Morphogenesis of joint β2-microglobulin amyloid deposits

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Introduction

The detection of amyloidosis in several patients on long-term dialysis came as a surprise [1]. The subsequent identification of beta-2-microglobulin (β2m) as the main component of this amyloid [2,3] demonstrated that we were faced with a new, specific complication of long-term renal replacement therapy.

The clinical picture of β2m amyloidosis (Aβ2m) emerged progressively. The carpal tunnel syndrome (CTS), reported initially by Assenat [1], was observed within 3 to 5 years after the onset of dialysis, its prevalence rising progressively with the duration of treatment, reaching 100% after 20 years on haemodialysis [4–6]. CTS was, however, not completely specific to Aβ2m: small β2m deposits were detected in only 70% of the cases [6].

The preferential involvement of the joints subsequently became apparent: it extended to bones [7] and synovia [8], and included arthralgias and destructive spondylarthropathy, whose prevalence also rose with dialysis duration [5,9]. Here again, the symptoms were not completely specific to Aβ2m. Of interest, joint ultrasonography and bone X-rays allowed a more accurate diagnosis of β2m amyloid deposition [10–13].

Finally, β2m amyloid was also identified in various organs, usually after >12 years of dialysis [14,15], with the prevalence rising progressively thereafter.

As yet, there is no fully adequate explanation to account for β2m precipitation into amyloid fibrils. A number of risk factors have been identified. As already pointed out, duration of dialysis is a critical determinant of several clinical manifestations of β2m amyloidosis [5,12,16]. Age at onset of dialysis has also emerged as an independent risk factor [12,17]. Finally, the role of membrane characteristics has been strongly suggested [12,18–21], but its mechanism remains disputed [22].

The actual determinants of β2m precipitation have been the object of a number of interesting studies; however, a final conclusion remains elusive. β2m retention is clearly a prerequisite, but the respective contributions of enhanced β2m production and limited removal by dialysis remain speculative [22]. Modification of β2m including partial proteolysis has been suggested but not confirmed [23,24]. Advanced glycation of β2m (AGE β2m) has been demonstrated by Miyata et al. [25,26], not only in circulating but also in precipitated β2m. The ability of several AGE-modified proteins to crosslink has led to the hypothesis that advanced glycation may enhance β2m amyloid fibril formation.

A number of studies have further elaborated on this hypothesis, some suggesting that AGE modification of synovial proteins enhances β2m precipitation [27] or that the presence of macrophages modifies β2m characteristics and increases fibril formation [reviewed in 28]. Histological evaluation has indeed demonstrated advanced glycation of the β2m amyloid fibrils [29–32], as well as the presence of macrophages [33] within and around the β2m amyloid deposits.

Morphogenesis of Aβ2m deposits

It is of note that histological and immunohistochemical observations were made on advanced, tumour-like deposits. Evidence obtained in early deposits remained scarce [34]. A multi-centre prospective collection of joint samples obtained at autopsy was therefore undertaken [35].

Aβ2m deposition in haemodialysis patients

In the first study [35], 54 patients who had been on haemodialysis (HD) for an average of 47 months were included. Only 4% of them had clinical evidence of β2m amyloidosis, carpal tunnel syndrome surgery and/or bone cysts. The age at HD onset ranged from 20 to 80 years (median 63 years), and at the time of death from 28 to 82 years (median 69 years). A total of 153 joint samples were obtained (range: 1–3; median 2 per patient) from sternoclavicular joints (n = 77), the shoulders (n = 35), knees (n = 28), hips (n = 8), wrists (n = 4) and the acromioclavicular joint (n = 1). Thirty-four control patients, of similar age but without

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history of end-stage renal disease (ESRD), who died during the same time interval were also submitted for post-mortem joint sampling.

This study led to three main conclusions. The first relates to the distribution of Aβ2m in the different joints. At least two different joints were available in 24 of the 26 HD patients with evidence of Aβ2m. The sensitivity of each joint type for the detection of Aβ2m was thus calculated. It was highest (97%) for sternoclavicular joints, followed by knees (91%) and shoulders (57%). In later studies, we therefore included only sternoclavicular joints. Interestingly, Zingraff et al. [36] also relied on synovial biopsies of the sternoclavicular joint, readily accessible during parathyroid surgery, to assess Aβ2m prevalence.

