Editorial

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The Removal of β2-Microglobulin in Spent Dialysate Cannot Be Monitored by Spectrophotometric Analysis

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Toxins are removed from plasma and filtered into the spent dialysate or ultra-filtrate, via diffusion and convection, or trapped inside the membrane by adsorption. It has been already demonstrated that the analysis of spent dialysate by spectrophotometry allows the monitoring of dialysis removal of small molecules and the noninvasive assessment of dialysis efficiency and Kt/V [1–5]. The removal of β2-microglobulin (β2M, MW 11.8 kDa) from plasma is considered an effective marker of the dialyzer clearance for 'middle molecules' and proteins with molecular weight ranging 10–30 kDa [6].

An interesting paper published in this issue of blood purification by Fredrik Uhlin et al. deals with the possibility to evaluate β2M elimination by monitoring the ultraviolet absorbance of spent dialysate. A high correlation was found between UV absorbance and β2M during HDF treatment but not for high-flux HD treatments. The authors conclude that UV absorbance could perhaps be used in solely HDF mode for the estimation of β2M removal, and that in any case, the possibility of optical estimation of β2M must be validated.

We synthetically present here unpublished results on β2M removal during HD treatments with dialysis membranes having different flux and adsorption capacities to clarify if the spectrophotometric analysis of spent dialysate may allow the possibility to monitor the removal of β2M during HD. These results were obtained from the analyses of serum and spent dialysate samples of the 22 MHD patients (16 men, 6 women) of a previous study, where we confirmed the possibility to monitor the removal of small molecules, namely uric acid, by spectrophotometric analysis (J Nephrol).

Serum and spent dialysate concentrations of β2M were measured with an immunonephelometric method (Siemens, BNAII) and compared with absorbance and fluorescence values. The sampling protocol, the analytical procedure, the instrumentation and the statistical methods have been previously described [5].

Absorbance and Fluorescence Spectra of β2-Microglobulin

In the range 200–600 nm, β2M shows an absorption peak at 280 nm (fig. 1a). The absorption peaks of urea, creatinine and uric acid are also shown for comparison (fig. 1a). An emission peak at 340 nm was observed in the fluorescence spectrum of β2M in the range 230–510 nm

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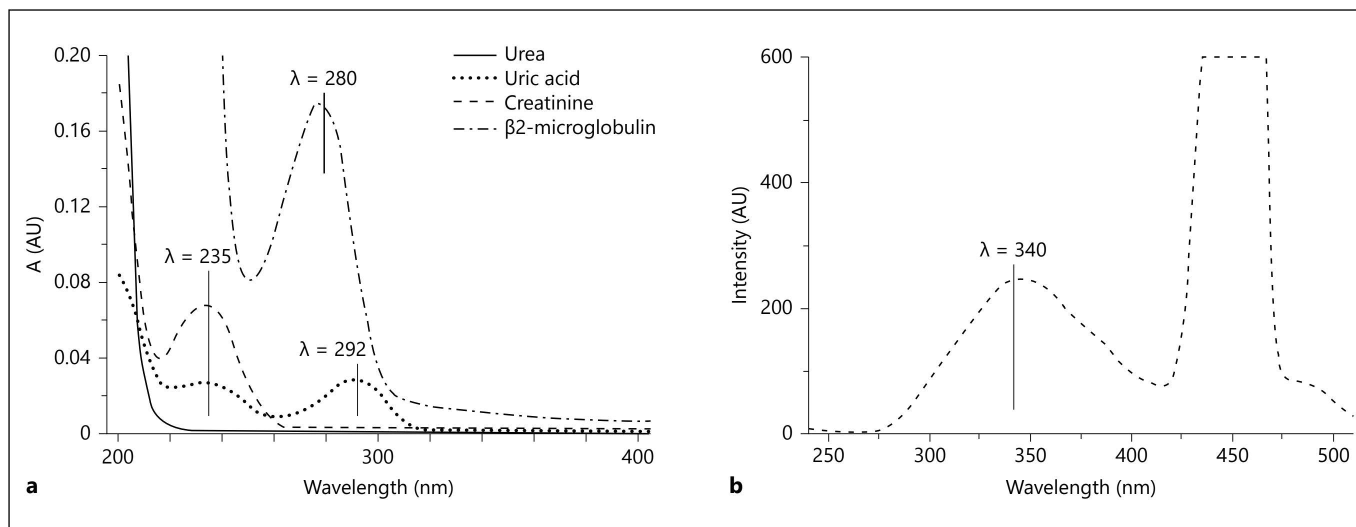

