Renal Replacement Therapy

REVIEW

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# Improved survival on super high-flux albumin-leaking hemodialysis and online hemodiafiltration with high albumin leakage in patients with mild hypoalbuminemia: evidence and a hypothesis

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## Abstract

It has been reported that survival on mild hypoalbuminemia due to high albumin leakage did not worsen in patients on hemodialysis (HD) or online hemodiafiltration (OHDF) even though the level of serum albumin is a classic nutrition marker associated with mortality. Survival was also equivalent on HD and OHDF for patients with similar levels of albumin leakage and serum albumin. Furthermore, survival on HD using a super high-flux (SHF) albumin-leaking membrane was better than that on HD using a SHF membrane, and survival on SHF albumin-leaking HD with high albumin leakage was better than that on OHDF with low albumin leakage. The following hypothesis regarding cross-talk between $\alpha_1$-microglobulin ($\alpha_1$MG) and albumin is proposed that can explain the mechanism by which the level of serum human mercaptoalbumin (HMA) increases postdialysis and decreases predialysis. At initiation of and during dialysis, the production of free $\alpha_1$MG in the liver increases by upregulation of the $\alpha_1$MG-bikunin precursor gene. The free $\alpha_1$MG rapidly reacts with some substances that are reversibly bound to human nonmercaptoalbumin (HNA)-1, resulting in the conversion to HMA and free $\alpha_1$MG with reduced activity (i.e., free $\alpha_1$MG with reduced or no antioxidant capacity) during dialysis and in the increased serum HMA level postdialysis. In addition, it is possible that both hypoalbuminemia and the conversion of HNA-1 to HMA increase the free form of indoxyl sulfate, which is removed by diffusion. The antioxidant capacity in serum after dialysis is mainly due to the very large amount of HMA, resulting in the conversion to HNA and the decreased serum HMA level before dialysis. However, the very small amount of free $\alpha_1$MG produced in the liver has strong antioxidant activity after dialysis.

**Keywords** Hypoalbuminuria, $\alpha_1$-Microglobulin, Super high-flux, Albumin leaking, Online hemodiafiltration

## Introduction

With the development and refinement of dialyzers and hemodiafilters, hemodialysis (HD) and online hemodiafiltration (OHDF) can now be used to remove large amounts of uremic toxins, thereby improving patients' clinical symptoms and survival. The ability to remove uremic toxins with larger molecules depends primarily on the pore size of the membrane used and convection volume. Accordingly, OHDF has been developed mainly

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for removal of a wide range of medium- to large-middle molecules that are not sufficiently removed by low-flux or high-flux HD.

Although high-volume postdilution OHDF is the norm in Europe, survival is similar between Japanese-style predilution OHDF and postdilution OHDF [1]. In Europe, high-volume postdilution OHDF using low-permeability membranes has been performed with albumin leakage not exceeding 3.4 g/session [2] or 5 g/session with a convection volume of 23 L/session/1.73 $m^2$ [3], suggesting that the performance of hemodiafilters is of little concern. In contrast, in Japan, either moderate- to high-volume predilution OHDF using low- to high-permeability membranes or low- to moderate-volume postdilution OHDF with low- to high-permeability membranes is performed, and albumin leakage is set to no more than 5 g/session in many facilities [4]. Since the acceptable serum albumin concentration varies depending on the facility, some facilities have used low-permeability membranes in patients with hypoalbuminemia and also in those with normoalbuminemia.

## Functional classification for blood purification equipment developed by the Japanese Society for Dialysis Therapy in 2013

In 2004, dialyzers were classified according to $\beta_2$-microglobulin ($\beta_2$MG) clearance under set conditions of a membrane surface area of 1.5 $m^2$, a blood flow rate of 200 mL/min, a dialysate flow rate of 500 mL/min, and a filtration flow rate of 15 mL/min in vitro into five functional types: type I, < 10 mL/min; type II, ≥ 10 and < 30 mL/min; type III, ≥ 30 and < 50 mL/min, type IV, ≥ 50 and < 70 mL/min; and type V, ≥ 70 mL/min. Type I was defined as a low-flux membrane, types II and III as high-flux membranes, and types IV and V as super high-flux (SHF) membranes [5]. In 2013, the Japanese Society for Dialysis Therapy (JSDT) extended these five types of dialyzer to include a further four types determined by $\beta_2$MG clearance and the sieving coefficient (SC) for albumin (type-Ia, < 70 mL/min and < 0.03; type-Ib, < 70 mL/min and ≥ 0.03; type-IIa, ≥ 70 mL/min and < 0.03; and type-IIb, ≥ 70 mL/min and ≥ 0.03) and a type S (a dialyzer membrane with special features, e.g., made from ethylene vinyl alcohol or polymethylmethacrylate) as presented in Table 1 [6]. Hemodiafilters were defined by a $\beta_2$MG clearance of ≥ 70 mL/min under set conditions of a membrane surface area of 2.0 $m^2$, a blood flow rate of 250 mL/min, a dialysate flow rate of 600 mL/min in predilution OHDF and 500 mL/min in postdilution OHDF, and a substitution volume rate of 240 mL/min in predilution OHDF and 60 mL/min in postdilution OHDF in vitro.

