

# Strategies for the removal of middle molecules in HD: Results of recent studies

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## Preface

Current estimates of haemodialysis adequacy are based on calculations of small solute (urea) clearance or changes. However, the number of clinical studies showing the contribution of middle- and large molecules to

long-term morbidity and mortality in HD patients has been growing. Recently a secondary analysis of the HEMO study revealed the importance of serum  $\beta_2$ -microglobulin ( $\beta_2$ -M) – a surrogate marker for middle molecules – as predictor for mortality. Besides the removal of small molecules, high-flux membranes, due to their larger pores, allow a considerable clearance of middle molecular weight substances.

The MPO (Membrane Permeability Outcome) Study performed by **Locatelli et al.** shows a clear clinical benefit of high-flux dialysis especially in HD patients with a poorer prognosis, i.e. the malnourished and diabetic HD patients, a growing population. **Okuno et al.** confirm in their study the relationship between  $\beta_2$ -M and mortality risk in HD patients. Moreover, in an additional secondary analysis of the HEMO study, **Cheung et al.** find a significant association between serum  $\beta_2$ -M levels and infectious mortality.

Several studies have stressed the importance of dialysis time in the removal of uraemic retention solutes, in particular of those with multicompartamental kinetic behaviour. **Eloot et al.** further investigated this issue by dialysing HD patients for 4, 6, or 8 hours while processing the same total blood and dialysate volume.

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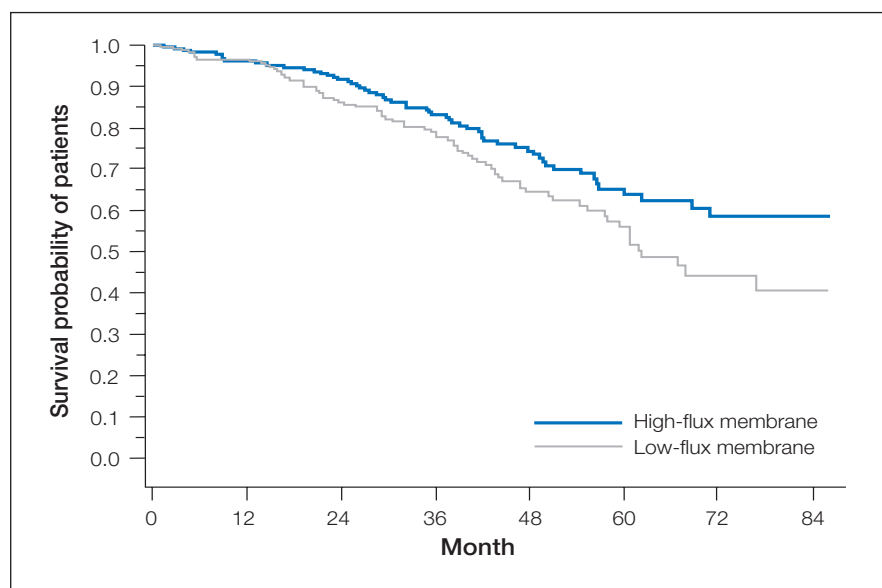


## 1. Effect of membrane permeability on survival of haemodialysis patients

Besides the removal of small molecules, high-flux membranes, due to their larger pores, allow a considerable clearance of middle molecular weight substances. It has not been unequivocally determined so far whether enhanced elimination of these solutes translates into long-term clinical benefits for HD patients. **Locatelli F et al.** therefore performed the Membrane Permeability Outcome (MPO) study in order to evaluate the effect of high-flux versus low-flux membranes on survival of HD patients.

The MPO study was a prospective, randomized, open, controlled study in incident HD patients with particular focus on patients at risk of worse outcome. For that reason the initial protocol aimed at including patients with a serum albumin  $\leq 4$  g/dl, as a hypoalbuminaemia often reflects malnutrition and inflammation and is associated with poor survival in dialysis patients. As after 11 of the planned 24 months of the recruitment period, the recruitment rate was lower than expected, the study protocol was amended, prolonging the recruitment period to 4.5 years and including also patients with serum albumin  $> 4$  g/dl at enrolment. These patients were assigned as a separate stratum in order to not jeopardize the original protocol. All patients had to be treated 3x/week with bicarbonate HD for a minimum treatment time of 180 minutes per session. To rule out influence of the dialysis dose as a confounding factor, spKt/V had to be maintained at a minimum of 1.2.

