Beta-2 microglobulin and short Ig chains and organ damage in dialysis patients: An update.

Bengt Lindholm
Baxter Novum and Renal Medicine
Karolinska Institutet
Stockholm
Sweden
Question: Does retention of proteins/protein fragments such as beta-2 microglobulin and light chains contribute to complications or mortality in CKD and dialysis patients?

Answer: Yes. Accumulation of proteins/protein fragments others than beta-2 microglobulin are receiving increased attention.
A Sad but Forgotten Truth: The Story of Slow-Moving Solutes in Fast Hemodialysis

Sunny Eloot, Wim Van Biesen, and Raymond Vanholder
Nephrology Section, Department of Internal Medicine, Ghent University Hospital, Gent, Belgium

ABSTRACT

When trying to optimize hemodialysis adequacy, it can be questioned whether one should focus on the dialyzer or on the patient. Another crucial question is whether the currently applied dialysis adequacy parameter, $Kt/V_{urea}$, is a reliable marker. For the small and water-soluble solutes, recent advances in convective strategies and/or new dialyzer designs do not add much removal capacity. Depending on their specific kinetics, generally quite different from those of urea, small solute removal benefits from longer or more frequent dialysis. Clearance of beta-2-microglobulin ($\beta_2$M), a marker of middle molecule removal pattern, is improved with dialysis using more open and permselective membranes, as well as by using high convective volume strategies. Furthermore, longer and more frequent dialyses have highly favorable removal characteristics plasmatic and extraplasmatic compartments over which these molecules are distributed. As $\beta_2$M may not be representative of other middle molecules, future kinetic analyses of alternative middle molecules will be of the utmost interest. Protein-bound solute clearance is improved by convective techniques, but not by more open dialyzer pores. Knowledge of their kinetics should be helpful in interpreting the observation that frequent (but not longer) dialysis enhances protein-bound solute removal. Hence, further technical improvements in dialyzers will have only a minor impact on dialysis adequacy, as retarded solute movement in the patient plays a decisive role. As urea kinetics is not representative of the kinetics of protein-bound compounds, middle molecules, nor even of other small and water-soluble solutes, it becomes self-evident that urea clear-
β2 microglobulin (B2M) is a prototype for middle molecular weight toxins
Beta 2 microglobulin: 11,800 Da component of the major histocompatibility complex (MHC) class I/human leukocyte antigen (HLA) molecule located on nucleated cells

β2 microglobulin is a slow-moving middle molecular solute and implicated in complications in dialysis patients

B2M is shed from effete cells carrying the HLA class I molecule and circulates as an unbound monomer.
Dialysis related amyloidosis: History

- Carpal tunnel syndrome (CTS) in HD patients was first observed by Warren and Otiendo in 1975.
- In 1978, Kenzora from Japan reported that amyloid deposits were present in carpal tunnel biopsy specimens, and Assenat et al. reported association between amyloid deposits and presence of CTS.
- In 1985, Gejyo et al. identified a novel protein, β2-microglobulin, as the primary component of the amyloid protein in DRA.

Dialysis-related amyloidosis

• β2-microglobulin is the major protein component of dialysis-related amyloidosis, which results from high extracellular concentration and posttranslational modification of B2M and a number of other promoters of amyloid fibril formation and deposition in osteoarticular tissues.

Key Processes in Dialysis Related Amyloidosis

Shed from the MHC I heavy chain of effete cells.

The glomerulus filters β2m, which is then catabolized in the proximal tubules.

Only a few micrograms of β2m appear in the urine in normal subjects.

Distributed in the extracellular space but in renal failure it is transported into tissues in which it eventually polymerizes to form characteristic amyloid.

