II.2 Haemodialysis dose quantification: middle molecules (MM)

Guideline II.2.1

A. β2-m is representative in its kinetic behaviour of other MM and peptides of similar size, and may be used as a marker for such molecules.

(Evidence level: B)

Guideline II.2.2

A. To enhance MM removal, synthetic high-flux membranes should be used. Additional strategies, such as adding a convective component, or increasing HD time or frequency, should be used to maximize MM removal.

(Evidence level: B)
Commentary on Guideline II. 2.1

**MM markers**

No surrogate molecule has been identified yet with the characteristics of an ideal marker for MM uraemic toxins.

*Vitamin B12.* Vitamin B12 (1350 Da), the most used marker for *in vitro* characterization of dialyzers [124], is not useful *in vivo*, because of its extensive binding to plasma proteins. Inulin (5200 Da), even widely studied [124] requires a methodology not suitable for clinical practice.

*Gentamicin.* Gentamicin (518 Da) [125], ofloxacin (361 Da) [126], and vancomycin (1448 Da) [127] more recently proposed as marker molecules in light of their appropriate MW, minimal protein binding, and small distribution volume, lack experimental and extensive clinical validation.

β2-m. β2-m is easily measured in blood. Even if its intradialytic kinetics has not been clarified definitely yet, its transport through different dialyzer membranes has been widely studied [128–137]. It is only removed through high-flux membranes, able to reduce the basal β2-m concentration by 23–30% with respect to cellulosic membranes [129,138–141]. The use of high-flux synthetic membranes has been associated with reduced incidence of bone amyloidosis [142], and carpal tunnel syndrome [140,143,144].

**Methods of MM removal quantification**

Methods used to evaluate the efficiency of a dialyzer, or a dialytic strategy in removing MM toxins, have substantial drawbacks.

*Reduction ratio.* The ratio between the final and initial plasma solute concentration of the session: \( R = \frac{1 - Ct}{Co} \) has been used in the past for β2-m [130], and recently applied to the novel uraemic retention solutes, like pentosidine [14,15,145,146], homocysteine [147,148], ADMA [35,37], and p-cresol [149].

Correction of the Ct value has been suggested to account for intradialytic change in distribution volume (the extracellular space) when calculating this ratio for β2-m [150].

This ratio overestimates the amount of solute removal, not accounting for the effect of post-HD rebound. As shown theoretically with two-pool modelling [151], intercompartmental concentration dysequilibrium during HD, and thus post-HD rebound, depends on the relationships between the dialyzer clearance \( Kd \), the intercompartmental clearance \( Kc \) and compartment volumes for each solute. Inulin is more efficiently removed from its smaller perfused volume (VP) than urea, as indicated by its higher ratio \( Kd/Vp \). Conversely, inulin refills at a slower rate from its non-perfused compartment, according to its lower \( Kc/Kd \) ratio [151]. In the more general case, these factors play in favour of a larger and more prolonged rebound for a MM than for a small solute. The *in vivo* kinetics of a MM during and after HD may deviate impredictably from that of a marker molecule, due to known and unknown factors. The extent and the rate of formation of protein bindings [146], removal of precursors/substrates or reduction of inhibitory activities against relevant enzymes [147] may modify the metabolic rate of a MM and interfere with its generation or removal. This might explain the flat post-HD concentration–time curve observed for pentosidine [146] and homocysteine [147], and the decrease in ADMA concentration up to 5 h after the end of HD [35]. All the above factors may impredictably affect the reliability of the reduction ratio as a method to quantify MM removal.

*Mean β2-m clearance of the session.* An equation based on a single compartment model has been proposed to estimate the β2-m clearance during a session [152]. Assuming extracellular β2-m distribution, negligible extra-dialytic clearance and solute generation during HD, the equation calculates an average clearance value for the whole session, including removal by diffusion, UF, and absorption.

\[
\frac{K}{\beta2-m} = \frac{Quf}{1 - \ln (Ct/Co)} / \ln (1 + Quf t/Vt)
\]

where: \( Co, Ct = \) start and end-session urea concentration; \( t = \) session time (minutes); \( Vt = \) end-session volume of the extracellular space.

This equation, accounting for treatment time, UF, and patient size, carries fewer drawbacks than other methods. However, disregarding the effect of post-HD rebound, it overestimates significantly the effective clearance of the session. By substituting the end-session concentration value with the value taken 30 and 60 min after the end, the equation yields results closer to those calculated from the arterial and venous concentration difference across the dialyzer [152]. However, much longer re-equilibration time has been reported by other authors in the case of β2-m [135].

*Dialysate quantification.* As a result of the extensive adsorption of most MM to synthetic membranes, direct quantification in the spent dialysate is not a precise method to quantify the removal of such compounds.

Apart from β2-m, the definition of an ideal marker of uraemic toxicity in the different ranges of the MM is far from being satisfactory. Lack of knowledge of the kinetics of these compounds also prevents to establish an adequate index to monitor the dialytic removal of MM toxins and compare different treatments and populations.

