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# Beta$_2$-microglobulin deposition in bone in chronic renal failure

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**Beta$_2$-microglobulin deposition in bone in chronic renal failure.** Recently, there have been reports of beta$_2$-microglobulin ($\beta_2$m) related amyloid deposition in perineural and periarticular tissues in patients receiving long-term hemodialysis, but it has been rarely described in bone. We, therefore, examined previously obtained bone biopsy specimens in patients receiving long-term hemodialysis to determine the prevalence of $\beta_2$m deposition in bone and to assess the relationship between $\beta_2$m deposits and bone histomorphometry. We found $\beta_2$m deposits in bone in 8% of 224 patients examined. Bone deposition of $\beta_2$m was absent in patients who were on dialysis for less than six years, but was present in 19% who dialyzed longer than 10 years. $\beta_2$m deposits were found in specimens from the iliac crest, femoral bone, tibia, vertebra and rib. In the iliac crest $\beta_2$m deposition was localized predominantly to the periosteum. Among these patients with $\beta_2$m in iliac crest periosteum, 62% had suffered a femoral neck fracture compared to only 4% of matched patients who had negative staining for $\beta_2$m in the iliac crest ($P < 0.001$). Histologically, osteitis fibrosa seemed more common in patients positive for $\beta_2$m than in patients negative for $\beta_2$m deposition. We conclude that $\beta_2$m deposition in bone is common in uremic patients who have received hemodialysis longer than 10 years. The high prevalence of femoral neck fracture in patients with $\beta_2$m localized to the periosteum of the iliac crest suggests that this involvement may be useful to predict susceptibility to femoral fracture.

Recently there have been reports characterizing the amyloidosis that occurs in patients treated with long-term hemodialysis. Beta$_2$-microglobulin ($\beta_2$m) has been identified as the major component of amyloid fibrils by biochemical and immunologic studies [1–4]. While amyloid deposits are found most frequently in perineural, periarticular and articular locations [5–10], it is unknown why amyloid preferentially deposits in these tissues. Less well studied are the cystic bone lesions thought to be secondary to local amyloid deposition [5, 6, 9–13], the likely result of $\beta_2$m accumulation [8, 14].

$\beta_2$m, an 11,800 dalton protein, may be an important determinant of bone cell function. In vitro studies have shown in one study that $\beta_2$m is produced in cultured bone cells [15] and is mitogenic in osteoblast enriched populations [15]. In another study [16], however, $\beta_2$m inhibited osteoblastic mineralization, while collagen synthesis was maintained. Whether the accumulation of $\beta_2$m in bone contributes to the variable histology of renal osteodystrophy is unknown. However, the high circulating concentrations of $\beta_2$m in dialysis patients (40 to 50 $\mu$g/ml) [1] could substantially affect osteoblast function and consequently, bone turnover.

The purpose of the present study was to determine the prevalence of amyloid deposition in bone of patients receiving long-term hemodialysis and to ascertain whether the presence of $\beta_2$m in bone correlates with bone histomorphometry.

## Methods

### *Materials*

Bone specimens were obtained from 224 patients (140 males, 84 females; age range, 14 to 75 years) who had received hemodialysis for a mean (± SD) of 9 ± 4 years (range, 1 to 22 years). All patients had received thrice weekly hemodialysis using cuprophane membranes for most of their dialysis experience. None had multiple myeloma or primary amyloidosis as the underlying cause of renal failure. Overall, there were 246 bone specimens for evaluation: 234 biopsies from the iliac crest, 7 from the femoral head in patients with femoral neck fracture, 1 from the tibia (biopsy of pathologic fracture), and 2 each from vertebra and rib (autopsy specimens). The iliac crest biopsies were obtained from patients being evaluated for renal osteodystrophy at several institutions throughout the United States between 1985 and 1988. The femoral heads were obtained from all the patients in three dialysis programs (total patient population approximately 600) who required femoral prostheses in a five-year period.

