Scintigraphic methods to detect β2-microglobulin associated amyloidosis (Aβ2-microglobulin amyloidosis)

Jürgen Floege, Jürgen Schäffer and Karl Martin Koch

Division of Nephrology, Department of Medicine, University of Aachen and Division of Nephrology, Department of Medicine, University of Hannover, Germany

Abstract
β2-Microglobulin-derived amyloidosis (Aβ2m) represents a major cause for morbidity in patients with end-stage renal disease. Symptoms of Aβ2m amyloid are mainly related to (peri-) articular amyloid deposition. Conventional non-invasive diagnostic techniques, i.e. clinical evaluation, joint ultrasonography or X-ray, computed tomography or magnetic resonance imaging findings, as well as conventional bone scans, suffer from relative non-specificity and/or low sensitivity. Two recent methods, namely scintigraphy with radiolabelled serum amyloid P component (SAP) or with the radiolabelled Aβ2m-precursor protein, β2-microglobulin (β2m), yield more specific information. Using 125I-labelled SAP, Aβ2m deposits have been visualized in several long-term haemodialysis (HD) patients. However, this scan did not show tracer accumulation in other frequently involved sites, such as hips or shoulders, but did frequently label the spleen, which is usually spared from Aβ2m deposits. Scanning with 131I-labelled β2m, in contrast, yielded tracer accumulations corresponding to the typical distribution pattern of Aβ2m. Specificity of this method was shown by several methods, and the sensitivity was found to markedly exceed that of combined clinical and radiological investigations. Recently, both the radiation exposure and the optical resolution of this latter scan have been further refined by substituting 131I with 111In. In a final step we generated recombinant human β2m (rh/β2m). 111In-rh/β2m again failed to show significant tracer accumulation over joint regions in patients on short-term HD without evidence of Aβ2m amyloidosis. In contrast, local tracer accumulations similar to those observed with natural, 111In-labelled β2m could be demonstrated in long-term HD patients with evidence of Aβ2m amyloidosis. In conclusion, scintigraphy for Aβ2m with 111In-labelled rh/β2m provides a homogenous and safe recombinant protein source, and allows for the sensitive and specific non-invasive detection of Aβ2m-amyloid deposits in dialysis patients.

Introduction
From the beginning of regular dialysis treatment in the late 1960s, it was evident that this replacement was incomplete with skeletal, cardiovascular, endocrine and neurological problems complicating the long-term course of the patients. In 1980, the list of complications of long-term renal replacement therapy was extended by yet another new clinical problem, carpal tunnel syndrome, due to deposition of an unknown type of amyloid [1]. Subsequently, it was realized that a new type of amyloidosis was present in these patients, symptoms of which were mainly related to (peri-) articular amyloid deposition [2,3]. In 1985, the precursor protein of this amyloidosis was identified as β2-microglobulin (β2m) [4], giving rise to the designation Aβ2m amyloidosis (synonyms include AB-amyloidosis, dialysis-related amyloidosis, and dialysis amyloidosis).

As in other types of amyloidosis, some suggestive but no pathognomonic clinical findings exist in Aβ2m amyloidosis. Radiology, computed tomography and magnetic resonance imaging have also been used to search for evidence of Aβ2m amyloidosis [5–12]. However, unless very strict diagnostic criteria are fulfilled [13], these methods suffer from non-specificity. Up to now, the definitive diagnosis of Aβ2m amyloidosis had to rely on histological findings: Congo Red staining showing green dichroism under polarized light, immunohistochemical demonstration of the precursor molecule and amyloid P component within deposits, and ideally the electron microscopical demonstration of typical (8–10 nm wide, non-branching, curvilinear) fibrils [2,3]. As discussed elsewhere in detail [14], histological confirmation of the clinical suspicion of Aβ2m amyloidosis is hampered by three problems: first, it is invasive and potentially harmful;
second, the information gained is punctual; and third, conventional, relatively safe methods to diagnose amyloid (fat aspiration, rectal biopsy) fail in Aβ2m amyloidosis [15–20]. Detritus in the synovial fluid may be used to diagnose Aβ2m amyloidosis [16,21], but again, aspiration of joint effusions is invasive and only yields punctual information.

