| FIX-U eco         | ATA               | 1.05| 0.02  |
| NVF-P             | PS Toray Medical  | 0.9 | 0.013 |
| NVF-H             | PS AsahiKASEI Medical | 0.9 | 0.009 |
| ABH-PA            | PS AsahiKASEI Medical | 0.78 | ≤ 0.01 |
| Polyflux-H        | PEAS GAMBRO       | 0.82| 0.0022|
| FX-HDF            | PS Fresenius Medical Care | 0.8 | 0.004 |

From each company’s catalog

![Graph showing the relationship between reduction rate and amount of albumin leakage.](image)
where the $\alpha_1$-MG reduction rate was >50%, and the maximum $\alpha_1$-MG reduction rate value was 60.0%. No significant correlation was observed between the amount of alb leakage and $\beta_2$-MG reduction rate (Fig. 1). There was a slightly strong significant correlation between the amount of alb leakage and $\alpha_1$-MG removal were almost identical (Fig. 1). There was no correlation between the $\beta_2$-MG reduction rate and the $\alpha_1$-MG reduction rate (Fig. 2).

Results of the regression analysis on reduction rates showed a significant correlation between the $\alpha_1$-MG reduction rate and the $\alpha_1$-MG removal amount (Table 3). Additionally, 17 out of the 435 total sessions (3.9%) were outlier values from the 95% prediction interval of the regression curve (Fig. 3). It was shown from these analyses that it was possible to appropriately evaluate the removal efficiency of OL-HDF by investigating the reduction rate without determining the removal amount.

**Discussion**

In this study, we evaluated the relationship between $\beta_2$-MG, $\alpha_1$-MG reduction rates, and amount of alb leakage, as well as the relationship between the reduction rates of $\beta_2$-MG and $\alpha_1$-MG. There was no correlation between the $\beta_2$-MG reduction rate and the $\alpha_1$-MG reduction rate, nor between the amount of alb leakage and $\beta_2$-MG reduction rate. We found that alb leakage was inevitable when removing $\alpha_1$-MG at high efficiency. Further, we revealed that there was a significant correlation between the $\alpha_1$-MG reduction rate and the $\alpha_1$-MG removal amount. Our study showed that $\alpha_1$-MG reduction rate was a suitable biomarker for evaluating the efficiency of removal of OL-HDF.

In Japan, $\alpha_1$-MG is widely used as a biomarker for evaluating the performance of removal efficiency of OL-HDF for several reasons: (1) optimal molecular size, (2) low likelihood of measurement errors due to its non-negligible blood concentration, (3) low likelihood of concentration changes under physiological conditions, (4) stable synthesis rate, (5) accumulation in renal failure, and (6) convection-based removal. $\alpha_1$-MG is primarily synthesized in the liver. Under physiological conditions, 50% of it is present in the blood in free form, while 50% is bound with dimeric immunoglobulin A (IgA) [6]. It is estimated that free $\alpha_1$-MG increases to around 70% because its excretion from the kidneys is reduced during renal dysfunction, but there are no detailed reports on these dynamics. $\alpha_1$-MG has strong antioxidant activity and has been reported as a protective molecule by scavenging free radicals, binding to heme, and undergoing reduction reactions when exposed to oxidative stresses.

[Fig. 2](#) Relationship between reduction rate of $\beta_2$-MG and $\alpha_1$-MG. Since there is no correlation between $\beta_2$-MG and $\alpha_1$-MG reduction rates, the advantages of OL-HDF cannot be evaluated with $\beta_2$-MG reduction rate alone. As efficient removal of larger LMWPs is an objective of OL-HDF, efficiency tests that use $\alpha_1$-MG as a biomarker are necessary.
The antioxidant activity of α1-MG in dialysis patients may be a topic of future studies.

The MW of IgA is 160,000; hence, the combined MW of the resulting α1-MG-IgA complex is 350,000, which is impossible to remove using dialysis. Since free α1-MG is the target in dialysis therapy and total α1-MG is usually measured, the reduction rate is affected by the binding affinity of α1-MG and IgA. Consequently, the α1-MG reduction rate calculated with the pre- and post-dialysis values do not appropriately indicate the removal efficiency of OL-HDF.