In the functional classification of blood purification equipment developed by the JSDT in 2023, English notation was added for four dialyzer types: type I-a, standard flux; type I-b, high-flux albumin-leaking; type II-a, SHF; and type II-b, SHF albumin-leaking (Table 1). It should be noted that prior to this, the SHF membrane referred to a membrane with $\beta_2$MG clearance of ≥ 50 mL/min.

**Table 1** Functional classification of dialyzers developed by the Japanese Society for Dialysis Therapy (JSDT) in 2013 [6]

| $\beta_2$-Microglobulin clearance (mL/min)[b] | Sieving coefficient for albumin[a] | | Type S |
|---|---|---|---|
| | < 0.03 | ≥ 0.03 | |
| ≥ 70 | Type II-a<br>Super high-flux[c] | Type II-b<br>Super high-flux<br>Albumin-leaking[c] | |
| < 70 | Type I-a<br>Standard flux[c] | Type I-b<br>High -flux<br>Albumin-leaking[c] | |

[a] In vitro using bovine saline test solution with a membrane surface area of 1.5 $m^2$, a blood flow rate of 200 ± 4 mL/min, and a filtration flow rate of 15 ± 1 mL/min for measurement of the albumin concentration of the filtrate using the bromocresol green method

[b] In vitro using bovine saline test solution with a membrane surface area of 1.5 $m^2$, a blood flow rate of 200 ± 4 mL/min, a dialysate flow rate of 500 ± 15 mL/min, and a filtration flow rate of 15 ± 1 mL/min

[c] English notation by the JSDT in 2023

## Survival on dialyzers and hemodiafilters according to $\beta_2$MG clearance and albumin leakage

Abe et al. analyzed the JSDT Renal Data Registry (JRDR) data collected between 2009 and 2011 and reported that mortality was significantly lower with the use of dialyzer membranes that had a $\beta_2$MG clearance of ≥ 50 mL/min but < 70 mL/min compared with a $\beta_2$MG clearance of < 10 mL/min, and that mortality was also significantly lower when $\beta_2$MG clearance was ≥ 70 mL/min compared with ≥ 50 but < 70 mL/min [5]. Furthermore, our group has demonstrated that survival is better on SHF albumin-leaking HD than on SHF HD and that survival is better on SHF HD and SHF albumin-leaking HD with albumin leakage ≥ 3 g/session compared with albumin leakage < 3 g/session [6]. In addition, survival was reported to be better on OHDF in patients with high albumin leakage than in those with low albumin leakage [7].

## Survival and serum albumin level

Albumin is a classical nutrition marker, and a serum albumin level < 4.0 g/dL is the parameter most strongly associated with mortality as a result of protein-energy wasting (PEW) in HD patients [8]. The Membrane Permeability Outcome study showed that survival was better in patients with a serum albumin level ≤ 4.0 g/dL on high-flux HD (SC for albumin < 0.01) than in those on low-flux HD (SC for albumin = 0) [9], suggesting that survival is

influenced by the degree of flux, albumin leakage, and serum albumin level. In dialyzed patients without inflammation, mortality after adjustment for confounding factors was not significantly different between those with hypoalbuminemia and those with normoalbuminemia [10]. Moreover, survival in patients with a combination of hypoalbuminemia, inflammation, and PEW was significantly worse than in those with any one of these factors alone [11]. Therefore, it has been suggested that mild hypoalbuminemia due to high albumin leakage is not an independent predictor of mortality in the absence of PEW or inflammation.

Serum albumin is the most important extracellular antioxidant in humans. It comprises a mixture of human mercaptoalbumin (HMA), the reduced form of albumin in which a free cysteine residue at position 34 from the N-terminus (Cys-34) has a thiol group, and human non-mercaptoalbumin (HNA), the oxidized form of albumin. Most of the Cys-34 in HNA-1 is reversibly reacted with cysteine, while the Cys-34 in HNA-2 is irreversibly oxidized to sulfinic acid or sulfonic acid. Terawaki et al. reported that the serum HMA level was higher post-HD than pre-HD (2.76 ± 0.43 versus 1.74 ± 4.10 g/dL, respectively) and that serum HNA-1 and HNA-2 levels were lower post-HD than pre-HD (1.18 ± 0.28 versus 1.96 ± 0.40 g/dL and 0.16 ± 0.05 versus 0.20 ± 0.07 g/dL, respectively) in patients without cardiovascular disease. The levels of serum albumin pre- and post-HD were 3.90 ± 0.32 g/dL and 4.08 ± 0.38 g/dL, suggesting that the increase in HMA concentration post-HD was due not only to blood concentration by ultrafiltration but also to production of HMA [12]. These findings suggest that reversibly reacted HNA-1 is converted to HMA during an HD session and that HMA, HNA-1, and irreversibly reacted HNA-2 are eliminated in the dialysate by albumin leakage. Furthermore, the respective adjusted odds ratios were found to be 2.5 and 25.6 for cardiovascular mortality in patients with a pre-HD HMA fraction of < 40% versus ≥ 40% and a post-HD HMA fraction of < 60% versus ≥ 60% [12]. In that study, albumin leakage was suggested to be below 3 g/session because of normoalbuminemia. Nagai et al. reported that survival was significantly improved by high albumin leakage ≥ 3.0 g/session, more than by low albumin leakage < 3.0 g/session, in HD patients using dialyzers with $\beta_2$MG clearance ≥ 10 mL/min [13]. They also reported that, when compared with low albumin leakage (1.0 g/session), high albumin leakage (9.1 g/session) significantly increased the HMA fraction (53.2 ± 4.9 versus 61.7 ± 5.1%, $P < 0.01$) and induced pre-HD hypoalbuminemia (3.8 ± 0.3 versus 3.4 ± 0.2 g/dL, $P < 0.01$) after 6 months without increasing the pre-HD serum HMA level [2.0 ± 0.3 versus 2.1 ± 0.2 g/dL, not significant (n.s.)] and while reducing the pre-HD serum HNA level (1.8 ± 0.2 versus 1.3 ± 0.2 g/dL, $P < 0.01$) [14]. These findings indicate that hypoalbuminemia resulting from high albumin leakage leads to production of HMA in the liver, which is albumin with normal antioxidant activity. Therefore, the acceptable level of hypoalbuminemia due to high albumin leakage needs to be determined in HD patients. Because cardiovascular events were significantly associated with serum HMA concentration but not with the HMA fraction in patients on peritoneal dialysis [15], high HMA concentration, and not the fraction, may be related to survival.