Given the above considerations, several groups have attempted to design a simple, specific and sensitive method by which a whole body screen for amyloid can be performed non-invasively. The diagnostic techniques that most closely approach these aims are scintigraphic methods of amyloid detection. To date, the three methods that have been evaluated in cases of Aβ2m amyloidosis include conventional bone scintigraphy, scintigraphy using radiolabelled serum-amyloid P-component (SAP), and finally scintigraphy using the specific amyloid precursor protein in Aβ2m amyloid, i.e. β2m.

**Conventional bone scintigraphy**

Since amyloid deposits, independent of the specific type, have a relatively high calcium content, conventional ⁹⁹ᵐTc-diphosphonate bone scans have been used for their visualization. Three groups have employed such scans in end-stage renal disease patients and described imaging of Aβ2m deposits by this method [22–24]. However, in two of these latter papers [22,23], visualization of amyloid deposits was incomplete as judged by the comparison with clinical and radiological findings. Furthermore, the scan could not distinguish between periarticular amyloid deposition and arthropathies of different origin. A third, more recent study [24] attempted to improve the value of bone scintigraphy by combining it with gallium-67 and thallium-201 whole body and single photon emission tomography (SPECT) images. Both of the latter detect inflammatory lesions. Indeed the authors noted a disappearance of tracer accumulation in biopsy-proven Aβ2m lesions following 3 months of melphalan, prednisone and colchicine therapy, while periarticular ⁹⁹ᵐTc-methylene-diphosphonate (MDP) accumulations persisted [24]. Given observations in transplanted patients, where rapid symptomatic relief from amyloid-associated pain is noted after the start of immunosuppression despite persistence of the amyloid [25], this finding is not unexpected. It implies, however, that gallium and thallium merely label inflammatory changes in and around amyloid deposits, which are a late occurrence in Aβ2m amyloid [26] and as such not specific for amyloid-induced arthropathy. Other notable findings of the study of Yen et al. [24] include the fact that abnormal (peri-) articular ⁹⁹ᵐTc-MDP accumulation were only detected in eight of 23 chronic haemodialysis (HD) patients, who had been on dialysis for 10–19 years (mean 13 years). This relatively low prevalence of abnormal findings in a long-term dialysis population again raises doubts about the sensitivity of conventional bone scintigraphy for the detection of Aβ2m amyloidosis. The addition of gallium-67 and thallium-201 whole body and SPECT images, apart from the additional radiation exposure, further decreased the rate of abnormal findings to six out of 23 patients [24].

Thus, clinical applicability of bone scans in cases of Aβ2m amyloidosis may largely be restricted to the follow-up of established cases of the disease.