### *Immunohistochemical studies*

Methyl methacrylate-embedded, undecalcified 5 $\mu$-thick sections were stained with alkaline Congo red and were examined by conventional and polarized light microscopy. Immunohistochemical studies were made with the peroxidase-antiperoxidase method [17]. Methyl methacrylate-embedded, 3 $\mu$-thick sections from Congo red-positive bone biopsy specimens were attached to slides by Tissue-Tack (Polysciences, Inc) and then treated in alcoholic sodium hydroxide for 10 minutes, and washed in absolute ethanol for 10 minutes. The sections were dipped in a 1% solution of hydrogen peroxide in absolute methanol for 30 minutes to inhibit endogenous peroxidase activity and washed three times for five minutes in phosphate-buffered saline. The non-specific reactivity of the secondary antibody was blocked by applying a 1% goat or donkey serum solution in phosphate-buffered saline supplemented with 4% bovine serum albumin for 15 minutes. The block solution was

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poured off the slides and the sections were incubated overnight at 4°C in a humid chamber with rabbit antihuman antisera using the following dilutions: 1:1000 for $\beta_2$-microglobulin, 1:500 for amyloid A component, 1:1000 for kappa and lambda immunoglobulin light chain antibody and goat antihuman antisera, 1:500 for prealbumin (Sigma Chemical Co., St. Louis, Missouri, USA). The primary antibody was washed off with phosphate-buffered saline, as described above. The secondary goat anti-rabbit IgG or donkey antigoat IgG was applied at a a dilution of 1:200 for 60 minutes at room temperature and then rabbit or goat peroxidase-antiperoxidase antiserum at 1:300 for 60 minutes at room temperature. Visualization of the bound peroxidase was achieved with the diaminobenzidine-$H_2O_2$ reaction for five minutes. As negative controls, tissue sections of kidney from patients with proven AA, AL and Aprealbumin amyloidosis (provided by Dr. T. Shirahama, Boston University, Massachusetts, USA) were used as comparisons. A strict positive control was not done as we did not have the capability to analyze tissue for $\beta_2$m biochemically. However, in one patient with extensive amyloid seen on both Congo red and $\beta_2$m immunohistochemical stains, electron microscopic studies were done. At five different sites that were positive by both staining techniques, the unique curvilinear fibrils said to be characteristic of $\beta_2$m amyloid [2] were seen.

### *Bone histomorphometry*

Histomorphometric analysis of Goldner stained sections was performed as previously reported [18]. The following classifications were made: osteitis fibrosa, if bone formation was normal or increased and there was significant marrow fibrosis; osteomalacia, if bone formation was below normal and there was an excess of unmineralized osteoid; mixed disease, if excess osteoid and marrow fibrosis were present; aplastic bone disease, if bone formation was below normal and osteoid area was normal or reduced; or mild hyperparathyroid disease, if bone formation and osteoid area were normal but osteoid covered surfaces were increased. Separate sections were also stained with aurintricarboxylic acid for the identification of aluminum [19] and Prussian blue for the detection of iron.

The Chi-square test was used for statistical analysis and a $P$ value $< 0.05$ was considered significant.

## Results

Congo red positive amyloid deposits in bone, as identified by apple-green birefringence under polarized light, were found in 19 of the 224 patients (Figs. 1A, B and 2). The mean (± SD) age of affected patients (9 males and 10 females) was 55 ± 9 years (range, 34 to 69 years) and the mean duration of hemodialysis was 13 ± 4 years (6 to 18 years). Immunohistochemical studies revealed that all the amyloid deposits reacted with anti-$\beta_2$m antibody (Figs. 1C, 3, and 4) and none reacted with antihuman AA, AL or Aprealbumin antibodies. None of the control kidney sections of AA, AL and Aprealbumin types of amyloid stained for anti-$\beta_2$m.

Figure 5 shows the prevalence of $\beta_2$m related amyloid in bone as a function of dialysis duration. $\beta_2$m deposits were not present in 45 patients who had received dialysis for less than six years, but were found in 3 of 94 patients treated for 6 to 10 years and in 16 of 85 patients treated longer than 10 years.