## Relationships among serum albumin level, albumin leakage, substitution volume, and survival in patients on HD and OHDF

In our facilities, hypoalbuminemia is tolerated up to approximately 3.0 g/dL as measured by a photometric method with bromocresol green, except in patients with obvious PEW or inflammation, considering that symptoms such as pruritus, restless legs syndrome, and fatigue can usually be improved by high albumin leakage. A study by our group found that high albumin leakage with hypoalbuminemia does not worsen survival in patients on HD with dialyzer membranes that had a $\beta_2$MG clearance of ≥ 50 mL/min or in patients on OHDF [7]. Table 2 shows the mean estimated albumin leakage and the serum albumin level divided by median values on HD and OHDF grouping. The mean serum albumin level in the high albumin leakage and high serum albumin concentration group was 3.5 ± 0.1 g/dL on HD and 3.6 ± 0.2 g/dL on OHDF and that in the high albumin leakage and low serum albumin concentration group was 3.2 ± 0.2 g/dL on HD and 3.2 ± 0.1 g/dL on OHDF. This suggests that mild hypoalbuminemia is acceptable, though careful observation is necessary. Significant differences in survival were observed between the high albumin leakage and high serum albumin concentration group and the low albumin leakage group and low serum albumin concentration on HD. We also found equivalent survival between HD and OHDF for patients with similar levels of albumin leakage and serum albumin. In addition, survival on predilution OHDF was influenced by albumin leakage but not by the substitution volume [7].

In 2022, the method used to measure serum albumin concentration at our facility was changed from bromocresol green to bromocresol purple, taking into account that a serum albumin level measured as ≤ 3.5 g/dL by bromocresol green would be reduced by 0.3 g/dL when measured by bromocresol purple. A nationwide survey is now needed to determine the effect on survival of accepting a lower serum albumin level resulting from high albumin leakage as measured using the bromocresol purple

**Table 2** Mean albumin leakage and serum albumin concentration (s-Alb) divided by median values on hemodialysis and online hemodiafiltration grouping by albumin leakage and s-Alb [7]

| Item | Group A versus Group B | | | Group A versus Group C | | | Group A versus Group D | | |
|---|---|---|---|---|---|---|---|---|---|
| | Group A | Group B | *P*-value | Group A | Group C | *P*-value | Group A | Group D | *P*-value |
| *Hemodialysis* | | | | | | | | | |
| Albumin leakage, g/session | 2.6 ± 1.2 | 2.7 ± 1.4 | *P* = 0.648 | 2.2 ± 1.1 | 1.1 ± 0.4 | *P* < 0.001 | 2.2 ± 1.0 | 1.1 ± 0.4 | *P* < 0.001 |
| s-Alb, g/dL | 3.5 ± 0.1 | 3.2 ± 0.2 | *P* < 0.001 | 3.5 ± 0.1 | 3.6 ± 0.2 | *P* = 0.245 | 3.5 ± 0.2 | 3.0 ± 0.3 | *P* < 0.001 |
| *Online hemodiafiltration* | | | | | | | | | |
| Albumin leakage, g/session | 7.1 ± 2.1 | 6.8 ± 1.7 | *P* = 0.621 | 6.8 ± 1.9 | 3.1 ± 0.8 | *P* = 0.478 | 7.1 ± 2.6 | 3.5 ± 0.7 | *P* < 0.001 |
| s-Alb, g/dL | 3.6 ± 0.2 | 3.2 ± 0.1 | *P* < 0.001 | 3.6 ± 0.2 | 3.6 ± 0.2 | *P* = 0.245 | 3.6 ± 0.2 | 3.2 ± 0.1 | *P* < 0.001 |

Group A, high albumin leakage and high s-Alb; Group B, high albumin leakage and low s-Alb; Group C, low albumin leakage and high s-Alb; Group D, low albumin leakage and low s-Alb

method in patients on dialysis without obvious PEW and inflammation.