**Serum amyloid P-component scintigraphy**

In 1988, Hawkins et al., based on extensive *in vitro* and animal experiments, injected ¹²³I-labelled SAP into patients with systemic amyloidoses of the AA-, AL- or Aβ2m-type [27]. SAP, a 250 kDa glycoprotein, is not an integral part of amyloid fibrils, but rather binds to established fibrils of almost any type, except for some cerebral amyloid fibrils [28]. Its function is presently unknown, but it has been speculated that SAP may render amyloid fibrils more resistant to proteolytic digestion [28]. Following the initial description of the scan in patients with systemic amyloidosis [27], subsequent papers by the same group described their experience in HD and peritoneal dialysis (PD) patients with Aβ2m [29,30]. In the 38 long-term HD patients with typical signs and symptoms of dialysis arthropathy, tracer accumulation was always noted in the wrist regions [29]. In contrast, despite the presence of knee and shoulder arthropathy in some of the patients, there was no tracer accumulation in 10 and 40% of these patient subgroups, respectively. Local deposition of tracer in the hip region was apparently very rare even in patients with clinical involvement of this region (Table 1). Splenic tracer uptake, as the only visceral deposits in 30% of the patients, was interpreted as indicating splenic amyloidosis, a notion which is not substantiated by histological findings of others [reviewed in 31]. Specificity of the scan was examined further by injecting ¹²³I-SAP into six short-term HD patients and by injecting ¹²³I-albumin into three HD patients with dialysis arthropathy. No tracer accumulation was noted in any of these cases. Regression or even normalization of local ¹²³I-SAP deposition upon serial scintigraphy in nine patients at 2–7.5 years after renal transplantation (of whom two had biopsy-proven Aβ2m amyloidosis) was interpreted as a striking reduction in the quantity of Aβ2m deposits [32]. However, based on histological and radiological findings, a number of other authors have held the opposite view, namely that Aβ2m amyloid shows little tendency to regress after transplantation [25]. These latter authors have argued that the SAP incorporated into Aβ2m amyloid fibrils may behave differently from the deposited β2m after renal transplantation, and that consequently ¹²³I-SAP scintigraphy may yield falsely negative findings in such cases [25]. Thus, although there is evidence that ¹²³I-SAP can accumulate in Aβ2m amyloid deposits, there are at present uncertainties about both the specificity (splenic uptake) and sensitivity (infrequent uptake in shoulder and hip regions).
of this scan in HD patients. The value of the $^{123}$I-SAP scan to follow the evolution of $\alpha$-2m deposits after renal transplantation currently remains unknown.

### $\beta$2m scintigraphy

Following a preliminary report in 1989 [33], we described our extended experience with $^{131}$I-$\beta$2m scintigraphy, purified from uraemic haemofiltrate, i.e. ‘natural’ $\beta$2m, in HD patients in 1990 [34] (Table 1). Local tracer accumulations were found to correlate well with clinical, radiological and histological findings. Specificity of the $^{131}$I-$\beta$2m scintigraphy had been demonstrated in our previous studies by the good correlation between clinical/radiological and scan findings, the demonstration of tracer enrichment in amyloid tissue and isolated amyloid fibres, and by the absence of tracer accumulation in short-term HD patients or HD patients with non-amyloid inflammatory disease [34]. In contrast to our findings, Kazama et al. reported that the tracer accumulation in amyloid tissue was mostly due to uptake by macrophages rather than by the amyloid fibres [35]. Sensitivity of the $^{131}$I-$\beta$2m scintigraphy was found to markedly exceed that of combined clinical and radiological examination [34]. Using the $^{131}$I-$\beta$2m scan it was also possible to label $\alpha$-2m amyloid deposits in long-term PD patients [36]. The method has been successfully reproduced in Japan using $\beta$2m derived from human uraemic plasma ultrafilterate [37] or urine [35,38], as well as in Spain [39] using $\beta$2m derived from human urine. A practical disadvantage of the $\beta$2m scan is that it cannot be performed in the presence of significant residual renal function, since in this case the radio-labelled $\beta$2m is rapidly excreted via the urine due to its small size of 11.8 kDa [40].

In the original method, we had labelled the purified $\beta$2m with $^{131}$I using the chloramine-T method [33,34]. This approach suffered from the disadvantage that chloramine-T induces some denaturing and/or cross-linking of the protein. More importantly, the usage of $^{131}$I appeared suboptimal given the long half-life (8 days) of $^{131}$I, the heterogeneous radiation spectrum ($\beta$- in addition to $\gamma$-radiation), and the resulting radiation exposure to the patient. Finally, particularly in the case of small joints such as those found in the hand, $^{131}$I-$\beta$2m scintigraphy yielded only blurred images because of limited resolution.