β2 microglobulin and dialys-related amyloidosis

• β2-M generated at a rate of about 2.4 mg/kg/day and is eliminated by kidneys.
• In ESRD patients, serum β2-M levels are in the range of 20–50 mg/l (and occasionally higher than 100 mg/l).
• **Important risk factors**: age, duration of chronic renal failure, and cumulative years on dialysis.
• **Enhanced production in response to**: inflammation, acidosis, calcitriol treatment, and dialysis technique modality.
• Elevated B2M levels are observed also in hematological, immunodeficiency, and autoimmune diseases.
Dialysis related amyloidosis: Current status

No fundamental treatment but elimination of ß2-MG is thought to be more effective, and the onset of DRA prevented, by using:

- High-flux dialysis membranes
- High-volume hemodiafiltration.
- Hemoadsorption therapy

Dialysis related amyloidosis: Current status

• Effective removal of B2M can be achieved with highly effective HD and hemodiafiltration techniques but **pre-dialysis session serum levels cannot be normalized**.

• The prevalence and severity of B2M amyloidosis appear to have decreased in the last 20 years, although its occurrence may simply be delayed.

Among 385 HD patients (33% biocompatible membranes, 45% high-flux dialysis, 45%), 31.7% had clinical signs of carpal tunnel syndrome.

Significant predictors of CTS were: age, female gender, serum β2M, total protein, dialysis with non-biocompatible high-flux dialysis compared to non-biocompatible low-flux dialysis, Kt/V and serum concentration of CML (OR 2.47 for the 3rd vs. 1st quartile, 95% CI 1.229–4.961, p = 0.011).

The prevalence of CTS (31.7%) as a possible manifestation of dialysis-related amyloidosis is still high.
Prevalence of CTS is still high

- Prevalence of CTS in this HD cohort was twice as high (31.7%) as in the general population (in which prevalence varies from 5 to 16% Aroori S, Spence RA: Ulster Med J 2008;77:6–17)

- Slightly lower β2M concentration in HD patients treated with biocompatible vs non-biocompatible membranes (median 38.2 vs. 43.2 mg/l, p = 0.026) but β2M concentration still up to 30 times higher than in healthy subjects.

Survival implications of retention of β2-M?

- In a subanalysis of the HEMO study mean cumulative predialysis serum β2-M levels were associated with all-cause mortality with optimal survival observed for levels below 27.5 mg/l.

- It is unclear whether aiming for even lower threshold serum B2M levels might provide additional benefit.

Drüeke TB, Massy ZA. Semin Dial. 22(4):378-80, 2009
Impact of convective therapies versus conventional HD

- A meta-analysis of 20 studies totaling 657 patients: No significant clinical advantage of convective therapies over HD in terms of mortality, treatment-related hypotension, and hospitalization.

- However, in a subset of these studies, pretreatment B2M was near-significantly lower among patients receiving convective therapies.

Plasma beta-2 microglobulin is associated with cardiovascular disease in uremic patients

Sophie Liabeuf\textsuperscript{1,2,3}, Aurélie Lenglet\textsuperscript{1,2,3}, Lucie Desjardins\textsuperscript{1,2,3}, Nathalie Neirynck\textsuperscript{4}, Griet Glorieux\textsuperscript{4}, Horst-Dieter Lemke\textsuperscript{5}, Raymond Vanholder\textsuperscript{4}, Momar Diouf\textsuperscript{6}, Gabriel Choukroun\textsuperscript{1,3,7} and Ziad A. Massy\textsuperscript{1,2,3,7}, on behalf of the European Uremic Toxin Work Group (EUTox)

\textsuperscript{1}INSERM U-1088, Amiens, France; \textsuperscript{2}Division of Clinical Pharmacology, Clinical Research Centre, Amiens University Hospital, Amiens, France; \textsuperscript{3}Jules Verne University of Picardy, Amiens, France; \textsuperscript{4}Nephrology–Dialysis–Transplantation, Department of Internal Medicine, University Hospital, Ghent, Belgium; \textsuperscript{5}EXcorLab GmbH, Obernburg, Germany; \textsuperscript{6}Clinical Research Department, Amiens University Hospital, Amiens, France and \textsuperscript{7}Division of Nephrology, Amiens University Hospital, Amiens, France

Higher B2M levels were independently associated with overall and cardiovascular mortality and cardiovascular events in the whole cohort and with cardiovascular events in the pre-dialysis cohort.
Plasma B2M vs CKD stage

*P<0.0001 vs. control group; **P<0.0001 vs. CKD stages 2 and 3; and ***P<0.0001 vs. CKD stages 4 and 5.