Furthermore, survival was found to be better on SHF albumin-leaking HD with high albumin leakage than on OHDF with low albumin leakage (Fig. 1) [6]. In that study, polyethersulfone (PES) was the membrane material in all the dialyzers but not in any of the hemodiafilters, suggesting that a SHF albumin-leaking membrane with PES may have a beneficial effect on survival [6]. The dialyzers and hemodiafilters using PES membranes with SC for albumin ≥0.03 are available only in Japan. In a subsequent preliminary study (unpublished data), the removal amount, reduction ratio of $\alpha_1$-microglobulin ($\alpha_1$MG), and the selective removal index of $\alpha_1$MG for albumin leakage were not significantly different between SHF albumin-leaking HD, predilution OHDF, and post-dilution OHDF using PES membranes with similar high albumin leakage, as shown in Additional file 1: Fig. S1. Because $\alpha_1$MG is a large-middle molecule such as lambda-free light chain and fibroblast growth factor 23, it is suggested that survival is equivalent between these modalities with similar albumin leakage.

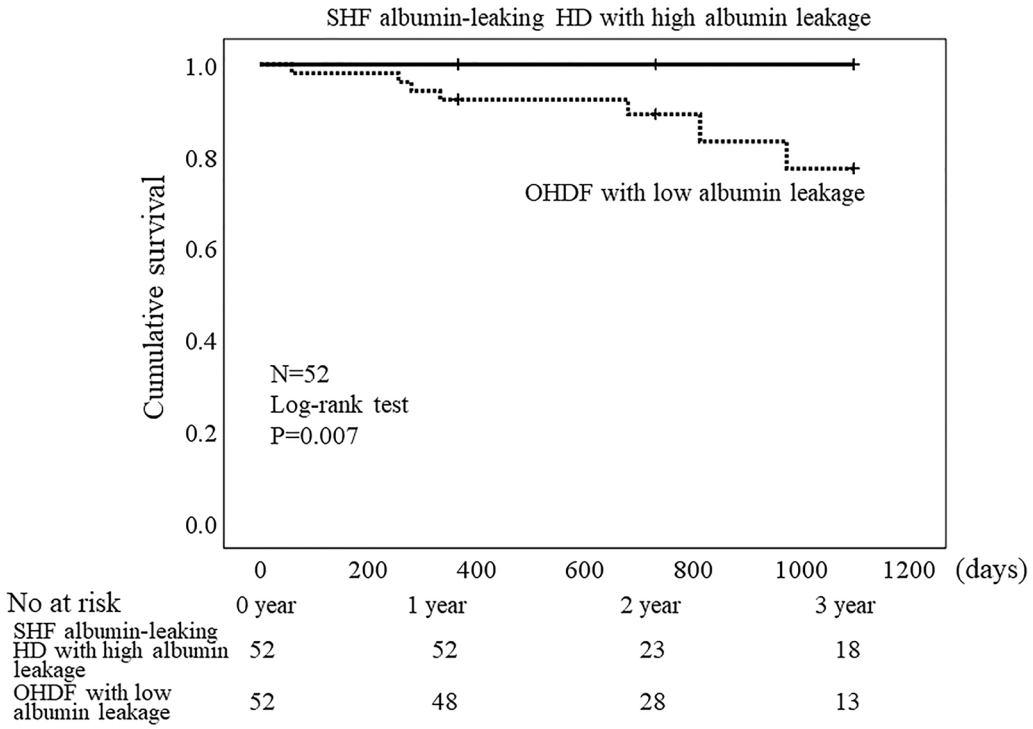


**Fig. 1** Comparison of survival outcomes between patients on super high-flux (SHF) albumin-leaking hemodialysis (HD) with high albumin leakage and those on online hemodiafiltration (OHDF) with low albumin leakage [6]

Although the mean serum albumin level on both HD and OHDF was 3.7 g/dL in the JRDR data for 2012 [16] and the annual crude mortality rate was 9.9% in the 2020 JRDR data report [17], the crude mortality rate at our facilities in 2020 was 7.1%, even though the mean serum albumin level was 3.3 g/dL on HD and 3.4 g/dL on OHDF [7]. This suggests that a certain level of hypoalbuminuria can improve survival.

There have been a number of studies of European-style high-volume postdilution OHDF with different mortality outcomes. In studies where no difference in mortality was observed, the mean serum albumin levels were in the range of 3.8–4.1 g/dL at baseline and during follow-up in all groups [18–20]. European-style postdilution OHDF failed to demonstrate a survival advantage over HD because of lack of sufficient albumin leakage. The fact that C-reactive protein was higher in European patients than in their Japanese counterparts between 2005 and 2008 [21] may also have influenced these results.