Overall, 738 HD patients from 59 centres in 9 European countries were enrolled from December 1998 until June 2003. The mean observation time was  $3.0 \pm 1.9$  years (maximum 7.5 years). After excluding patients who did not start the study treatment, who had major protocol deviations, or who did not achieve the required dialysis dose, 647 patients entered the survival analysis: for patients with serum albumin  $\leq 4$  g/dl, 250 patients on high-flux and 243 on low-flux HD; for patients with serum albumin  $> 4$  g/dl, 68 patients on high-flux and 86 on low-flux HD. Patient characteristics at baseline were comparable. Mean ultrafiltration coefficient of the dialysers was  $44.7 \pm 9.1$  ml/mmHg/h in the patients treated with high-flux membranes, and  $9.8 \pm 3.5$  ml/mmHg/h in those treated with low-flux membranes. High-flux HD resulted in a lower accumulation of serum  $\beta_2$ -microglobulin than low-flux HD (increase by  $4.4 \pm 7.8$  mg/dl and by  $8.0 \pm 12.3$  mg/l, respectively,  $p < 0.05$ ). The authors found for the patients with serum albumin  $\leq 4$  g/dl a lower mortality in the high-flux HD group compared to the low-flux HD group,  $p = 0.032$  (see **Figure 1**). In the Cox proportional hazards model, high-flux versus low-flux revealed a 37% RR reduction of mortality (HR 0.63; 95% CI 0.45 to



**Fig. 1:** Kaplan-Meier survival curves for incident HD patients with serum albumin  $\leq 4$  g/dl (Log-rank test  $p = 0.032$ )

0.90;  $p = 0.010$ ). In the overall patient population including the patients with normal serum albumin levels no significant survival difference between the high-flux and low-flux group could be observed. In a subgroup analysis a higher survival rate in the diabetic patients treated with high-flux HD ( $n = 83$ ) compared to the diabetic patients treated with low-flux HD ( $n = 74$ ) was detected,  $p = 0.039$ . The rate of hospital admissions was comparable in the high-flux and low-flux HD groups.

The authors discuss that the causal relation between treatment with high-flux membranes and survival in patients with hypoalbuminaemia and diabetes - an increasing proportion of dialysis patients - could lie in their enhanced removal capacity of uraemic retention solutes with higher molecular weight. Earlier studies and also their own data now have proven that high-flux membranes more effectively remove  $\beta_2$ -microglobulin, considered to be a surrogate marker of middle-molecule uraemic toxins. Predialysis serum  $\beta_2$ -microglobulin levels have been associated with all-cause mortality. A confounding effect of residual kidney function on survival would be unlikely because there were no differences between the high-flux and low-flux groups.

*In conclusion, the study adds further evidence to the beneficial effects of high-flux membranes, especially in those patients with more comorbidities as reflected by hypoalbuminaemia. Moreover, a subgroup analysis demonstrated a survival advantage in diabetics treated with high-flux HD.*

CL

Locatelli F, Martin-Malo A, Hannedouche T, Loureiro A, Papadimitriou M, Wizemann V, Jacobson SH, Czekalski S, Ronco C, Vanholder R, for the Membrane Permeability Outcome (MPO) Study Group: Effect of membrane permeability on survival of hemodialysis patients; *J Am Soc Nephrol*, 20 (3): 645 - 654, 2009

## 2. Serum $\beta_2$ -microglobulin level is a significant predictor of mortality in maintenance haemodialysis patients

$\beta_2$ -microglobulin ( $\beta_2$ -M) is used as a surrogate marker of middle-molecular weight uraemic toxins. Elevated concentration of circulating  $\beta_2$ -M has been demonstrated to be a potential risk for the onset and/or development of dialysis-related amyloidosis. The study of **Okuno S. et al.** examined the relationship between serum  $\beta_2$ -M and clinical outcome in dialysed patients.