- 142 patients at different CD stages
- Baseline B2M levels were associated with vascular calcification but not with arterial stiffness or bone density.
B2M vs eGFR in 96 predialysis CKD stages 2–5 patients

$r^2=0.789, \, P<0.0001$}

Liabeuf S et al. Kidney Int. 2012 Aug 15
Probability of cardiovascular event-free survival for pre-dialysis patients as a function of median plasma B2M

Results were adjusted for propensity score, which included age, albumin, C-reactive protein, hemoglobin, phosphate levels, aortic calcification score, and estimated glomerular filtration rate.

- During a mean follow-up of 969 days, 44 pts died and 49 suffered a cardiovascular event.
- Higher B2M levels were independently associated with overall and cardiovascular mortality and cardiovascular events in the whole cohort and with cardiovascular events in the predialysis cohort.
- B2M appeared to be a better predictor than well-established factors associated with outcomes in this population, such as eGFR and inflammation biomarkers.

Liabeuf S et al. Kidney Int. 2012 Aug 15
Higher serum B2M levels associated with *better* survival in chronic HD patients


- Retrospective and cross-sectional study with 95 all-cause deaths among the 289 patients.
- Serum B2M higher in survivors vs non-survivors (36.8 ± 12.3 vs. 32.6 ± 13.2 µg/ml, p = 0.009)
- Cox: Elevated B2M levels associated with *lower* mortality rate (relative risk: 0.608; 95% CI: 0.37 to 0.99; p = 0.046).
- B2M was positively correlated with protein intake (nPNA), duration of HD, BMI, and concentrations of creatinine, albumin, BUN, and hs-CRP
Other proteins/peptides than B2M may also cause disease
Incidence and outcome of patients starting renal replacement therapy for end-stage renal disease due to multiple myeloma or light-chain deposit disease: an ERA-EDTA Registry study

Dimitrios J. Tsakiris, Vianda S. Stel, Patrik Finne, Emily Fraser, James Heaf, Johan de Meester, Sabine Schmaldienst, Friedo Dekker, Enrico Verrina and Kitty J. Jager

- Of 159,637 patients on RRT, 1.54% had multiple myeloma (MM) or light-chain deposit disease (LCDD).
- Increasing incidence
- Poor survival
- Unadjusted median survival on RRT was 0.91 yrs in MM and LCDD patients and 4.46 yrs in non-MM patients.
Incidence of RRT for ESRD due to MM per million population, by cohort, standardized for age and gender using the European standard population of 1995 as reference (upper panel) and the percentage of new patients on RRT for ESRD due to MM, by cohort (lower panel).

1.8% of new patients in the 2001-2005 cohort

Unadjusted patient survival on RRT (upper panel) and on dialysis (lower panel) since day 1 in MM and non-MM patients.

Pathophysiology of amyloidosis: Many different precursors

- Increase production
  - Proteolysis
  - Mutation
  - Aging
  - Decrease catabolism

- Circulating precursor of amyloid protein:
  - Immunoglobulin light chain
  - SAA
  - TTR
  - β2M
  - ApoA-I or apoA-II

- Partial proteolysis (extracellular or intracellular) processing
- Blood vessel deposition
  - Mechanical toxic effects
  - Apoptosis

- Misfold protein oligomers
- Amyloid fibril deposition

- Tissue damage and dysfunction
  - Kidney
  - Musculoskeletal system
  - Heart
  - Gut
  - PNS
  - Liver
  - Spleen

- GAGs, SAP

Perfetto, F. et al. Systemic amyloidosis: a challenge for the rheumatologist
Nat. Rev. Rheumatol. 2010
At least 27 precursor proteins have been identified to result in either local tissue or systemic amyloidosis, with 9 of them manifesting in cardiac deposition and resulting in a syndrome termed "cardiac amyloidosis" or "amyloid cardiomyopathy.