Table 1 summarizes the histologic location of $\beta_2$m related amyloid in bone. $\beta_2$m was identified in 17 (7%) of the 234 bone specimens from the iliac crest (periosteum in 16, cartilage in 1 and bone marrow in 2), in six of seven femoral bone specimens (synovium in 4, superficial articular cartilage in 3, trabecular bone in 1 and bone marrow in 5) and in one specimen from the tibia (involving the synovium, cortical bone and bone marrow).

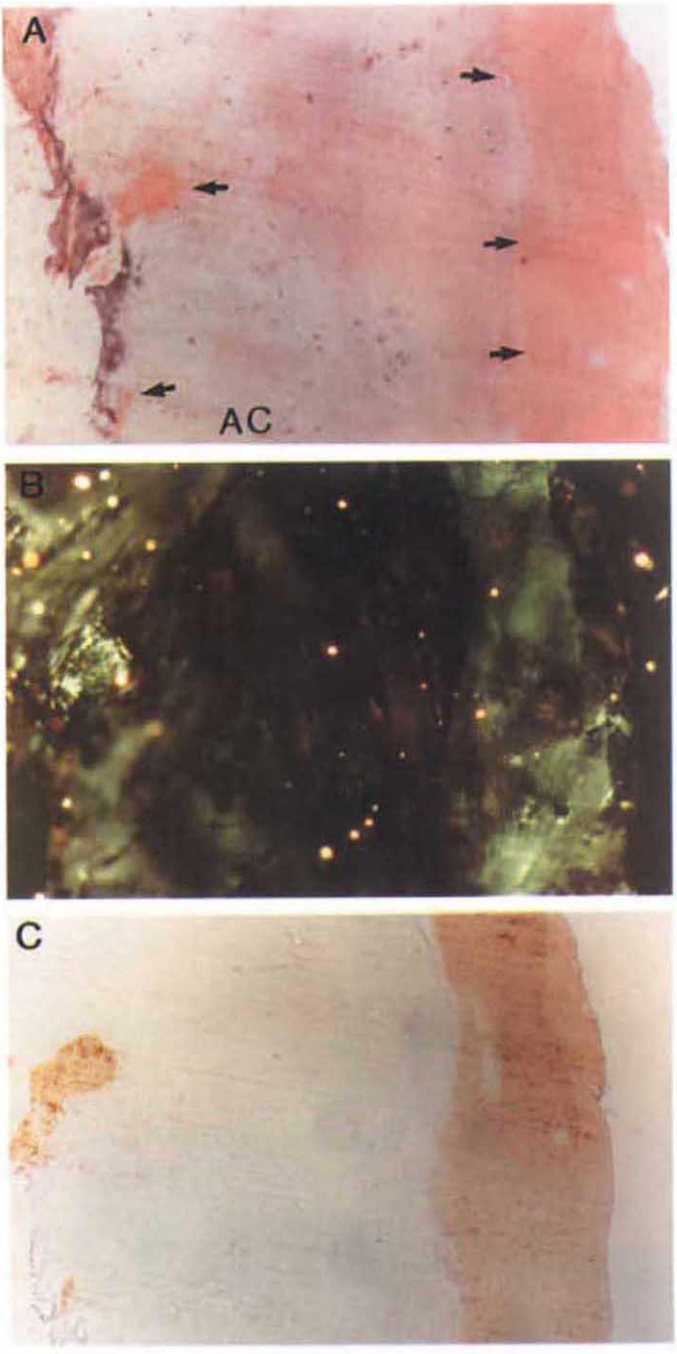


**Fig. 1.** *Serial sections of femoral bone from a dialysis patient.* **A.** Congo red stain. Amyloid deposits (arrow) are seen in the superficial articular cartilage (C) (×115). **B.** The same area under polarized light. Congo red stained amyloid deposits demonstrate green birefringence (×115). **C.** Immunohistochemical staining with anti-$\beta_2$m. Amyloid deposits stain brown for $\beta_2$m (×115).

