Dialysis-related amyloidosis

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Introduction

Dialysis-associated amyloidosis is a complication of end-stage failure patients which has been recognized a decade ago. It generally occurs after several years of renal replacement therapy [1,2]. However, it may be seen occasionally in patients with long-standing, severe renal failure who are not yet treated by dialysis or related procedures [3,4]. It is mainly localized in osteoarticular structures. It is often associated with the occurrence of the carpal tunnel syndrome and painful, chronic arthropathies that may evolve to progressive joint destruction.

In 1985, two groups of workers showed that β2-microglobulin (β2M) was the major constituent protein of dialysis-associated amyloidosis [5,6], even though a number of other proteins have been found since in the amyloid deposits as well, including serum-derived amyloid P component (SAP), glycosaminoglycans, proteases, antiproteases, ubiquitin, and immunoglobulin light chains [7,8]. The β2M protein accumulates in the plasma of chronically uremic patients, and this accumulation is almost certainly a major contributing factor in the formation and tissue deposition of β2M amyloid and associated pathological conditions. However, several other factors appear to play an important role as well which are discussed below.

Clinical features of β2-amyloidosis and diagnostic procedures

Carpal tunnel syndrome

The carpal tunnel syndrome (CTS) is a prominent, relatively early feature of β2M amyloidosis. An increasing prevalence of shoulder pain and CTS with time on haemodialysis treatment was initially reported by Charrat et al. [9]. It is of note that nearly all of their patients suffered from CTS after 15 years of intermittent haemodialysis treatment with cuprophane membrane and particularly long dialysis sessions. This observation has been confirmed subsequently by many others, albeit often with a lower incidence of CTS.

Amyloid arthropathy

The deposition of β2M amyloid clearly is associated with the syndrome of chronic arthralgias and arthropathy in dialysis patients, although the precise pathogenetic role played by amyloid deposits still needs to be clarified. Its incidence augments with increasing time on dialysis and with ageing [10]. The condition has been described mainly in patients dialysed with the cuprophone membrane but the use of synthetic, highly permeable dialysis membrane offers no absolute long-term protection. It would appear that once the amyloidogenic process has started it is difficult, if not impossible, to stop it completely, even by changing the dialysis procedure.

The chronic arthralgias are usually bilateral and often involve the shoulders initially. Other joints, in particular the knees, wrists and small joints of the hands, may be involved as well. Chronic joint swelling is another important feature of the disease, as may be recurrent haemorrhage and chronic tenosynovitides of the finger flexors.

Destructive arthropathies of large peripheral joints and of the spine may ensue, causing major incapacity. Massive amyloid deposits are almost constantly observed at the site of such lesions [11,12]. They are observed in haemodialysis patients and in CAPD patients as well. Destructive spondylarthropathies involve most frequently the cervical spine. They may be asymptomatic or lead to mild spinal pain. In rare instances, however, they may cause nerve root compression [13,14].

Diagnostic procedures

The histological demonstration of β2M amyloid deposits remains the gold standard of the diagnosis. Clinical evidence grossly underestimates the degree of
Table 1. Clinical features of β2-M amyloidosis and diagnostic procedures