Although cardiac amyloidosis has been traditionally considered to be a rare disorder, as clinical appreciation and understanding continues to grow, so too has the prevalence, suggesting that this disease may be greatly underdiagnosed.

The most common form of cardiac amyloidosis is associated with circulating amyloidogenic monoclonal immunoglobulin light chain proteins.
Current perspectives on cardiac amyloidosis


• Cardiac amyloidosis, particularly amyloidogenic immunoglobulin light chain protein cardiomyopathy (AL cardiomyopathy), results in a rapid decline in cardiac function, with symptomatic heart failure and development of amyloid cardiomyopathy.

• Most standard heart failure therapies, such as angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, and digoxin have low efficacy in treating this disease, partly because they are all poorly tolerated.

• 1.3-yr median survival from diagnosis in AL cardiomyopathy, comparable with the most aggressive forms of cancers
## Common types of cardiac amyloidosis: precursors, prevalence and prognosis

<table>
<thead>
<tr>
<th>Precursors/ Disease type</th>
<th>Prevalence / Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Immunoglobulin light chain</strong>/Primary amyloid cardiomyopathy</td>
<td>50% in pts with AL amyloidosis. Median survival-11 mo</td>
</tr>
<tr>
<td><strong>Mutant transthyretin</strong>/Familial amyloid cardiomyopathy</td>
<td>30% in pts with familial amyloidosis. Median 9–13 yr</td>
</tr>
<tr>
<td><strong>Wild-type transthyretin</strong>/Senile systemic amyloid cardiomyopathy.</td>
<td>In most of pts with senile amyloidosis. Median survival-75 mo</td>
</tr>
<tr>
<td><strong>Serum amyloid A</strong>/Secondary amyloid cardiomyopathy</td>
<td>Rare, only 2% in pts with AA amyloidosis. Good</td>
</tr>
<tr>
<td><strong>Atrial natriuretic factor</strong>/Isolated atrial amyloidosis</td>
<td>Very common (&gt;40% in people &gt; 50 years). Good prognosis</td>
</tr>
</tbody>
</table>

Amyloid fibril deposition in heart tissue affected by amyloidogenic immunoglobulin light chain protein cardiomyopathy (AL cardiomyopathy)

Amyloid fibrils
nonbranched rigid fibrils
10 nm diameter

Collagen fibrils
much larger diameter
Immunoglobulin light chains

• Overproduction, release, retention and deposition of immunoglobulin free light chains from B cells

• These light chains come together to form amyloid deposits in different organs.
The antibody is composed of 2 immunoglobulin heavy chains and 2 light chains. There are two types of light chains:

- kappa (κ) chain MW 22.5 kDa,
- lambda (λ) chain: MW dimeric λ 45 kDa.
Measurement of immunoglobulin free light chains in serum

- Alternative to the analysis of Bence Jones proteins in urine.
- **The ratio of kappa-to-lambda free-light chains** indicates whether the individual might have a plasma cell tumour such as multiple myeloma or AL amyloidosis.
Ratio of kappa to lambda Ig light chains

Antibodies are produced by B lymphocytes, each expressing only one class of light chain. Once set, light chain class remains fixed for the life of the B lymphocyte.

- In a healthy individual, the total kappa to lambda ratio is roughly 2:1 in serum (measuring intact whole antibodies) or 1:1.5 (ratio of 0.66) if measuring free light chains, with a highly divergent ratio indicative of neoplasm.