Therefore, we investigated whether the α1-MG reduction rate and the α1-MG removal amount obtained from the 435 OL-HDF sessions were significantly correlated. Results showed that there was a significant correlation between the two measures. However, there were 17 test values outside the 95% prediction interval of the regression curve. Specifically, outliers were observed below the lower limit in seven patients across seven sessions, whereas outliers were observed over the upper limit in five patients across ten sessions. Test conditions when outlier values were obtained may be a topic of future studies.

Measuring the amount of a given solute removed during dialysis is complicated due to the need for the installation of a device that is capable of continuously collecting waste fluid from a drainage line of the patient monitoring system. However, the reduction rate can be calculated simply by collecting blood before and after dialysis and calculating the reduction rate using the following equation:

\[
RR = \frac{1 - \frac{(1 - Ht_{post})C_{post}}{(1 - Ht_{pre})C_{pre}}}{100}\%
\]

Where Ht is hematocrit and C is concentration. This enables the evaluation of removal efficiency of middle- and large-MW solutes.

It is essential in the evaluation of high-efficiency OL-HDF to investigate the removal efficiency of solutes with

### Table 3

Results of regression analysis on reduction rates. To evaluate the association between removal amount and reduction rate, and to identify cases that fell outside of the association, we performed a regression analysis with reduction rate as the dependent variable and removal amount as the independent variable. The removal amount was log-transformed.

| Parameter                  | Regression coefficient (β) | S.E.   | t      | p       |
|----------------------------|----------------------------|--------|--------|---------|
| (Constant)                 | -42.467                    | 3.490  | -12.17 | < 0.0001|
| Ln (reduction rate)        | 15.638                     | 0.713  | 21.93  | < 0.0001|

**Fig. 3** Relationship between Reduction rate and removal amount of α1-MG. From the results of the regression analysis, a logarithmic approximation curve including the 95% prediction interval is created with the removal amount as the horizontal axis and the reduction rate as the vertical axis. Values with reduction rates that fell outside the 95% prediction interval are defined as outlier cases. There are 17 outliers (3.9%) in this study.
a MW of 30,000–50,000 using α1-MG as a biomarker. Our study supported the use of α1-MG reduction rate to be an appropriate evaluation method indicating that past accumulated data can be applied to future studies.

Several reports investigated the effects of OL-HDF on a patient’s survival [12–14]. Kikuchi et al. [15] reported that pre-HDF with high-replacement fluid volume had a more favorable effect on patient’s survival than hemodialysis and pre-HDF with low-replacement fluid volume. However, both reports only investigated the impacts of replacement fluid volume on a patient’s survival, and neither mentioned specific numerical values for removal efficiency. In Europe, α1-MG has been used for over five years as a biomarker for removal performance when evaluating OL-HDF or high-performance dialyzers, and the evaluation of dialysis efficiency using α1-MG reduction rate has become increasingly common [16, 17]. The groundbreaking JAMREDS study, which was started in Japan this spring, examined the effect of not only replacement fluid volume but also α1-MG reduction rate on patient survival of OL-HDF. Hence, the usefulness of removing middle- and large-MW toxins is anticipated to become even more apparent in the future.

The limitation of this study was that it was performed in a single facility, which can lead to selection bias.

Conclusions
We found that α1 reduction rate may be used as a valid biomarker to evaluate the removal efficiency of OL-HDF. Furthermore, some albumin leakage is inevitable when removing α1-MG at high efficiency.

Abbreviations
alpha: Albumin; HD: Hemodialysis; IgA: Immunoglobulin A; LMWP: Low molecular weight protein; MW: Molecular weight; OL-HDF: Online hemodiafiltration; pre-HDF: Pre-dilution online hemodiafiltration; post-HDF: Post-dilution online hemodiafiltration; SD: Standard deviation; Qb: Blood flow rate; Qd total: Total dialysate flow rate; α1-MG: α1-microglobulin; β2-MG: β2-microglobulin

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Authors’ contributions
KS designed the study and drafted the manuscript. HH and YK acquired and analyzed the data. TS designed the study. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets during and/or analyzed during the current study available from the corresponding author on reasonable request.