Fig. 1. Absorption (**a**) and fluorescence spectra (**b**) of β2M (dash-dotted line). The absorption spectra of urea (continuous line), uric acid (dotted line), creatinine (dashed line) are reported for comparison. The wavelengths of β2M (280 nm), creatinine (235 nm), and uric acid (235 and 292 nm) absorption peaks as well as the β2M fluorescence emission peak (340 nm) are highlighted.

(excitation wavelength 220 nm) (fig. 1b). On the basis of these data, the UV absorbance was measured at 280 nm, and the fluorescence emission at 340 nm (excitation wavelength 220 nm).

Serum and Spent Dialysate Concentrations, Absorbance and Fluorescence Values in Spent Dialysate Over Time

During the dialysis sessions with polysulfone low-flux (LF) membrane, β2M concentration showed a slight increase in serum (table 1; fig. 2, left). No detectable concentrations of β2M were observed in spent dialysate, since this molecule is not cleared from blood by LF membranes. Surprisingly, both UV absorbance at 280 nm and fluorescence emission at 340 nm decreased in spent dialysate samples according to exponential functions. During the dialysis sessions with the HF membrane (table 1; fig. 2, middle), the serum and spent dialysate absorbance at 280 nm showed similar exponential decreases over time, which were much faster than that observed for the fluorescence emission at 340 nm. During treatments with the high-flux and adsorption (HF-ADS) membranes, which has additional adsorption properties, serum β2M decreased according to an exponential function (table 1; fig. 2, right), whereas the concentrations in spent dialysate samples had an irregular time course, probably due to absorption into the membrane. The variations of spectrophotometric signals (absorbance at 280 nm and fluorescence at 340 nm) in spent dialysate over time were completely independent from β2M concentrations in dialysate.

The Contribution of β2-Microglobulin to UV Absorption and Fluorescence Signals Was Insignificant

Indeed, in dialysate samples (n = 86) obtained during treatment with HF membranes, the contribution of β2M to the absorbance measured at 280 nm and to the fluorescence at 340 nm was lower than 0.2%.

All these data indicate that the removal of β2M in spent dialysate may be hardly assessed under the experimental conditions used by absorbance or fluorescence measurements, even if β2M has an absorption peak at 280 nm and a fluorescence emission at 340 nm. Indeed, the absorbance peak found for β2M at 280 nm is the same as the one that is currently used to evaluate the removal of small molecules. Furthermore, the contribution of β2M to the absorbance was relatively less and its contribution to fluorescence was absolutely negligible. Thus, the absorbance at 280 nm is mainly due to other substances, namely, uric acid [5]. In fact, significant UV absorption and fluorescence emission signals were also measured treatments with the LF membrane, which do not allow any β2M transfer, as demonstrated by the absence