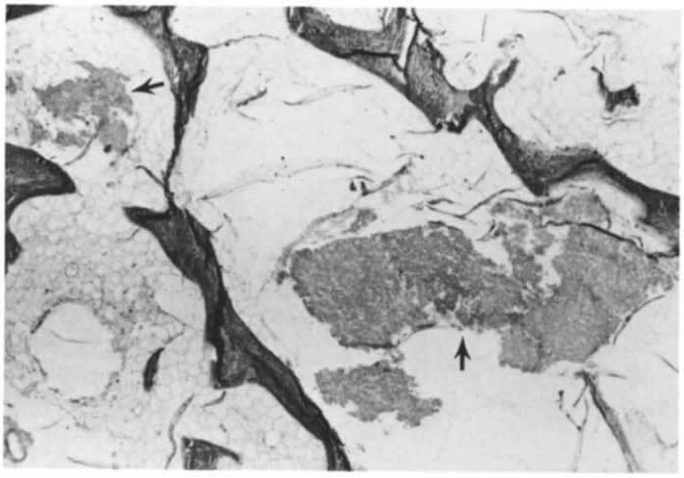

**Fig. 2.** *A section under polarized light showing the bone marrow of the same patient as in Fig. 1.* Amyloid deposits (arrow) adjacent to the bone trabeculae demonstrate green birefringence (×29).

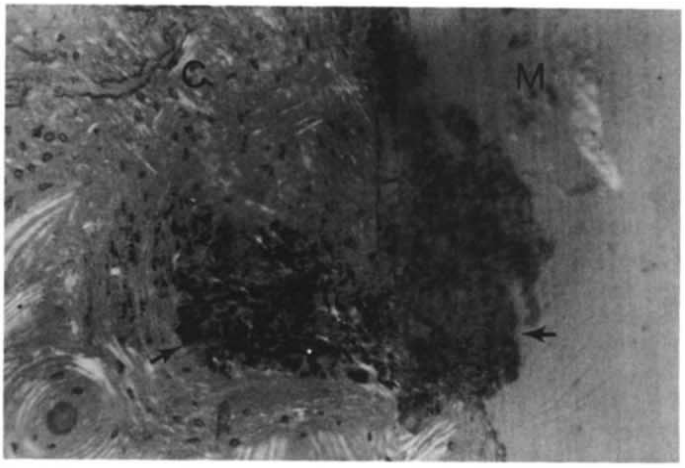


**Fig. 4.** *Bone section from the tibia with anti-$\beta_2$m and methylene blue counterstain.* $\beta_2$m positive deposits (arrows) appear to invade the cortical bone (C) from the marrow (M) (polarized light; ×115).

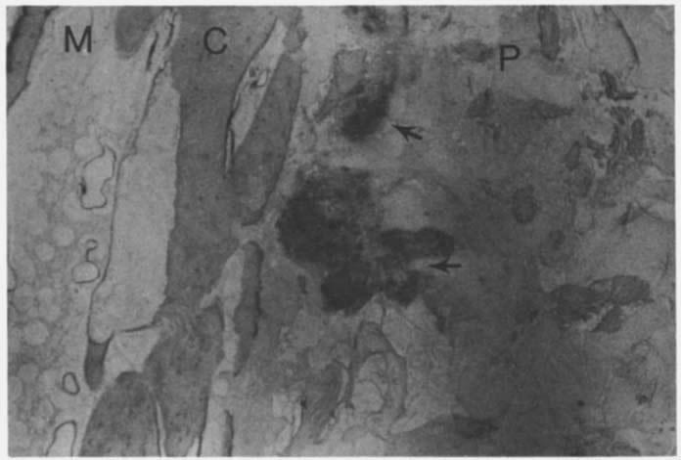


**Fig. 3.** *Iliac crest bone biopsy stained with anti-$\beta_2$m and hematoxylin counterstain.* $\beta_2$m deposits (arrows) are located in the iliac periosteum (P) (×115). C, cortical bone; M, marrow.