One of the reasons for the low C-reactive protein level in Japan is thought to be the purification of the dialysis fluids. According to the JSDT Standard of Fluids for Hemodialysis and Related Therapies, ultrapure dialysis fluid for HD and OHDF should have a viable bacterial count < 0.1 CFU/mL and endotoxin < 0.001 EU/mL (below the lower limit of the assay sensitivity), and online prepared substitution fluid should be sterile and nonpyrogenic (endotoxin free) [22]. The recently published CONVINCE study demonstrated that high-volume postdilution OHDF (mean 25.2 L/session) improved survival with higher *Kt*/*V* more than high-flux HD during the COVID-19 pandemic [23]. The mean level of C-reactive protein during follow-up in that study decreased to a level similar to that in Japan.

## Proposed functional classification for dialyzer membranes and classification of HD with reference to the European definition

Differences in measurement conditions should be borne in mind when comparing the function of dialyzers between countries. In the European functional classification, $\beta_2$MG clearance is measured by conventional HD and the SC for albumin is calculated in vitro. Low-flux, high-flux, medium cut-off, protein-leaking, and high cut-off membranes are briefly stated to have the following respective $\beta_2$MG clearance and SC for albumin: < 10 mL/min and 0, 20–80 mL/min and < 0.01, ≥ 80 mL/min and < 0.01, ≥ 80 mL/min and 0.01–0.03, and undefined and < 0.2 [24]. The medium cut-off and protein-leaking membranes correspond to the SHF membrane in Japan. We propose adding the SHF albumin-leaking membrane ($\beta_2$MG clearance ≥ 80 mL/min and SC for albumin ≥ 0.03 and < 0.1) to the functional classification of dialyzer membranes and adding SHF albumin-leaking HD to the classification of HD with reference to the European definition (Table 3) considering the following: (1) HD with the SHF albumin-leaking membrane ($\beta_2$MG clearance ≥ 70 mL/min and SC for albumin ≥ 0.03 measured in vitro) is superior in terms of survival to HD with the SHF membrane ($\beta_2$MG clearance ≥ 70 mL/min and SC for albumin < 0.03); (2) $\beta_2$MG clearance with the SHF albumin-leaking membrane is ≥ 80 mL/min in conventional HD, similar to the European measurement method; and (3) no dialyzer with SC for albumin ≥ 0.1 is required for maintenance HD.

## Advantages and disadvantages associated with high albumin leakage and hypoalbuminemia

The main advantage of hypoalbuminemia due to high albumin leakage is production of HMA with normal antioxidant activity, as mentioned earlier. Furthermore, high albumin leakage can increase removal of uremic

**Table 3** Proposed functional classification of dialyzer membranes and classification of hemodialysis (HD) with reference to the European definition

| Classification of dialyzer membranes | Classification of HD | Sieving coefficient for albumin[a] | $\beta_2$-Microglobulin clearance (mL/min)[b] |
|---|---|---|---|
| Low-flux (LF) | LF HD | 0 | < 10 |
| High-flux (HF) | HF HD | < 0.01 | ≥ 20 and < 80 |
| Medium cut-off (MCO) | MCO Expanded HD (HDx) | < 0.01 | ≥80 |
| Protein leaking (PL) | PL HD | ≥ 0.01 and < 0.03 | ≥80 |
| Super high -flux (SHF) albumin -leaking (AL) | SHF AL HD | ≥ 0.03 and < 0.1 | ≥80 |
| High cut-off (HCO) | HCO HD | < 0.2 | Undefined |

[a] In vitro

[b] For conventional HD with a blood flow rate of 200–400 mL/min

toxins with larger molecules such as lambda free light chain (45 kDa) and fibroblast growth factor 23 (32 kDa) [25, 26], resulting in improved survival. Although albumin leakage is significantly correlated with dialysate removal of large-middle molecules such as $\alpha_1$MG [7], there is no significant correlation between albumin leakage and dialysate removal of large molecules that have a high binding rate with albumin, such as indoxyl sulfate [27]. The disadvantages of hypoalbuminemia are edema, dialysis-induced hypotension as a result of hypovolemia, and increases in fibrinogen and lipoprotein(a). However, hypoalbuminemia can increase the free form of highly albumin-bound uremic toxins, such as indoxyl sulfate, which are removed easily by diffusion.

## Evidence for antioxidant capacity of $\alpha_1$MG in patients on dialysis

The free form of $\alpha_1$MG has strong antioxidant capacity through reductase and dehydrogenase activity, heme-binding and degradation, covalent radical scavenging, and physiological upregulation of the $\alpha_1$MG-bikunin precursor gene in plasma and in interstitial fluids of all tissues with a high turnover rate (half-life of approximately 3–4 h) in healthy individuals [28–30]. However, complexes of free $\alpha_1$MG with IgA, prothrombin, and albumin, which are not eliminated by dialysis because of their larger molecular size, may have no or weak antioxidant capacity [31]. Free $\alpha_1$MG, which has a strong scavenger action, shows a higher turnover rate (half-life of approximately 2–10 days) in dialyzed patients, suggesting that it becomes free $\alpha_1$MG with reduced activity (i.e., free $\alpha_1$MG with reduced or no antioxidant capacity). A reduction ratio of over 35% in $\alpha_1$MG can decrease clinical symptoms such as bone and/or joint pain, reduced activity, pruritus, restless legs syndrome, and irritability [32].