The second conclusion is derived from the control group. Whereas Aβ2m was detected in almost half (26/54) of the HD patients, minute non-β2m amyloid deposits were found in a third of the elderly control patients. This observation suggests that serum amyloid P component (SAP) scintigraphy used to detect Aβ2m has a lower specificity than anticipated as it may bind to amyloid deposits of non-β2m type, present in a third of patients without renal failure [17].

The third and most striking observation was the presence of Aβ2m in 48% of the HD patients despite a rather short mean follow-up. Aβ2m was detected as early as within 23 months of HD. The prevalence increased with duration of HD, from 21% within the first 2 years to 100% after >13 years (Figure 1). Histological evidence of Aβ2m is thus present much earlier than anticipated on the basis of arthralgias or CTS [5].

**Aβ2m deposition in patients on chronic peritoneal dialysis**

Although symptomatic Aβ2m had been reported occasionally in patients treated solely by peritoneal dialysis [37,38], the actual prevalence of this complication remained unknown. On the basis of lower serum β2m levels, it had been suggested that prevalence should be lower in CAPD than in HD patients [22]. Still, convincing data remained unavailable as the clinical manifestations used to assess Aβ2m (CTS and bone cysts) develop late after the onset of renal replacement therapy [39].

We therefore extended our prospective post-mortem study to 26 patients given solely peritoneal dialysis [40] for a median of 27 months (range: 4.5–126 months). Only one of them had been operated on for CTS prior to the onset of CAPD, but Aβ2m was not found in the tissue removed during surgery. Age at CAPD onset ranged from 36 to 91 years (median 69 years), and at the time of death from 44 to 93 years (median 73 years). Aβ2m was diagnosed in 31% of the patients, here again as early as within 21 months of CAPD. The prevalence ranged from 20% after 1–24 months to 50% after 25–48 months. In order to compare the prevalence in CAPD and HD patients, each of the 26 CAPD patients was paired for age and duration of dialysis with a patient treated solely by HD. Prevalence did not differ significantly between the two groups, at 31 and 50% in CAPD and HD patients, respectively. Here again, the discovery that Aβ2m affected 31% of the patients after a median of only 27 months of CAPD revealed a much earlier onset of Aβ2m than previously appreciated.

**The evolution of Aβ2m deposits**

Both in CAPD and in HD patients, histological evidence antedates markedly the clinical symptoms of Aβ2m: amyloid lesions observed after 2 years of dialysis are certainly not equivalent to those discovered after >10 years. It therefore appeared useful to re-analyse several controversies with respect to the onset of amyloidosis in light of the age of the deposit. The evolution of early into late amyloid deposits was therefore evaluated, with the hope of finding some clues on amyloidogenesis.

We reviewed 54 dialysed and 24 control patients included in our prospective autopsy studies [27]. A large (>2 cm diameter) sternoclavicular joint was available in each patient. Among the dialysed patients, 34 had received solely HD, 16 solely CAPD, and four both methods. Dialysis had lasted from 3 to 244 months (median 46 months). Age at dialysis onset

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**Fig. 1.** Prevalence of Aβ2m as a function of haemodialysis duration. Data from Jadoul et al. [35].
ranged from 42 to 91 years (median 64 years) and at
time of death from 43 to 93 years (median 68 years).

Aβ2m was identified by Congo Red (birefringence
under polarized light) and an anti-β2m antibody (ab)
staining, whereas Anonβ2m was defined by Congo
Red positivity but with negative anti-β2m ab staining.

Among the 54 dialysed patients, 32 had Aβ2m, eight
had Anonβ2m, and 14 had no amyloid deposits at all.
Interestingly, among the 24 control patients, 12 had
Anonβ2m and 12 had no amyloid at all. The Anonβ2m
deposits were always small. These data confirm those
previously reported in HD patients [35] and stress
again that SAP scintigraphy may yield false positive
results, both in uraemic and non-uraemic subjects.

An important further observation was that Aβ2m
precipitates first on the surface of the cartilage as a thin
layer, which will eventually thicken. No macrophages
were ever present around cartilaginous deposits.