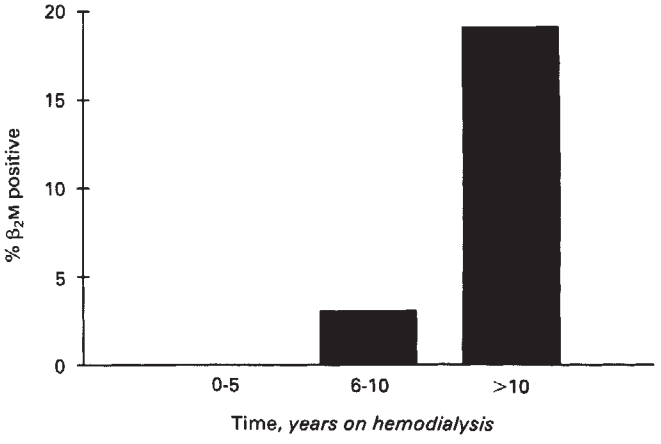


**Fig. 5.** *The prevalence of $\beta_2$m related amyloid in bone as a function of hemodialysis duration.*

Sections from vertebra and rib had $\beta_2$m deposits in the periosteum only. Among the six patients with $\beta_2$m deposition in the femoral bone, there were four who also had iliac crest biopsies, three of which were positive for $\beta_2$m in the periosteum.

Table 2 shows the percent of tissue area occupied by amyloid. The iliac crest contained relatively small amounts of amyloid while the other tissues contained considerably more. This is not surprising since the other specimens were obtained from areas with symptoms while none of the patients had symptoms referable to the iliac crest. There was no difference in dialysis duration between the patients with femoral fracture ($\bar{x}$ 14 years, range 7 to 20) and iliac crest biopsy ($\bar{x}$ 15 years, range 8 to 20). Patients with biopsies from other sites had been on dialysis for 6, 16 and 23 years when their bone was obtained from the tibia, vertebra and rib, respectively.

Shown in Table 3 is the relationship between $\beta_2$m deposition in the periosteum of iliac crest and the prevalence of femoral neck fracture in matched dialysis patients. Among the 13 patients with $\beta_2$m deposition in the iliac crest periosteum, 8 (62%) had a femoral neck fracture. In contrast, only 1 of 25 patients who were matched for age, sex, and dialysis duration and who were negative for $\beta_2$m had sustained a femoral neck fracture ($P < 0.001$). Cortical thickness in the eight patients with femoral fracture measured 338.6 ± 139.9 $\mu$, while in 19 of the 25 patients who were controls (in 6 patients cortex was removed for chemical measurements) it measured 437 ± 378.5 $\mu$ (mean ± SD, $P$ = NS). Cancellous bone volume also did not differ.

Because recent reports have suggested an association between aluminum and/or iron accumulation and dialysis-related amyloid deposition, stains for aluminum and iron were performed in all amyloid positive bone specimens and compared to 67 amyloid-negative bone specimens obtained from patients who were matched for age, sex, and dialysis duration (Table 4). Aluminum on the bone surface was present in 92% of amyloid positive specimens and bone marrow iron was present in 77%. In the amyloid negative specimens, bone surface aluminum was

**Table 1.** Histologic sites of $\beta_2$m related amyloid in bone of dialysis patients

| Source of biopsy | | Site of stained $\beta_2$m | | | | |
|---|---|---|---|---|---|---|
| | | Periosteum | Synovium | Cartilage | Bone | Bone marrow |
| Iliac crest | (17/234) | 16 | NA | 1 | 0 | 2 |
| Femoral head | (6/7) | NA | 4 | 3 | 1 | 5 |
| Tibia | (1/1) | NA | 1 | NA | 1 | 1 |
| Vertebra | (1/2) | 1 | NA | NA | 0 | 0 |
| Rib | (1/2) | 1 | NA | NA | 0 | 0 |

Numbers in parentheses indicate the number of positive specimens per total number examined. NA, not applicable.

**Table 2.** Amyloid % of tissue area

| | Iliac crest | Femoral head | Vertebra | Rib | Tibia |
|---|---|---|---|---|---|
| 1 | 0.13 | | | | |
| 2 | 0.11 | | | | |
| 3 | 0.73 | 5.40 | | 0.18 | |
| 4 | 0.01 | | | | |
| 5 | 0.007 | | | | |
| 6 | 0.09 | | | | |
| 7 | 0.007 | | | | |
| 8 | 0.86 | 0.04 | | | |
| 9 | 0.004 | | | | |
| 10 | 0.01 | | | | |
| 11 | 0.004 | | | | |
| 12 | 0.001 | | | | |
| 13 | 0.002 | | | | |
| 14 | 0.001 | | | | |
| 15 | 0.004 | | | | |
| 16 | 0.02 | | | | |
| 17 | 0.004 | 4.83 | | | |
| 18 | | 1.62 | | | |
| 19 | | 1.61 | | | |
| 20 | | 0.43 | | | |
| 21 | | | | | 13.38[a] |
| 22 | | | 0.07 | | |

[a] Area of pathologic fracture

present in 94% and bone marrow iron was present in 75%. Neither metal was found in proximity to the $\beta_2$m deposits.