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<td>Chronic arthralgias (usually starting in the shoulder)</td>
<td>Carpal tunnel syndrome</td>
<td>Capsulol synovial thickening (shoulder rotator cuff, supraspinatus tendon, biceps tendon, femoral neck capsule)</td>
<td>Subchondral bone erosions and cysts</td>
<td>Bone scan: increased focal uptake of tracer (99mTc-labelled diphotonate, 123I-labelled P component, 131I-labelled β2-microglobulin)</td>
<td>Bone scan: increased focal uptake of tracer (125I-SAP) as a tool for the evaluation of disease activity</td>
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<td>Decreased joint mobility</td>
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<td>Localization and extension of infiltrative amyloid masses</td>
<td>Destructive arthropathy and spondylarthropathy</td>
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<td>Periarticular soft tissue swelling</td>
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<td>Visualization of the occipito-cervical junction</td>
<td>Subchondral bone erosions and cysts</td>
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<tr>
<td>Carpal tunnel syndrome</td>
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<td>Bone fractures</td>
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<td>Pathological fractures (especially in hip region)</td>
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<td>Destructive arthropathy and spondylarthropathy</td>
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<td>Subcutaneous amyloid mass (rare)</td>
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<tr>
<td>Bone scan using radiolabelled isotope uptake in areas of radiologically involved joints [23]. However, such articular hyperfixation is not specific for dialysis amyloidosis since it may be observed in synovitides of other origin as well. Scintigraphic procedures using radiolabelled P component (SAP) [24] or radiolabelled β2M [25] are able to localize amyloid deposits more specifically. However, they provide at best similar, though frequently less clear-cut, scintigraphic features compared with that obtained by radiolabelled diphosphonates. Moreover, the site-specificity is relatively poor. Since scintigraphy yields limited quantitative information concerning the progression of the disease, our group recently applied the technique of radiolabelled SAP plasma kinetics to dialysis-associated amyloidosis in an attempt to quantify the disease process [26]. We found that whereas healthy subjects and patients on hemodialysis treatment for short time periods had a monoeponential shape of the plasma [125I]SAP curve, patients on prolonged haemodialysis therapy and suffering from severe dialysis arthropathy had a biexponential decay. The latter allowed to derive an additional extravascular distribution volume, probably corresponding to the amyloid mass or the activity of the amyloidogenic process. Such radionuclide methods could allow one to evaluate the extent of amyloid deposits and to monitor their progression objectively, without having to rely on invasive exploration procedures.</td>
<td>Bone scan: increased focal uptake of tracer (125I-SAP) as a tool for the evaluation of disease activity</td>
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Table 1 summarizes the main clinical and paraclinical features of β2M amyloidosis as well as the main presently available imaging techniques.

Differential diagnosis of cystic lesions

Brown tumours of secondary hyperparathyroidism may be difficult to differentiate from amyloid cysts in dialysis patients. However, they are usually associated with other features of severe ostestitis fibrosa and may expand to the bone cortex, in contrast to amyloid tumoral cysts. Their distribution is frequently different since they usually affect the metaphysis or diaphysis of tubular bones, the jaws, ribs and iliac wings, whereas the distribution of amyloid cysts is restricted to the vicinity of synovial joints. Although brown tumours may also affect the epiphysseal bone in the vicinity of synovial joints, subchondral bone cysts in long-term dialysis patients are far more frequently due to amyloid cysts.

Systemic amyloid deposits

Systemic β2M amyloid deposits may occur in long-term haemodialysis patients. Most often they are faint and of no clinical importance [27] but in exceptional patients they may cause serious complications such as bowel infarction and perforation [28–30]. The involved organs are the heart, the gastrointestinal tract, and the lungs, and smaller deposits may be seen in medium-sized blood vessels of virtually all visceral organs [27,31]. Surprisingly, only the spleen appears to be devoid of β2M amyloid deposition [31,32]. The observations of systemic deposits raise the concern that the development of β2M amyloidosis in dialysis patients may be a threat not only to their well-being but also to their life in rare instances. The possibility has also
to be considered that another type of amyloid fibril may coexist in the exceptional case. This can occur either at the same location [33] or at different tissue sites, according to the usual predilection site of each form of amyloidosis [34].

Plasma β2-microglobulin, renal failure and renal replacement therapy

Retention of β2M due to renal failure

During chronic renal insufficiency, the decrease in glomerular filtration, tubular reabsorption and tubular catabolism contributes to a progressive increase of β2M in plasma, in parallel with the ascension of plasma creatinine [35]. At the time when dialysis treatment is started, plasma concentrations are usually greater than 15 mg/l. Such high levels are never attained in non-uraemic inflammatory conditions in which plasma β2M generally does not increase beyond 5 mg/l. Extremely high plasma β2M levels are generally reached in the oligo-anuric state. It is possible that in addition to enhanced β2M generation (see below) the accumulation in plasma of β2M homologues such as granulocyte inhibitory proteins GPI and GPII, which cross-react with commercially available assays for intact β2M [36,37], account at least in part for the excessive values in anuric subjects.