The thin cartilaginous layer probably represents the
earliest stage of Aβ2m deposition since it was
often observed in the absence of synovial or capsular
deposits, whereas capsular deposits were never
observed in the absence of cartilage deposits. The fact
that Aβ2m deposits first in the cartilage has to be
taken into consideration in all attempts to understand
the initial steps of amyloid deposition. For instance,
udies claiming that AGE modification of synovial
collagen [26] accounts for the initial formation of β2m
amyloid fibrils should be re-interpreted, since Aβ2m
appears first on the cartilage rather than in the
synovium. Of equal importance is the observation
that macrophages are absent at this initial stage. Their
presence is therefore not required for β2m amyloid
formation, in contrast to previous suggestions [33]:
modifications of the β2m molecule by macrophages are
not implicated in the initial stage of Aβ2m formation.

Aβ2m deposits were present in the capsule and/or
in the synovia, in 60 and 58% of the subjects with
cartilaginous deposits, respectively. Clusters of macro-
phages were not uniformly associated with these
deposits: they were identified only ca. 75 and 25% of
the synovial and capsular deposits, respectively.

On the basis of these observations we concluded
that, after its initial deposition in the cartilage, Aβ2m
expanded to synovia and capsules. Macrophages were
evolvedly attracted to these deposits in the final stage.

This sequence of events was supported by a quanti-
tative analysis of Aβ2m deposits in the cartilage.
Their thickness proved to be highly correlated to
dialysis duration and was thus utilized for the timing
of Aβ2m deposits. The early, cartilage-restricted
Aβ2m deposition was called stage 1. It was associated
with small deposits (median 0.03 mm²) and occurred
after a mean duration of 39 months. The further
development of synovial and/or capsular deposits in
the absence of macrophages was called stage 2.
Cartilaginous deposits were larger (median 0.19 mm²)
and dialysis duration averaged 56 months. In the last
stage, called stage 3, macrophages appeared around
capsular or synovial deposits. The size of the cartil-
aginous deposits was even larger (median 0.27 mm²)
and dialysis duration averaged 111 months. Figure 2
illustrates schematically the three stages.

Of interest, the prevalence of subchondral cysts was
similar (ca. 40%) in patients with Aβ2m, in patients
with Anonβ2m, and in control subjects with or without
Anonβ2m. Thus, the cysts appear to develop independ-
ently from Aβ2m. By contrast, marginal bone
erosions were observed only in stage 3 Aβ2m. They
were characterized by a cystic dilation of the synovial
reflection, including variable amounts of fibrous tissue
with small vessels and macrophages (Figure 3). We
believe that these cysts are the forerunners of the bone
cysts identified by X-ray in long-term dialysis patients
[12,13].

It is of note that the pattern of Anonβ2m deposition
in dialysed patients is similar to that found in control
patients. Early deposits were identified in the cartil-
age of all Anonβ2m subjects, and in the synovia or
capsule of only a small minority of them (in the
absence of macrophages).

The various stages identified in this study reconcile
various patterns of Aβ2m deposition. Athanasou et al.
[41] previously reported the absence of macrophages
around small Aβ2m deposits in the cartilage in a heterogeneous series of joint samples. The size of the deposits, however, was not quantitated and macrophages were not detected by specific immunostaining. By contrast, Argiles et al. reported large clusters of macrophages around massive amyloid deposits [33].

**Conclusion**

Several implications for future studies accrue from the current observations. First, the presence of Aβ2m in a sternoclavicular joint sample should not be ruled out unless cartilage is available. Secondly, as early Aβ2m deposition does not require the participation of macrophages, further studies should concentrate on the cartilage as a nidus for Aβ2m fibril deposition.

Our study has not addressed the last issue on Aβ2m precipitation, i.e. the role of advanced glycation of β2m and other joint proteins. The possibility that AGE modification leads to protein cross-linking and fibril formation, and the potential effect of AGE modification on the recruitment of macrophages in the late stage of amyloidogenesis remain to be explored.

**References**


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**Fig. 3.** Marginal bone erosion delineated by the arrowheads. Large Aβ2m deposits are present in the cartilage (arrows) and small Aβ2m deposits within the area of erosion (Congo Red; magnification 2 × 20). C, capsule (reprinted with permission from Garbar et al. [28]).
Joint \( \beta_2 \)-microglobulin amyloid deposits