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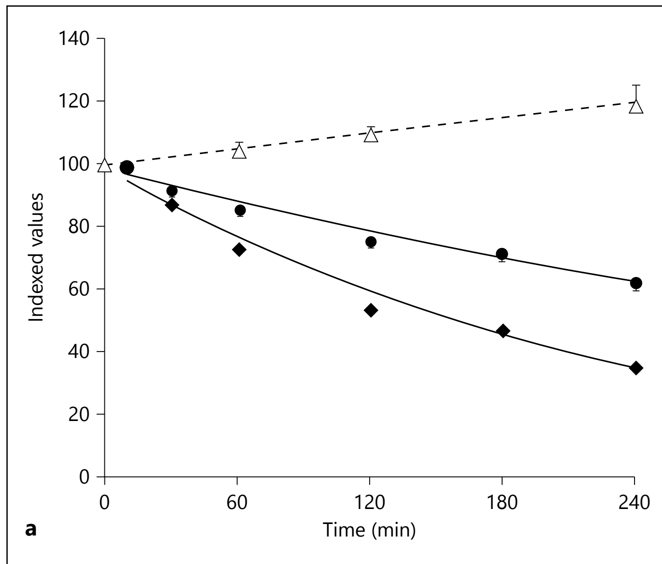

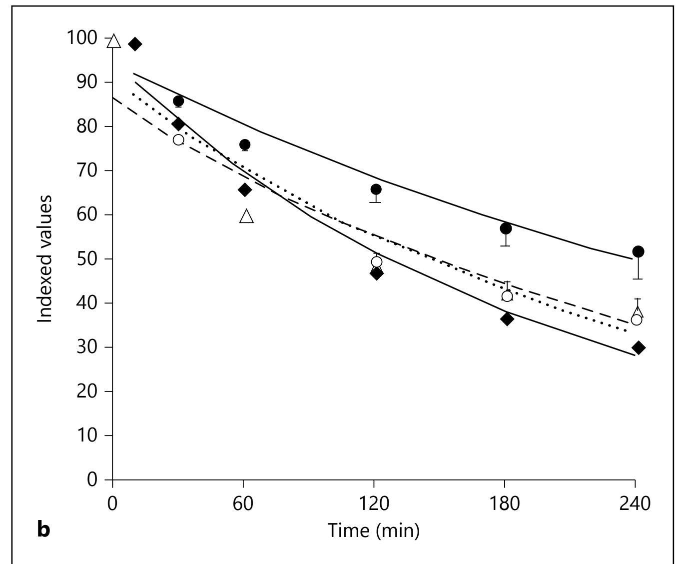

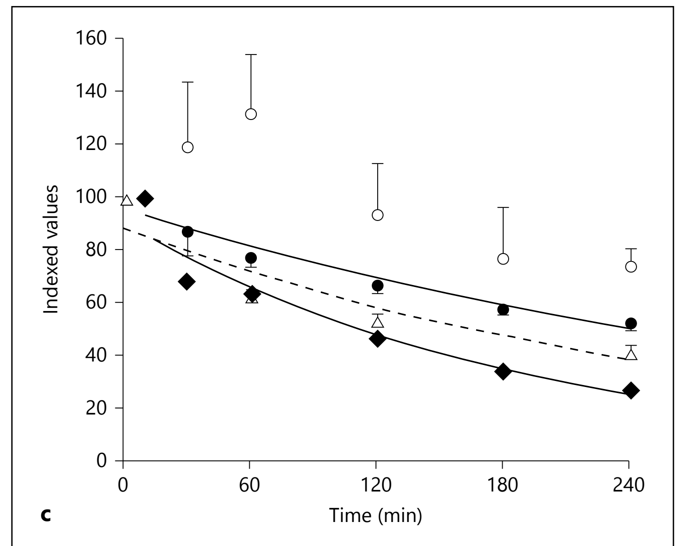

Fig. 2. Serum (△) and spent dialysate (○) concentrations of β2M over time compared with absorbance (◆) at 280 nm and fluorescence emission (●) at 340 nm in spent dialysate. Values are scaled to maximum (time 0 min for serum; time 10 min for spent dialysate); at each time, mean values ± standard error of the mean are reported. The first plot (**a**) shows the results in the 11 patients treated with LF membrane; the second plot (**b**) the results in the 8 patients treated with HF membrane, and the third plot (**c**) the results in the 3 patients treated with HF membrane with adsorption properties. Correlation coefficients and regression equations are reported in table 1.