This observational, single-centre study was performed at Shirasagi Hospital Kidney Centre in Osaka, Japan. 490 prevalent HD patients ( $> 3$  months HD duration,  $60.1 \pm 11.8$  years, 288 males) were enrolled and data collected from 1999 – 2003. Patients were dialysed 3x/week for 3.5 – 4.5 h with high-flux membranes, bicarbonate dialysate, a dialysate flow of 500 ml/min and a blood flow between 160 – 220 ml/min. Patients were divided into 2 groups according to the median value of the baseline  $\beta_2$ -M concentration (32.2 mg/l): lower serum  $\beta_2$ -M group:  $< 32.2$  mg/l ( $n = 245$ ) and higher serum  $\beta_2$ -M group:  $\geq 32.2$  mg/l ( $n = 245$ ).

The mean follow-up was  $40 \pm 15$  months. During this period, 91 all-cause deaths occurred. As shown in

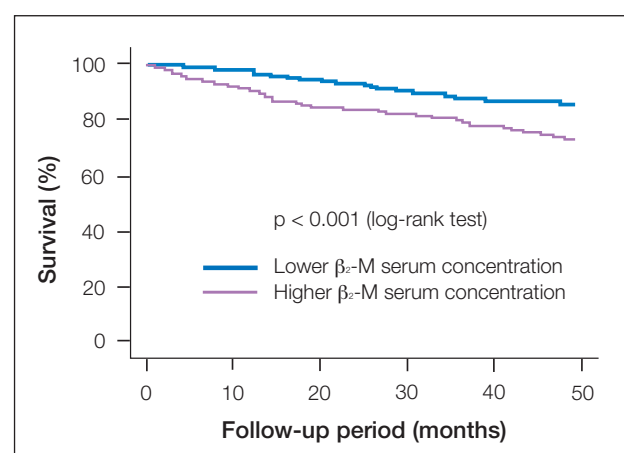


Fig. 2: Kaplan-Meier analysis of all-cause mortality of 490 HD patients

**Figure 2**, patients with higher serum  $\beta_2$ -M concentrations exhibited a significantly higher death rate than those with lower serum  $\beta_2$ -M concentrations ( $p < 0.001$ ). Moreover, multivariate Cox proportional hazards analysis showed serum  $\beta_2$ -M concentration as a significant, independent predictor of all-cause mortality (hazard ratio per 1 mg/l increase: 1.05, 95% CI: 1.01 – 1.08,  $p = 0.005$ ) and for non-cardiovascular mortality (HR: 1.06, 95% CI: 1.02 – 1.10,  $p = 0.006$ ). However, for cardiovascular mortality there was no significant association. The duration of HD was significantly longer in the lower  $\beta_2$ -M group than in the higher  $\beta_2$ -M group ( $96.3 \pm 85.3$  vs  $80.4 \pm 64.8$  months,  $p = 0.020$ ). Serum albumin was significantly higher in the lower  $\beta_2$ -M group than in the higher  $\beta_2$ -M group ( $4.1 \pm 0.3$  vs  $4.0 \pm 0.4$  g/dl,  $p < 0.001$ ). CRP levels were significantly lower in the lower  $\beta_2$ -M group than in the higher  $\beta_2$ -M group ( $0.10$  vs  $0.16$  mg/dl,  $p = 0.002$ ).

The authors compared the results with the HEMO study, where serum  $\beta_2$ -M concentration correlated significantly with mortality as well. However, serum  $\beta_2$ -M levels only predicted mortality in the subgroup of patients who had been on dialysis for  $< 3.7$  years but not in patients who had been on dialysis for more than 3.7 years. These results were in contrast to the current study showing that serum  $\beta_2$ -M level was also a significant predictor for all-cause mortality in patients with longterm HD ( $> 5$  years,  $n = 259$ ). Although the reason for the difference remains unknown, an explanation could be the longer HD duration ( $87.4 \pm 75.7$  months) in the current study compared to the mean duration of 3.7 years in the HEMO study. Patients with lower serum  $\beta_2$ -M concentration and longer duration of HD may have lived longer. Another explanation may be the use of high-flux membranes only in the current study, whereas in the HEMO study about 40% of the patients were treated with low-flux membranes and also reuse was widespread.