In 164 patients treated at our facilities, the predialysis and postdialysis serum $\alpha_1$MG levels, the removal amount of $\alpha_1$MG, and the $\alpha_1$MG reduction ratio corrected for hematocrit were 103.1 ± 18.4 mg/L, 68.4 ± 15.3 mg/L, 186.5 ± 65.2 mg/session, and 42.0 ± 12.3%, respectively [7]. The serum $\alpha_1$MG level in healthy individuals is 10–20 mg/L, which corresponds to 5.2–10.4 times the predialysis level and 3.4–6.8 times the postdialysis level. Furthermore, assuming that 25% of $\alpha_1$MG exists in the free form and 75% in the complex form in healthy individuals and that 75% exists in the free form and 25% in the complex form predialysis, the free form is 15.5–31.0 times higher and the complex form is 1.7–3.4 times higher in patients on dialysis.

Therefore, the liver may be producing more free $\alpha_1$MG than the maximum daily amount in healthy individuals as a result of high oxidative stress in these patients. The liver is unlikely to produce a greater amount of free $\alpha_1$MG during dialysis by decreasing the serum $\alpha_1$MG level because it does not decrease below the normal range, which may be a stimulating factor. It is possible that interstitial $\alpha_1$MG migrates into the blood because of the reduction in serum $\alpha_1$MG concentration during dialysis, and the activity of interstitial free $\alpha_1$MG might also be reduced in an environment of high oxidative stress. There is a possibility that the complex form of $\alpha_1$MG converts to the free form with normal antioxidant capacity. However, there is no evidence of changes in the serum concentrations of the free form with reduced activity or in the complex form during dialysis.

The Stokes radii of free $\alpha_1$MG and albumin are similar, at 28.6 Å [33] and 35.5 Å [34], respectively, despite having different molecular weights (33 kDa and 66 kDa, respectively). The reduction ratio of $\alpha_1$MG is strongly correlated with albumin leakage [7]. There is no benefit in removal of free $\alpha_1$MG with reduced activity, which still has some antioxidant capacity. Therefore, unlike with high albumin leakage, there is presently no evidence that free $\alpha_1$MG with reduced activity is a uremic toxin or that the high removal of free $\alpha_1$MG with reduced activity itself improves antioxidant capacity.

## Hypothesis regarding crosstalk between $\alpha_1$MG and albumin

High albumin leakage with mild hypoalbuminemia can improve survival in patients on HD or OHDF [7] by increasing the serum HMA level and HMA fraction and by decreasing serum HNA-1 and HNA-2 levels [12–14]. However, the mechanism underlying the increase in HMA during dialysis is unknown because HMA, HNA-1, and HNA-2 are eliminated in the dialysate.

There is no evidence that removal of free $\alpha_1$MG with reduced activity improves survival in patients on dialysis. Although a reduction ratio of over 35% in $\alpha_1$MG can decrease clinical symptoms [32], it is necessary to confirm that the free $\alpha_1$MG is produced in the liver in response to a high $\alpha_1$MG reduction ratio. Free $\alpha_1$MG and free $\alpha_1$MG with reduced activity are eliminated in the dialysate, but complex $\alpha_1$MG is not.

Our hypothesis for crosstalk between $\alpha_1$MG and albumin is summarized in Figs. 2 and 3. The liver synthesizes free $\alpha_1$MG with upregulation of the $\alpha_1$MG-bikunin precursor gene induced by elevated concentrations of free hemoglobin, heme, and reactive oxygen species [29]. Strong oxidative stress and hemolysis at initiation of and during dialysis induces an increase in production of free $\alpha_1$MG. This free $\alpha_1$MG rapidly reacts with substances that are reversibly bound to the 34 residues on HNA-1, resulting in conversion to HMA and free $\alpha_1$MG with reduced activity during dialysis. HNA-2 is irreversibly reacted and cannot be converted to HMA. The serum HMA level is