To date, β2M amyloid deposits have been observed exclusively in uraemic patients. This indicates that a longstanding, marked increase in β2M plasma concentration is probably a prerequisite for dialysis amyloidosis to occur. However, no relation exists between the height of the plasma β2M concentration and the apparent extent and degree of severity of β2M amyloidosis [38]. Factors other than the increase in circulating β2M are almost certainly involved in formation and the deposition of amyloid fibrils (see below).

Influence of haemodialysis treatment

The influence of the haemodialysis procedure on the kinetics of circulating β2M levels under various conditions has been examined in many studies. The physical characteristics of the dialysis membrane, namely its structure, surface area, permeability and biocompatibility, play an important role in the change of plasma β2M during the dialysis session, allowing to obtain either no removal or a more or less marked subtraction via diffusion, convection, and/or adsorption. Thus, the use of standard cuprophane and cellulose acetate membranes does not allow to remove significant amounts of β2M whereas the use of highly permeable membranes, such as the polysulphone F-60 or the polycrylonitrile AN-69 membrane, allows to clear more or less marked amounts of the polypeptide [39,40] and to decrease the predialysis plasma β2M level consistently [41,42].

Production of β2PM

Whether the daily β2M production rate is increased or not in stable dialysis patients is still uncertain. It has been estimated to be comparable to that of healthy subjects, based on radioisotope dilution methods [43–45]. However, methodological limitations would not allow to detect small differences in vivo. In addition, the production rate probably differs from patient to patient, and even in a given individual it may vary, for instance in association with inflammatory events. This could explain discrepant results between studies done in vivo and in vitro. Some of the latter were suggestive of an increase in β2M production by mononuclear cells in contact with the cuprophane membrane, but not after exposure to more biocompatible membrane [46,47]. The amount of β2M which is produced day by day must be eliminated through extrarenal, still unknown pathways. It is possible that most of the accumulated protein is deposited as amyloid fibrils in osteocartilagi structures. An alternative possibility would be that in advanced chronic renal failure the relative contribution of extrarenal catabolic pathways to the overall elimination of the protein is increased.

Pathophysiological considerations of β2M-amyloidosis

At present, the pathogenesis of β2M amyloidosis is still incompletely understood. Theoretically, amyloidogenesis can result from enhanced production of amyloid fibrils, from diminished degradation, or from both. It is generally believed that in all types of amyloid disease each specific β-pleated fibril protein has a serum precursor molecule [48]. Two key processes could be involved, namely a mechanism whereby a stimulus invokes alterations in the serum concentration or the primary structure of amyloid precursor proteins, and in addition a step that involves processing or conversion of the precursors to amyloid fibrils [48]. Whether amyloid fibrils, once deposited, can subsequently undergo degradation in vivo has never been conclusively demonstrated.

Formation of β2M amyloid fibrils in vitro and contribution of local factors

The application of such general rules of amyloidogenesis to the particular type of dialysis-associated amyloidosis poses some difficulties. The marked increase of serum β2M in advanced renal failure certainly is a minimum requirement. In fact, Connors et al. [49] described the in vitro formation of amyloid fibrils, using an extremely high concentration of native β2M protein, in the absence of any proteolytic treatment. Subsequently, Campistol et al. [50] succeeded in inducing the formation of β2M amyloid fibrils in the supernatant of peripheral blood mononuclear cells maintained in culture. Spontaneous fibril generation was observed with cells from dialysis patients, but not with cells from healthy volunteers. In a recent
experimental study, Ono and Uchino obtained the formation of amyloid-like substance in vitro from human urine-derived β2M in the presence of serum amyloid P (SAP) component, even though white blood cells were absent [50a]. When omitting SAP or leaving only glucosaminoglycans in the incubation medium, no amyloid fibrils were formed. The latter two experiments indicate that β2M modified by the uremic state as well as SAP are required for amyloid fibrillogenesis.