To improve the safety and resolution of the scan, we recently labelled the natural $\beta$2m with $^{111}$In (halflife 2.8 days, exclusive $\gamma$-radiation) following the prior conjugation of the chelator diethylenetriaminepentaacetic acid (DTPA) to $\beta$2m [41,42]. In a second step we evaluated the usage of recombinant human $\beta$2m (rh$\beta$2m) for scintigraphy [42]. Using natural $^{111}$In-labelled $\beta$2m, eight HD patients without signs of $\alpha$-2m amyloid were scanned. Scintigraphy at 48–72 h post injection did not reveal significant tracer accumulation over joint regions. In contrast, nine patients on HD for 10–21 years with evidence of $\alpha$-2m showed selective tracer uptake over various joint regions. Compared with the previous $^{131}$I-$\beta$2m scan, scintigraphy with $^{111}$In-labelled $\beta$2m offered highly improved image contrast and increased sensitivity. Radiation exposure during the $^{111}$In-$\beta$2m scintigraphy was now reduced to ca. 5.3 mSv (from 11–15 mSv with $^{131}$I-$\beta$2m), which is comparable to that of a conventional bone scintigraphy [43] or of the $^{123}$I-SAP scan [27]. Next, we generated recombinant human $\beta$2m (rh$\beta$2m) [42]. $^{111}$In-rh$\beta$2m again failed to show significant tracer accumulation over joint regions in two patients on short-term HD without evidence of $\alpha$-2m amyloidosis. In contrast, local tracer accumulations similar to those observed with natural, $^{111}$In-labelled $\beta$2m could be demonstrated in four long-term (10–27 years) HD patients with evidence of $\alpha$-2m amyloidosis [42].

### Conclusion

In conclusion, the available data suggest that scintigraphy using radiolabelled $\beta$2m currently represents the most specific method to non-invasively detect $\alpha$-2m amyloid deposits in long-term dialysis patients. The change of the $\beta$2m labelling method from $^{131}$I to DTPA-conjugation with subsequent chelation of $^{111}$In

<table>
<thead>
<tr>
<th>Reference Nos</th>
<th>Imaging of deposits in large joints</th>
<th>Imaging of deposits in vertebral column</th>
<th>Imaging of deposits in small joints</th>
<th>Demonstration of tracer accumulation in AB amyloid fibrils</th>
<th>Radiation exposure (mSv)</th>
<th>Time from tracer injection to image (h)</th>
<th>Injected protein (µg)</th>
<th>Injected radioactivity (MBq)</th>
</tr>
</thead>
<tbody>
<tr>
<td>22–24, 43</td>
<td>+</td>
<td>+/−</td>
<td>+</td>
<td>Not done</td>
<td>About 5</td>
<td>3</td>
<td>600</td>
<td>900</td>
</tr>
<tr>
<td>29, 30, 32</td>
<td></td>
<td></td>
<td>+</td>
<td>Not done</td>
<td>4.5</td>
<td>24</td>
<td></td>
<td>190</td>
</tr>
<tr>
<td>33–39</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11–15</td>
<td>72</td>
<td>20–25</td>
<td>60–70</td>
</tr>
<tr>
<td>41</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>48–72</td>
<td>150</td>
<td></td>
<td>37</td>
</tr>
</tbody>
</table>

*Using natural or rh$\beta$2m.
led to a significant reduction in radiation exposure and a markedly improved image quality of the β2m scintigraphy, which now allows for the imaging of small joints and thereby enhances the sensitivity of the diagnostic method. In addition, the availability of homogenous rh/β2m provides a safe and stable protein source. Given the similarity of the findings obtained with the three scan methods, i.e. with 131I-β2m, 111In-β2m and 111In-rh/β2m scintigraphy, we infer that the scan specificity, as described extensively previously [33,34], was maintained. Thus, 111In-β2m scintigraphy has achieved the potential to serve as a more widely applicable technique for early, specific and non-invasive diagnosis of Aβ2m amyloidosis in end-stage renal failure patients.

References