- The normal ratio of kappa to lambda, according to a novel polyclonal free light chain assay, ranges from 0.26 to 1.65\(^a\).

- Both the kappa and the lambda chains can increase proportionately, maintaining a normal ratio. This is usually indicative of something other than a blood cell dyscrasia, such as kidney disease.

\(^a\)Katzmann JA et al. Clin Chem 48 (9): 1437-44
Serum immunoassays for the measurement of immunoglobulin free light chains

- Resulted in **paradigm shift in the diagnosis, assessment and monitoring of patients with plasma cell dyscrasias**

- **Plasma cell dyscrasias**: monoclonal gammopathy, multiple myeloma, solitary plasmacytoma of bone, extramedullary plasmacytoma, Waldenström's macroglobulinemia, primary amyloidosis, light chain deposition disease and heavy-chain disease.

Immunoglobulin light-chain amyloidosis needs to be considered in any patient presenting with cardiomyopathy with:

- preserved systolic function,
- heavy albuminuria,
- an unexplained sensorimotor peripheral neuropathy,
- hepatomegaly,
- atypical MGUS (monoclonal gammopathy of undetermined significance) or multiple myeloma.

Monoclonal Gammopathy of Undetermined Significance (MGUS)

An asymptomatic premalignant condition characterized by production of immunoglobulin from a clone of bone marrow plasma cells.

Differs from the associated malignant conditions by the following criteria:

• monoclonal protein (termed the M-protein or paraprotein) measures $<3 \text{ g/dL}$,
• $<10\%$ monoclonal plasma cells on bone marrow examination,
• absence of end organ damage.

Screening for a monoclonal protein is a common part of the assessment of patients presenting with a renal injury.

Monoclonal proteins can be associated with **cast nephropathy**, **amyloidosis**, and **light chain deposition disease**.

Light chain deposition disease

• Abnormal light chains (= "M-protein" or "Bence Jones protein").

• Light chain deposition disease (LCDD): deposition in organs of light chains.

• The kidneys are almost always affected and this often leads to kidney failure.

• About 50% of patients with light chain deposition disease also have multiple myeloma.

• Unlike in AL amyloidosis with characteristic amyloid deposits, light chains are deposited in non-amyloid granules in LCDD.

Immunoglobulin light-chain amyloidosis: Treatment

- The prognosis is determined by the levels of cardiac biomarkers and immunoglobulin free light chains.

- All patients with systemic light-chain amyloid require therapy.

- Stem-cell transplantation produces a high response rate but is a viable option in only 20% of patients.

- Corticosteroids, alkylating agents, immunomodulatory drugs, and proteasome inhibitors all have shown activity in this disorder, and combinations are currently being explored in clinical trials.

- Despite advances in the past decade, 30% of patients still die within a year of diagnosis.
Immunoglobulin free light-chain (Ig-FLC) and allergic disease

• Immunoglobulin free light-chain (Ig-fLC) might be involved in the pathophysiology of allergic disease atopic dermatitis, cow's milk allergy, allergic rhinitis, or asthma.

• Increased plasma kappa and lambda Ig-fLC concentrations were found in children with atopic dermatitis.

Non-clonal serum immunoglobulin free light chains predict survival in the general population

- 15,859 US residents >50 years in whom unmasked data and samples for κ and λ FLC sum (Σ FLC) testing were available.
- **A high Σ FLC predicted worse overall survival;** the risk ratio for death for those with the highest decile of Σ FLC (≥ 4.72 mg/dL) was 4.4 (95% confidence interval, 4.1-4.7) relative to the remaining study participants.
- Multivariate analyses demonstrated that this excess risk of death was independent of age, sex, and renal insufficiency, with a corrected risk ratio of 2.1 (95% confidence interval, 1.9-2.2).
- Conditions resulting in overactivation of the immune system may have contributed to the excess risk of death.
- Free light chains may be a link in the connections between inflammation and atherosclerosis.