Several local factors may be involved, including the local enrichment, generation, polymerization, and/or failure of degradation of native or modified amyloid precursor molecules, the contribution of a chronic inflammatory process, changes of other amyloid components such as glucosaminoglycans [51,52], and disturbed interactions between proteinases and proteinase inhibitors such as s2-macroglobulin [53].

**Modified β2M**

Linke et al. [54] described β2M truncation by limited proteolysis occurred, and suggested that such changes might be required for amyloid formation. However, their finding could not be confirmed by others [55,56], thus rendering the hypothesis of this type of amyloidogenic transformation of the protein unlikely.

In the search for a better understanding of the amyloidigenic process, a new mechanism has been proposed recently, based on the observation of β2M protein modified with advanced glycation end-products (AGE) [57,58]. The modified protein could correspond to β2M molecules with a more acidic isoelectric point found by others [55,59]. However, AGE-modified β2M is only one of several constituents of acidic β2M which are present in the circulation and in amyloid deposits [60]. The AGE-transformed protein has been found to be modified with pentosidine by one group [61], and with N4-(carboxymethyl)lysine by another [62]. AGE-β2M binds to specific receptors, named RAGE [60], and thereby elicits various cellular reactions. The latter include the capacity to enhance directed migration (chemotaxis) and random cell migration (chemokinesis) of human monocytes in vitro [58], and to induce an enhanced secretion by activated monocytes of the bone resorbing cytokines, IL-1β and TNF-α [58,60]. The authors suggest that AGE-β2M could participate in the pathogenesis of dialysis-associated amyloidosis by enhancing focal monocyte/macrophage accumulation and initiating an inflammatory process which would ultimately lead to bone and joint destruction. This suggestion is in keeping with a report of augmented in vitro cytokine production (IL-1 and IL-6) by tenosynovium obtained from long-term haemodialysis patients [63].

Another type of β2M modification which might play a role as well, is the formation of advanced oxidation protein products (AOPP). They are markedly increased in uremia, in close association with plasma AGE-pentosidine and dityrosine levels [64]. The occurrence of enhanced oxidative stress during the haemodialysis session was reported by the Necker group many years ago [65], and exposure of β2M to hydroxyl radicals has been shown to lead to aggregation and dityrosine formation [66]. Oxidant-induced protein damage in dialysis patients, as reflected by high AOPP levels, could be an important component in uremia-related inflammatory processes, including the dialysis-associated arthropathy of the β2M type.

In vivo conditions of β2M amyloid formation

Since β2M amyloidosis has initially been described only in patients treated by long-term, intermittent haemodialysis a relation between the dialysis technique and the deposition of β2M amyloid fibrils has been hypothesized [1]. Subsequently, several pieces of indirect evidence have been provided for a role of the still widely used cuprophane membrane. Thus the prevalence of dialysis amyloidosis, either in terms of subchondral erosions and destructive arthropathy [10,22] or in terms of the carpal tunnel syndrome [67] has been reported in retrospective, carefully conducted studies to be higher in patients treated exclusively with standard cuprophane membranes than in those dialysed predominantly or exclusively with the more permeable and biocompatible polycrylonitrile AN-69 membrane. In contrast, others have not been able to identify a difference in the prevalence of dialysis amyloidosis with the use of different haemodialysis membranes [68,69], probably because of a less rigorous definition of the criteria used for the diagnosis of dialysis-associated amyloidosis and because of a relatively small population size in one of these two negative studies [70].

However, several observations argue against a major, lest unique, role of a particular dialysis membrane. First of all, rare cases of β2M amyloidosis have been reported to occur even in patients exclusively haemodialysed with the AN-69 polycrylonitrile membrane [70]. Secondly, uraemic patients treated by CAPD [71] or long-term intermittent haemofiltration [72] may develop β2M amyloidosis as well. Thirdly, our attempts to stimulate β2M generation from blood cells in vitro, in the presence of cuprophane membrane fragments, have been unsuccessful [40] even though more recently others have been able to obtain such a stimulation [46]. Fourthly, the degree of bacteriological quality of the dialysis fluid may also play a role, possibly by endotoxin contamination of the dialysate [73].