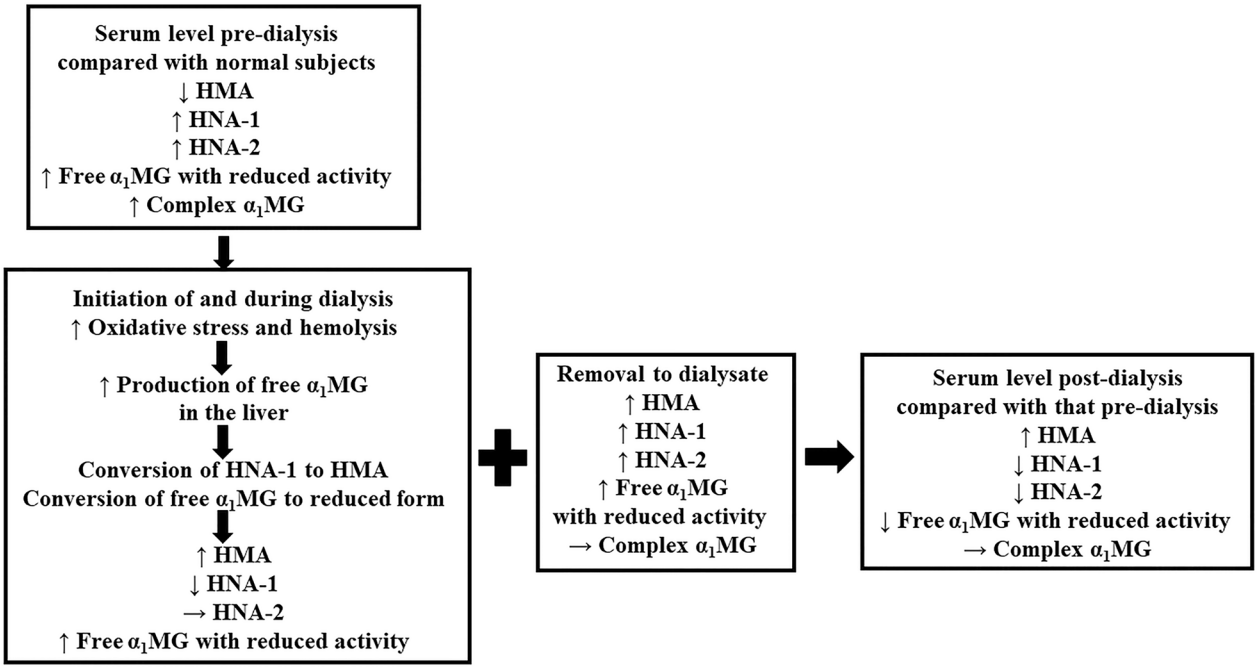


**Fig. 2** Hypothesis for crosstalk between $\alpha_1$-microglobulin ($\alpha_1$MG) and albumin at initiation of and during dialysis. HMA, human mercaptoalbumin; HNA-1, human nonmercaptoalbumin-1; HNA-2, human nonmercaptoalbumin-2

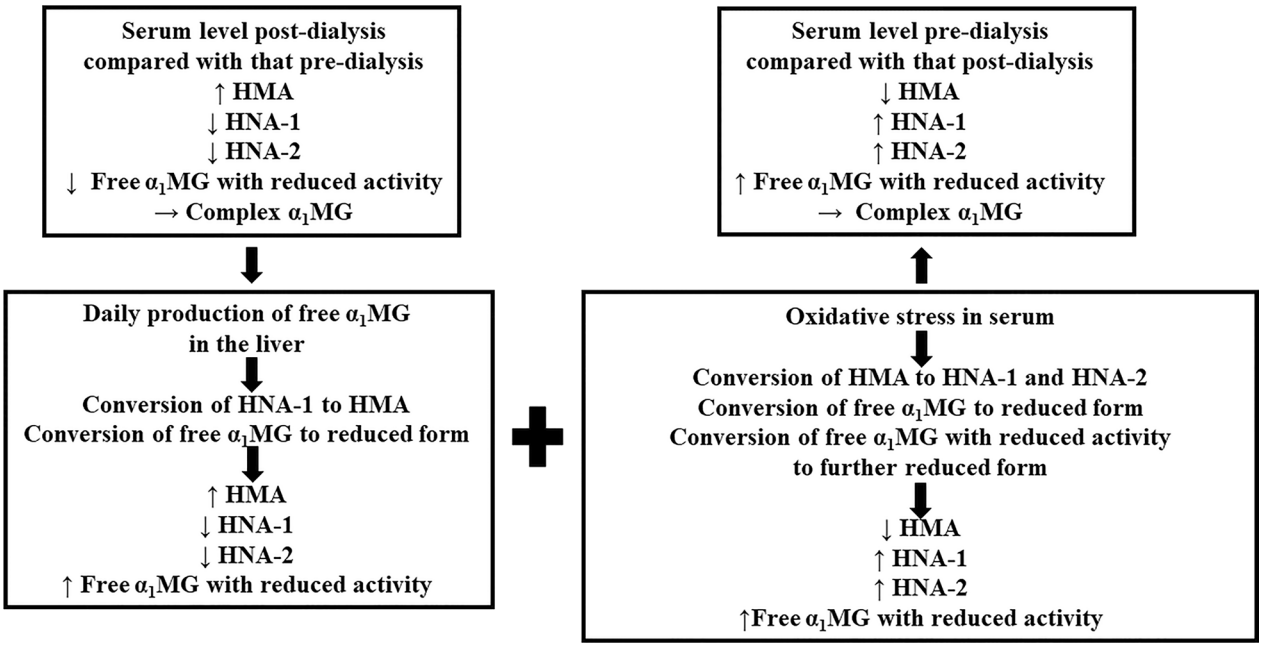


**Fig. 3** Hypothesis for crosstalk between $\alpha_1$-microglobulin ($\alpha_1$MG) and albumin after dialysis. HMA, human mercaptoalbumin; HNA-1, human nonmercaptoalbumin-1; HNA-2, human nonmercaptoalbumin-2

higher postdialysis than predialysis even though HMA is eliminated in the dialysate, and the serum HAN-1, HNA-2, and free $\alpha_1$MG with reduced activity levels are lower postdialysis than predialysis because they are removed in the dialysate (Fig. 2).