**Commentary on Guideline II.2.2**

**MM removal: effects of flux**

Partial removal of solutes in the size of the smaller MM may be achieved by diffusion even in conventional HD
with unsubstituted cellulose membranes. This has been proven in an acute in vivo controlled study using ofloxacin [126] (361 Da) as a surrogate MM. A reduction ratio by 82% has been found for free pentosidine (379 Da) concentration during an acute controlled study with cuprophan membrane [146].

Similar removal (~70%) of this low MM AGE has been observed by comparing a cellulose membrane with high-flux polysulphone (PS), polymethylmetacrilate (PMMA), and polyacrilonitrile (AN69) membranes [145]. Reduction by ~30% in homocysteine (135 Da) concentration has been shown with low-flux membranes [147], and in a randomized study comparing low-flux with high-flux PS [148]. Reduction ratios varying from 20 to 65% have been reported for ADMA (202 Da) during conventional HD [34,37,153]. In these studies the time of sampling was not specified. When reported, an increase in post-HD ADMA concentration was observed, followed by a 65% reduction 5 h after the end of the session [35].

High-flux HD with highly permeable membranes yields more substantial removal of larger solutes, as shown for low MW AGEs (<6 kDa) and for AGE peptides (<12 kDa) in acute controlled studies [154,155], and for AGE-apolipoprotein-B in a randomized study comparing AN69 vs low-flux PS [156]. A significant reduction in triglycerides and increase in high-density lipoprotein concentration and lipoprotein lipase activity have been reported as an acute effect of high-flux HD with PS membranes, not shown with a cellulose membrane [157].

Further enhancement and widening of the molecular spectrum of the removed uraemic compounds may be obtained with all the available highly permeable and biocompatible membranes on both haemofiltration (HF) and HDF. This has been demonstrated for β2-m [130,136,158–162], for some of the AGE compounds [14], for ADMA [163], for complement fractions, such as factor D (24 kDa) [161,164,165], fraction Ba (33 kDa) [165], C3a (8.9 kDa), C5a (11 kDa) [166], and for pro-inflammatory cytokines as TNF-α (17 kDa) [167,168], interleukin-1 (IL-1, 17 kDa) [167], IL-6, and IL-8 [168].

The mechanism by which MM removal occurs through high-flux membranes largely depends on the membrane itself. Several studies support some general conclusions [128,130,134,136,164,166–172]. The predominant mechanism with AN69 and PMMA is adsorption [128,136,168,169,171], while cellulose triacetate shows minor adsorption, and removes MM mainly by diffusion [127,130,168]. In the middle, polyamide shows intermediate characteristics and combines diffusion and convection with a minor adsorption capacity [168]. Polysulphone shows minor adsorption and removes MM mainly by filtration [173]. In general, the degree to which convection augments total solute removal is proportional to the MW of the solute and to the rate of UF [160]. The pore diameter, structure, and chemical properties of the membrane play also an important role [130,134,170,174].

Interactions between flux and biocompatibility

As quoted above, high-flux biocompatible membranes used in convective and mixed techniques seem to extend the amount and the molecular range of toxins removed. However, the role of the high flux is often difficult to dissociate from the biological effects of biocompatibility. In spite of similar dialytic removal, basal levels of pentosidine were lower in patients treated with high-flux PS membranes than with other high-flux or cellulose membranes, possibly as an effect of minor oxidative stress [145]. High-flux and low-flux PS resulted in similar plasma homocysteine levels in a 3-month longitudinal study, in spite of a significantly greater removal per session obtained with the high-flux membrane [148]. On the other hand, a significant reduction in homocysteine levels was obtained with superflux PS and cellulose triacetate [175]. The use of low-flux polyamide resulted in the most favourable ratio arginine/ADMA concentration when compared with high-flux polyamide during different dialytic strategies [163]. The role of flux in the improvement of lipid profiles with the use of synthetic membranes [176–179] has been claimed by some authors [178] and challenged by others [177]. As reported in a recent prospective randomized study, the use of low-flux and high-flux biocompatible membranes resulted in similar effects on lipoprotein and lipid profiles [148].

No studies have been published on the long-term biological effects of an increased AGE removal with high flux, highly biocompatible membranes. Instead, it is well established that patients treated with synthetic membranes show a reduction in basal β2-m compared with patients treated with cellulose membranes [129]. Biocompatible low-flux membranes induce a slower increase in plasma β2-m with time, independent of the influence of residual renal function [180]. Pre-HD β2-m concentrations are lower in high-flux HD and HDF than in conventional HD with cuprophan and low-flux biocompatible membranes [139]. Plasma β2-m is further reduced in high efficiency on-line HDF vs high-flux HD [161]. The last two studies point to a prominent role of flux in the reduction of plasma β2-m.

Even if an intensive extracorporeal treatment fails to return β2-m concentrations to normal [181], a reduction in plasma β2-m reduces the incidence of bone amyloidosis [141,142] and carpal tunnel syndrome [140,143,144] (discussed in Section III). The long-term use of synthetic membranes results in a better prevention of cardiovascular events [182] inflammation, malnutrition, and improves the outcome of therapy [140,144,183–187]. It remains unclear whether biocompatibility, or high-flux, or both, may explain these results. Two historically prospective studies on large database suggest that the reduced risk of mortality is associated with the enhanced MM removal promoted by high-flux membranes, independently from the effects related to their biocompatibility [186,188].