*In conclusion, the data of the study identify serum  $\beta_2$ -M levels as a significant predictor of mortality in HD patients independent of HD duration, diabetes, malnutrition and chronic inflammation.* KB

Okuno S, Ishimura E, Kohno K, Fujino-Katoh Y, Maeno Y, Yamakawa T, Inaba M, Nishizawa Y: Serum  $\beta_2$ -microglobulin level is a significant predictor of mortality in maintenance haemodialysis patients; *Nephrol Dial Transplant*, 24: 571 – 577, 2009

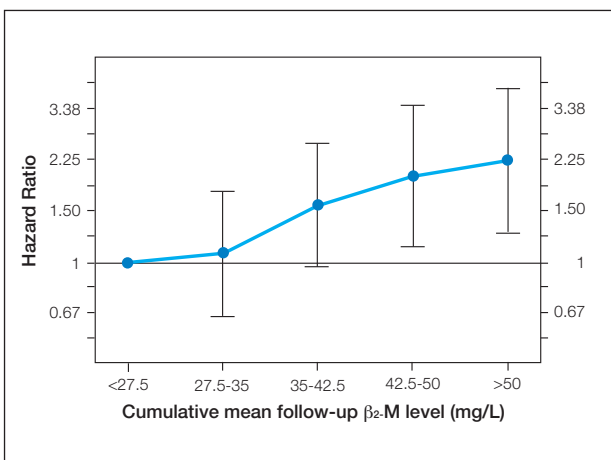
### 3. Association between serum $\beta_2$ -microglobulin level and infectious mortality in haemodialysis patients

In a secondary analysis of the HEMO (haemodialysis) Study a decrease of cardiac deaths could be observed in patients who were randomized to the high-flux arm, especially in patients with dialysis for  $> 3.7$  years. In another secondary analysis of the HEMO Study, the cumulative mean predialysis serum  $\beta_2$ -microglobulin ( $\beta_2$ -M) level during follow-up was statistically significantly lower in favour of the high-flux arm and predicted lower all-cause mortality. In-vitro studies have identified proteins with homology or molecular weight similar to  $\beta_2$ -M that have neutrophil-inhibitory effects. The accumulation of these proteins leads to a higher serum  $\beta_2$ -M concentration and may predispose the patients to infectious complications. In this observational analysis of the HEMO Study, **Cheung AK et al.** examined the association of serum  $\beta_2$ -M levels and dialyzer  $\beta_2$ -M kinetics with cause-specific mortality, focussing on cardiac and infectious deaths.

A cohort of 1813 patients of the HEMO Study was included, the mean follow-up was 2.6 years, 56.0% were female, 62.9% were black, 44.5% had diabetes and the mean age was  $57.6 \pm 14.1$  years. The association between serum  $\beta_2$ -M levels and the risk for *infec-*

tious or cardiac mortality was investigated using a time-dependent Cox-regression model, controlled for baseline demographics, comorbidity, residual kidney function and dialysis-related variables.

In the entire cohort, each 10 mg/l increase in serum  $\beta_2$ -M level was associated with a 21% increase in the rate of infectious mortality (HR: 1.21, 95% CI: 1.07 – 1.37,  $p = 0.002$ ), see **Figure 3**. Similar associations, although with lower statistical significance, were also observed in the two subgroups ( $\leq 3.7$  years on dialysis and  $> 3.7$  years on dialysis). In contrast, serum  $\beta_2$ -M levels were not associated with an increased risk for cardiac death. Kt/V of  $\beta_2$ -M correlated significantly with cardiac mortality in the subgroup of patients with  $> 3.7$  prestudy years of dialysis. Each 0.1 unit increase in  $\beta_2$ -M Kt/V was associated with a 7% decrease in the rate of cardiac mortality (95% CI: 0.87 – 1.00,  $p = 0.036$ ). No statistically significant association between  $\beta_2$ -M Kt/V and cardiac mortality could be seen in the entire cohort or those with  $\leq 3.7$  prestudy years of dialysis. Kt/V of  $\beta_2$ -M was not significantly associated with infectious mortality, in both the full cohort and within the two subgroups. However, a trend for lower infectious mortality in the group of  $> 3.7$  prestudy years of dialysis could be seen (HR: 0.93,



**Fig. 3:** Association of predialysis serum  $\beta_2$ -M levels with infectious mortality

95% CI: 0.86 – 1.01,  $p = 0.10$ ) for each 0.1 unit increase in  $\beta_2$ -M Kt/V. The authors conclude that the data generally support the role of  $\beta_2$ -M as a marker of uraemic middle molecules. In addition, the association between serum  $\beta_2$ -M levels and infectious death further validates the importance of serum middle molecular weight proteins in the pathogenesis of immunodeficiency in advanced kidney failure.