PEW and inflammation, which worsen survival, decrease HMA production in the liver. Since the

adjusted odds ratio was found to be 25.6 for cardiovascular mortality in patients with a post-HD HMA fraction of < 60% versus ≥ 60% [12], it is suggested that the amount of HMA produced during dialysis is important. The hypothesis is that HMA production depends on the amount of free $\alpha_1$MG produced upon stimulation by dialysis, suggesting that the decrease in ability to produce free $\alpha_1$MG in the liver during dialysis worsens survival. Therefore, it is suggested that some reasons for the difficulty in producing free $\alpha_1$MG are PEW and inflammation, similar to HMA production.

After dialysis, free $\alpha_1$MG that is produced daily in the liver becomes free $\alpha_1$MG with reduced activity by converting HNA-1 to HMA, resulting in a decrease of HNA-1 and increases of HMA and free $\alpha_1$MG with reduced activity. Oxidative stress in serum induces conversion of HMA to HNA-1 and HNA-2, free $\alpha_1$MG to free $\alpha_1$MG with reduced activity, and free $\alpha_1$MG with reduced activity to a further reduced form, resulting in a decrease of HMA and increases of HNA-1, HNA-2, and free $\alpha_1$MG with reduced activity. Finally, predialysis levels of serum HMA are lower than postdialysis levels, while those of serum HNA-1, HNA-2, and free $\alpha_1$MG $\alpha_1$MG with reduced activity are higher (Fig. 3). Assuming the serum HMA and $\alpha_1$MG levels postdialysis are 2.76 g/dL [12] and 68.4 mg/L [7], respectively, the level of HMA in serum is 406.4 times higher than that of $\alpha_1$MG. Given that $\alpha_1$MG consists of complex, free, and many free forms with reduced activity, the antioxidant capacity of free $\alpha_1$MG present in the blood after dialysis is almost negligible compared with that of HMA. In addition, daily production of HMA and free $\alpha_1$MG in healthy individuals is 10–15 g and 200–300 mg, respectively, which is 33.3–75.0 times higher for HMA. Therefore, the antioxidant capacity in serum is mainly attributable to the very large amount of HMA, although the very small amount of free $\alpha_1$MG produced in the liver also has strong antioxidant activity.

Indoxyl sulfate is one of the highly albumin-bound uremic toxins. There is a significant increase in the serum level of the free form of indoxyl sulfate 1 h after starting dialysis, and the level post-HD is significantly lower than that pre-HD [35]. Although the serum free indoxyl sulfate level decreases during dialysis by diffusion, it is unclear why serum free indoxyl sulfate is increased after 1 h. The bond between indoxyl sulfate and albumin site II is reversible and can be broken by administration of drugs that bind to site II [36] or increased plasma ionic strength [37]. Therefore, it is possible that the increase in serum free indoxyl sulfate level results from loss of binding at site II by conversion of HNA-1 to HMA.

## Conclusions

Survival is better with high albumin leakage than with low albumin leakage, and high albumin leakage with hypoalbuminemia does not worsen survival in patients on HD or OHDF. In addition, survival on HD and OHDF is equivalent in patients with similar albumin leakage and serum albumin levels, and survival is better on SHF albumin-leaking HD with high albumin leakage than on OHDF with low albumin leakage.

The crosstalk between $\alpha_1$MG and albumin in patients on dialysis could be explained by the hypothesis that free $\alpha_1$MG reacts with certain substances from HNA-1 at the initiation of and during dialysis, resulting in the conversion to HMA and free $\alpha_1$MG with reduced activity and the increased serum HMA level postdialysis. The antioxidant capacity in serum after dialysis is mainly due to the very large amount of HMA, resulting in the conversion to HNA and the decreased serum HMA level predialysis. However, the very small amount of free $\alpha_1$MG produced in the liver has strong antioxidant activity after dialysis.

### Abbreviations

| | |
|---|---|
| $\alpha_1$MG | $\alpha_1$-Microglobulin |
| $\beta_2$MG | $\beta_2$-Microglobulin |
| Cys-34 | Cysteine residue at position 34 from the N-terminus |
| HD | Hemodialysis |
| HMA | Human mercaptoalbumin |
| HNA | Human nonmercaptoalbumin |
| JRDR | Japanese Society for Dialysis Therapy Renal Data Registry |
| JSDT | Japanese Society for Dialysis Therapy |
| PES | Polyethersulfone |
| PEW | Protein-energy wasting |
| OHDF | Online hemodiafiltration |
| SC | Sieving coefficient |
| SHF | Super high-flux |

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s41100-024-00543-1.

**Additional file1: Fig. S1.** Comparison of albumin leakage, removal amount of $\alpha_1$-microglobulin ($\alpha_1$MG), reduction ratio of $\alpha_1$MG, and selective removal index of $\alpha_1$MG removal for albumin leakage (SRI) between dialysis modalities. SHF albumin-leaking HD, super high-flux albumin-leaking hemodialysis using PES-25Daeco; predilution OHDF, predilution online hemodiafiltration with a substitution volume of 84 L using MFX-25Ueco; postdilution OHDF, postdilution online hemodiafiltration with a substitution volume of 10 L using MFX-25Ueco. n = 8. ANOVA tests

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### Author contributions

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**Availability of data and materials**
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## Declarations

**Ethics approval and consent to participate**
Not applicable.

**Consent for publication**
Not applicable.

**Competing interests**
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