The question of whether β2M amyloid deposits may regress under certain conditions, in particular after renal transplantation, remains matter of controversy. One group recently found a decreased uptake of radio-labelled P component on scintigraphy after successful kidney grafting [74]. They claimed that this would indicate regression of dialysis amyloidosis. However, three other groups, including our own, suggested that no regression occurs after renal transplantation, based on radiographic and histological findings [75–77]. It is probable that the scintigraphic findings using radio-labelled P component reflect amyloid disease activity rather than actually present amyloid deposits.
Table 2 summarizes the various mechanisms which are potentially involved in the pathogenesis of β2-M amyloidosis.

Is β2M amyloid an innocent bystander or an active player?

This question is of major pathophysiologic interest. Several experimental and clinical findings are in favour of an active role. Thus native β2M, and even more so AGE-β2M, are capable of directly enhancing bone resorption via the induction of IL-1, IL-6 and TNF [78,79]. The growth factor-like activity ascribed to β2M could also be mediated by insulin-like growth factor-I and its receptor [80]. However, the claimed active role of β2M as a bone cell mitogen has been questioned by another group of authors [81]. A preferential collagen binding affinity of β2M has been reported which was dependent on the concentration of β2M and also on the amount of collagen present in the in vitro preparation [82]. The latter finding may explain the predilection of β2M amyloid for collagen-rich tissues such as joints. Another effect of β2M with potential relevance for the pathogenesis of dialysis arthropathy is its capacity of inducing the synthesis of fibroblast collagenase, similarly to the effect of serum amyloid A, thus regulating collagen breakdown [83].

Finally, in a recent clinical study, we have found an association of high serum β2M levels with increased bone cell number and serum markers of bone turnover in chronic haemodialysis patients [84]. A similar association between plasma β2M and indices of bone resorption has been observed in osteoporotic post-menopausal women without renal failure [85]. These observations suggest that β2M is either an activator of bone cells or at least another marker of bone cell activity.

Although all of the above mentioned actions of β2M in vitro and the pathophysiological relevance of various studies in vivo need further confirmation, these findings are in favour of the hypothesis that the polypeptide, especially after post-translational modification, is not an inert molecule but an active player which exerts biological activity on bone and joint tissues, particularly in concentrations as high as those found in end-stage renal failure patients.

References


Table 2. Mechanisms potentially involved in the pathogenesis of β2-M amyloidosis

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<th>Systemic factors</th>
<th>Local factors</th>
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<td>High precursor (β2-M) concentration in the circulation</td>
<td>Local generation and/or degradation of normal or modified β2-M</td>
</tr>
<tr>
<td>Proteolytic changes of β2-M</td>
<td>Deposition and/or modification of P component</td>
</tr>
<tr>
<td>Modification of β2-M by advanced glycation end-products (AGE) or oxidation (AOPP)</td>
<td>Crystal deposits (Al, Fe, calcium apatite, calcium oxalate)</td>
</tr>
<tr>
<td>Induction of inflammatory state by uremic state or dialysis technique (dialysis membrane, endotoxin transfer, complement activation, cytokines, oxygen radicals)</td>
<td>Local micro-inflammatory state (role of Aceβ-β2-M?)</td>
</tr>
<tr>
<td>Secondary hyperparathyroidism?</td>
<td>Globin chains, immunoglobulin light chains</td>
</tr>
<tr>
<td>Glucosaminoglycans (heparan sulfate), inactive proteinases, proteinase inhibitors (x2-macroglobulin)</td>
<td>Ubiquitin</td>
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70. van Ypersele de Strihou C. Influence of dialysis techniques on β2M amyloidosis. Rev Rhum (Engl Ed) 1994; 61 (9, suppl): 67S–69S