*To sum it up, this secondary analysis of the HEMO Study underlines the importance of removal of middle molecules like  $\beta_2$ -M by high-flux HD, since there is a significant association between serum  $\beta_2$ -M level and infectious mortality.* KB

Cheung AK, Greene T, Leypoldt JK, Yan G, Allon M, Delmez J, Levey AS, Levin NW, Rocco MV, Schulman G, Eknoyan G for HEMO Study Group: Association between serum  $\beta_2$ -microglobulin level and infectious mortality in hemodialysis patients; Clin J Am Soc Nephrol, 3: 69 – 77, 2008

#### 4. Impact of haemodialysis duration on the removal of uraemic retention solutes

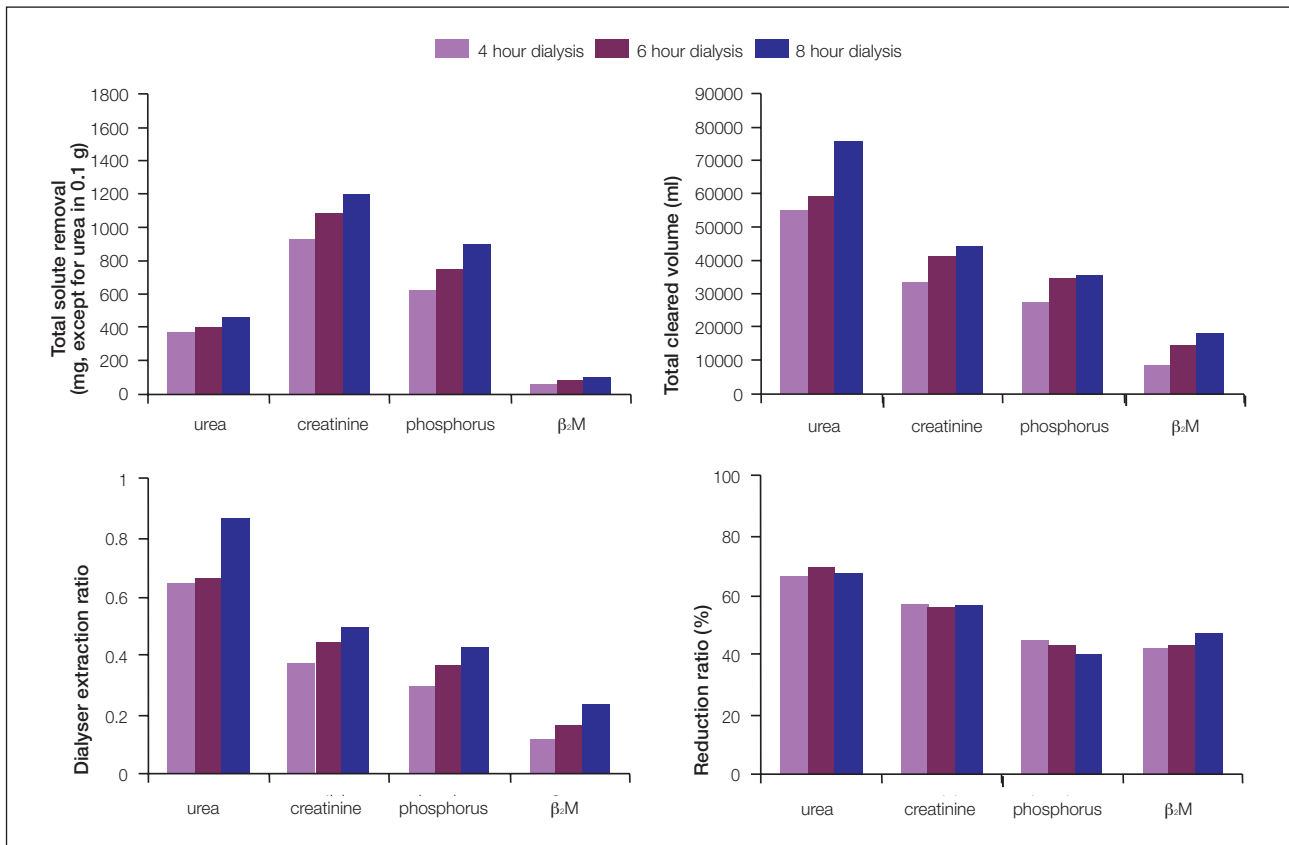
Although several studies have already emphasized the importance of time in the removal of uraemic retention solutes following a multicompartamental model of kinetics, the factor “time” was not the only parameter with a potential impact on adequacy that was modified in those studies. Therefore **Eloot S et al.** investigated the isolated effect of the time factor on the removal and kinetic behaviour of the different molecules urea, creatinine, phosphorus, and  $\beta_2$ -microglobulin ( $\beta_2$ M).