Increased concentrations of free light chain lambda in sera from HD patients.


- Serum levels of FLC were determined in 5 pre-HD CRF patients, 63 CRF patients receiving HD therapy, 15 CAPD and 40 healthy volunteers.
- The highest serum FLC concentration was observed in patients receiving HD.
- Concentrations of kappa- and lambda FLC were about 4-fold and 1.8 fold greater than control values in HD patients, with a predominance of lambda chains.
- This HD-associated elevation of serum FLC strongly suggests a role for FLC in the etiology of light chain deposit disease and AL-amyloidosis.
- Lambda-FLC and beta 2-m exhibited similar patterns of change in the serum during the course of HD therapy.

• A sensitive, quantitative immunoassay was used to analyze serum and urinary polyclonal FLC in 688 patients with CKD of various causes.
• Results: Serum κ and λ FLC concentrations increased progressively with CKD stage (both $P < 0.001$) and strongly correlated with markers of renal function, including cystatin-C ($κ: R = 0.8, P < 0.01$; and $λ: R = 0.79, P < 0.01$).
• Urinary FLC concentrations varied significantly between disease groups ($κ: P < 0.001; λ: P < 0.005$) and also rose significantly with increasing CKD stage (both FLC $P < 0.0001$).
• **Urinary FLC concentrations were positively correlated with their corresponding serum concentration** ($κ: R = 0.63; λ: R = 0.65$; both $P < 0.001$) and urinary albumin creatinine ratio ($κ: R = 0.58; λ: R = 0.65$; both $P < 0.001$).
Serum free light chain (FLC) concentrations in patients with CKD. Both $\kappa$ and $\lambda$ FLC increased progressively with each CKD stage (both $P < 0.0001$).

Hutchison C A et al. CJASN 2008;3:1684-1690
Serum FLC ratio in patients with CKD. The FLC ratio increased progressively through stages 1 through 5 CKD.

Hutchison C A et al. CJASN 2008;3:1684-1690
Can light chains be removed by dialysis?
HD using high cut-off dialysers for treating acute renal failure in multiple myeloma

• Six patients diagnosed with MM and ARF requiring dialysis and with serum free light chain levels above 500mg/l were treated with 8-hour HD sessions with an HCO-HD filter.
• Before and after each session, serum free light chain levels were measured by nephelometry; other parameters were recorded as well.
• At the same time, patients underwent chemotherapy according to protocols.

HD using high cut-off dialysers for treating acute renal failure in multiple myeloma

- Free light chain levels decreased by a mean of 65% between treatment onset and completion.
- The mean percentage of reduction of light chain levels per session was $54.98\% \pm 17.27\%$.
- There were no major changes in pre-dialysis albumin, calcium, phosphorous or magnesium levels.
- Renal function was recovered in 3 patients.

Vallée et al analyzed free light chain removal in 10 HD and 17 HDF sessions in 6 patients with multiple myeloma.

Superiority of high-efficiency HDF over high-flux HD for serum FLC removal in multiple myeloma patients on RRT.

No negative impact on total plasma proteins was noted.
HD and especially HDF resulted in significant reduction (%) of FLC in kappa (KL) and lambda (LL) FLC after correction for fluid removal.

Beta-2 microglobulin and short Ig chains and organ damage in dialysis patients: Summary

- **B2M** is still a concern.
- Other proteins/protein fragments such as light chains are **important risk factors for mortality** due to organ damage (kidneys, heart and other organs).
- At least **27 proteins/protein fragments** from various cell types act as **amyloidogenic precursors** causing heart and kidney damage. Implications in general population and in CKD are not fully known.
- Improved diagnostic tools such as determination of serum light chains, and improved therapeutic options including also extracorporeal removal of protein fragments provide new opportunities to address this problem.
Thank you