Ethics approval and consent to participate
This study has been approved by our institutional committee on human and/or animal research. All patients provided informed written consent.

Consent for publication
Not applicable

Competing interests
The authors declare that they have no competing interest

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References
1. Sakurai K. Biomarkers for evaluation of clinical outcomes of hemodiafiltration. Blood Purif. 2013;35(suppl 1):64–8.
2. Sakurai K, Saito T, Hosoya H, et al. Therapeutic effect of high-efficiency online hemodiafiltration for recurrent restless legs syndrome in dialysis patients. J Artif Organs. 2020;23:296–301.
3. Yamashita CA, Sakurai K. Clinical effect of pre-dilution hemodiafiltration based on the permeation of the hemofilter. Contrib Nephrol. 2015;185:1–7.
4. Masakane I, Sakurai K. Current approaches to middle molecule removal: room for innovation. Nephrol Dial Transplant. 2018;33(suppl_3):ii12–21.
5. Sakurai K, Saito T, Yamauchi F, et al. Alpha-1-microglobulin is an important marker and some albumin leakage increases therapeutic effects in hemodiafiltration therapy. Nephrol Dial Transplant. 2018;33(suppl_1):i185 https://doi.org/10.1093/ndt/gfy104.
6. Berggård T, Thelin N, Falkenberg C, et al. Prothrombin, albumin and immunoglobulin A form covalent complexes with alpha1-microglobulin in human plasma. Eur J Biochem. 1997;253(3):576–83.
7. Althorn M, Berggaard T, et al. Processing of the lipocalin α1-microglobulin by hemoglobin induces hemo-binding and heme-degradation properties. Blood. 2002;99(6):1994–901.
8. Meining W, Berggaard T, et al. The crystal structure of human α1-microglobulin reveals a potential haem-binding site. Biochem J. 2012;445:175–82.
9. Olsson MG, Gram M, et al. Pathological conditions involving extracellular hemoglobin: molecular mechanisms, clinical significance, and novel therapeutic opportunities for α1-microglobulin. Antioxid Redox Signal. 2012; 17:813–45.
10. Åkerström B, Gram M, et al. A1M, an extravascular tissue cleaning and housekeeping protein. Free Radic Biol Med. 2014;74:274–82.
11. Kristiansson A, Bergwick J, et al. Human radical scavenger α1-microglobulin protects against hemolysis in vitro and α1-microglobulin knockout mice exhibit a macrocytic anemia phenotype. Free Radic Biol Med. 2020; In press.
12. Canaud B, Bragg-Gresham JL, Marshall MR, et al. Mortality risk for patients receiving hemodiafiltration versus hemodiafiltration: European results from DOPPS. Kidney Int. 2006;69:2087–93.
13. Grooteman MP, van den Dorpel MA, Bots ML, et al. Effect of online hemodiafiltration on all-cause mortality and cardiovascular outcome. J Am Soc Nephrol. 2012;23:1087–96.
14. Maduell F, Moreso F, Pons M, et al. ESOHOL Study Group: High-efficiency postdilution online hemodiafiltration reduces all-cause mortality in hemodialysis patients. J Am Soc Nephrol. 2013;24:487–97.
15. Kikuchi K, Hamano T, Wada A, et al. Pre-dilution online hemodiafiltration is associated with improved survival compared with hemodialysis. Kidney Int. 2019;95:929–38.
16. Maduell F, Broseta JJ, et al. Comparison of solute removal properties between high-efficiency dialysis modalities in low blood flow rate. Apher Dial. 2020;24(1):387–92.
17. Maduell F, Rodas L, et al. Evaluation of the influence of the surface membrane and blood flow in medium «cut-off» (MCO) dialyzers. Nefrologia. 2019;36(5):623–8.