Table 1. Correlation coefficients and regression equations of serum and spent dialysate concentrations of β2M over time, compared with absorbance at 280 nm and fluorescence emission at 340 nm in spent dialysate

	LF membrane	HF membrane	HF-ADS membrane
Serum β2M			
Correlation coefficient	0.999	0.940	0.944
Regression equation	$Y = 0.08X + 99.9$	$Y = 86.2e^{-0.0037X}$	$Y = 87.5e^{-0.0034X}$
Spent dialysate β2M			
Correlation coefficient	Not measurable	0.971	Not evaluable
Regression equation	Not measurable	$Y = 90.7e^{-0.0041}$	Not evaluable
Spent dialysate absorbance 280 nm			
Correlation coefficient	0.990	0.986	0.981
Regression equation	$Y = 98.9e^{-0.0042X}$	$Y = 95.0e^{-0.005X}$	$Y = 89.9e^{-0.0053X}$
Spent dialysate emission 340 nm			
Correlation coefficient	0.989	0.979	0.979
Regression equation	$Y = 98.8e^{-0.0019X}$	$Y = 95.1e^{-0.0027X}$	$Y = 95.1e^{-0.0027}$

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of β2M in spent dialysate. Moreover, the time courses of UV absorption and fluorescence emission of spent dialysate during dialyses with HF-ADS membrane were completely independent from that of β2M measured with biochemical methods. The fluorescence emission is possibly determined by naturally fluorescent compounds [7] or by other molecules still unknown. In addition, our data indicate that even biochemical determinations in spent dialysate cannot be used to monitor the plasma disappearance of substances mainly removed by adsorption to dialysis membrane, like β2M [8].

We conclude that the removal of β2M cannot be evaluated by spectrophotometric analysis of spent dialysate.

Disclosure Statement

The authors declare nothing to disclose.

References

1 Daugirdas JT: Second generation logarithmic estimates of single-pool variable volume Kt/V: an analysis of error. J Am Soc Nephrol 1993;4:1205–1213.
2 Fridolin I, Lindberg LG: On-line monitoring of solutes in dialysate using wavelength-dependent absorption of ultraviolet radiation. Med Biol Eng Comput 2003;41:263–270.
3 Uhlin F, Fridolin I, Lindberg LG, Magnusson M: Estimation of delivered dialysis dose by on-line monitoring of the ultraviolet absorbance in the spent dialysate. Am J Kidney Dis 2003;41:1026–1036.
4 Castellarnau A, Werner M, Günthner R, Jakob M: Real-time Kt/V determination by ultraviolet absorbance in spent dialysate: technique validation. Kidney Int 2010;78:920–925.
5 Donadio C, Calia D, Ghimenti S, Onor M, Colombini E, Fuoco R, Di Francesco F: Uric acid is the major determinant of absorbance in spent dialysate allowing spectrophotometric evaluation of dialysis dose. J Nephrol 2014;27:331–337.
6 Vanholder R, De Smet R, Glorieux G, Argilés A, Baurmeister U, Brunet P, Clark W, Cohen G, De Deyn PP, Deppisch R, Descamps-Latscha B, Henle T, Jörres A, Lemke HD, Massy ZA, Passlick-Deetjen J, Rodriguez M, Stegmayr B, Stenvinkel P, Tetta C, Wanner C, Zidek W: Review on uremic toxins: classification, concentration, and interindividual variability. Kidney Int 2003;63:1934–1943.
7 Swan JS, Kragten EY, Veening H: Liquid-chromatographic study of fluorescent materials in uremic fluids. Clin Chem 1983;29:1082–1084.
8 Randoux C, Gillery P, Georges N, Lavaud S, Chanard J: Filtration of native and glycated beta2-microglobulin by charged and neutral dialysis membranes. Kidney Int 2001;60:1571–1577.

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