Each patient was dialysed on 3 different occasions using the Genius single-pass batch system (Fresenius Medical Care, Bad Homburg, Germany) with high-flux dialysers (Fresenius FX80). In this study the Genius

system consisted of a closed dialysate tank of 90 l. The system uses a double-sided roller pump that generates equal blood and dialysate flows of up to 350 mL/min. In general, dialysis ends when the entire volume of dialysate present in this system has passed the dialyzer. As a consequence, dialysis sessions - in spite of markedly different duration - still will operate with an identical blood and dialysate volume, hence offering the opportunity to evaluate the impact of time as the only variable. Dialysis sessions were each time performed on the same day of the week, whereas a washout period of 2 weeks was applied in between the experimental dialysis sessions. The experimental sessions lasted 4, 6, or 8 hours (h) and were assigned in random order. Blood flows were set at 350, 250, and 180 mL/min with the 4, 6, and 8 h session, respectively. UF rates were set to the needs of the patients and approximated  $0.36 \pm 0.19$  L/h

(4 h session),  $0.24 \pm 0.16$  L/h (6 h session), and  $0.21 \pm 0.11$  L/h (8 h session).

9 HD patients (5 women) with a mean age of  $71 \pm 10$  years were studied. Total solute removal, total cleared volume and dialyzer extraction ratio were statistically significantly higher with a prolonged dialysis session for urea, creatinine, phosphorus, and  $\beta_2$ M (see **Figure 4**). The reduction ratios progressively increased at different time points [5, 15, 30, 60, 120, 240 min (4, 6, and 8 h session), 360 min (6 and 8 h session), and 480 min (8 h session)] for urea, creatinine, and  $\beta_2$ M, and a difference in post-dialysis reduction ratio compared to the value at 120 min was found during the 4, 6, and 8 h dialysis, respectively, for urea (all  $p = 0.008$ ), creatinine (all  $p = 0.008$ ), and during the 4 h dialysis for  $\beta_2$ M ( $p = 0.031$ ). For phosphorus (all sessions) and  $\beta_2$ M (6 and 8 h dialysis), however, reduction ratios



**Fig. 4:** Total solute removal, total cleared volume, dialyzer extraction ratio, and reduction ratio of urea, creatinine, phosphorus, and  $\beta_2$ -microglobulin for the 4h, 6h, and 8h dialysis session

remained constant from the 120th minute on for all sessions, and in individual patients, even an intradialytic rebound of phosphorus was observed. No significant differences were found for the reduction ratios of all the studied solutes between the 4, 6 and 8 h schedules (see **Figure 4**). Kt/V urea values were  $1.39 \pm 0.28$ ,  $1.60 \pm 0.59$ , and  $1.51 \pm 0.49$  for the 4, 6, and 8h dialysis (not significant).

The most striking result of this study was that prolonged dialysis time results in a higher removal of the total amount of solute from the patient's body. This positive effect was not only seen for  $\beta_2$ M and phosphorus but also for the small and water soluble solutes urea and creatinine. However, Kt/V urea and the urea reduction ratio did not detect the difference. As slowing down dialysis flow allows more shifts of solute out of the extraplasmatic compartments, compartmental behavior of the different solutes will be different when dialysis duration is extended, allowing a higher absolute amount of solute removal in spite of no differences in reduction ratios and Kt/V urea.

*The authors conclude that, though prolonged dialysis compared to a standard 4h dialysis lead to a significantly larger removal of solutes, Kt/V urea or urea reduction ratio did not differ. Thus, care should be taken when using these parameters as the only parameters to quantify dialysis adequacy of dialysis sessions with different time durations.* CL

Eloot S, van Biesen W, Dhondt A, Van de Wynkele H, Glorieux G, Verdonck P, Vanholder R: Impact of hemodialysis duration on the removal of uremic retention solutes; *Kidney Int*, 73: 765 – 770, 2008



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