## Discussion

In patients treated with long-term hemodialysis, amyloid deposition is preferentially localized to periarticular tissues [5–10]. Although a few reports [7, 8, 14] have described amyloid involvement in bone, little is known about the prevalence of bone amyloid deposition in chronic uremia. In the present study, we found that amyloid in bone stained exclusively for $\beta_2$m and that the periosteum was the predominant location when $\beta_2$m was present in the iliac crest. While the deposition of $\beta_2$m in bone was not apparent until after five years of hemodialysis, its presence increased in frequency after that time reaching a prevalence of 19% in patients who had dialyzed longer than 10 years.

The occurrence of $\beta_2$m in bone may be much more frequent than we find in iliac crest biopsies. Although the most common location to do bone biopsies, the iliac crest may not be a "favored" location for deposition of this material. It seems unlikely that we failed to detect (false negative error) any substantive amount of amyloid since we screened a minimum of 20 $mm^2$ of bone and were able to detect as little as 0.004% of tissue area of amyloid (0.08 $mm^2$).

**Table 3.** Relationship between $\beta_2$m deposition in the iliac periosteum and the prevalence of femoral fracture in matched dialysis patients

| Iliac periosteum | Femoral fracture |
|---|---|
| $\beta_2$m positive ($N$ = 13) | 8 (62%)[a] |
| $\beta_2$m negative ($N$ = 25) | 1 (4%) |

[a] $P < 0.001$

**Table 4.** Relationship between $\beta_2$m deposition in bone and the location of aluminum and iron deposits

| $\beta_2$m deposition | Bone surface aluminum | Bone marrow iron |
|---|---|---|
| Positive ($N$ = 26) | 24 (92%) | 20 (77%) |
| Negative ($N$ = 67) | 63 (94%) | 50 (75%) |

The prevalence of carpal tunnel syndrome, a proven complication of amyloidosis [5–8] is greater than 50% in patients on dialysis over 10 years. Thus, our finding of a 19% prevalence in the iliac crest underestimates the actual incidence of this systemic illness. A true prevalence can only be established by a detailed autopsy study. Nonetheless, the demonstration of an increased frequency of amyloid deposits with time on dialysis and the apparent relation to femoral fracture are worth noting. It is possible that amyloid deposition in the iliac crest tells us more about the severity than the prevalence of this condition.

Until recently, pathological hip fractures in dialysis patients have been assumed to be secondary to hyperparathyroidism, aluminum accumulation, or other metabolic abnormalities such as osteoporosis. The last problem does not appear to be an important contributor in these patients, since iliac crest bone volume and cortical thickness did not differ between patients and controls. Admittedly, osteoporosis may not be well distinguished with iliac crest histology.

Our histologic studies demonstrate that when present amyloid is diffusely deposited in bone supporting the concept that extensive deposition may contribute to bone weakness in specific areas [12, 20], and possibly predispose to fractures. The finding of amyloid in six of seven femoral specimens from patients with femoral neck fractures further supports this hypothesis. Interestingly, there was a higher prevalence of femoral fracture in patients with periosteal deposition of $\beta_2$m in the iliac crest when compared to matched patients with negative staining for $\beta_2$m. It is not clear why $\beta_2$m preferentially accumulates in periosteal tissue. However, in affected patients the appearance of $\beta_2$m in the iliac periosteum may indicate that substantial amounts of $\beta_2$m have also accumulated in femoral bone, thereby increasing the risk for fracture in that location.

While it is unknown how $\beta_2$m in bone might relate to fractures, observations in patients with primary amyloidosis suggest that localized erosions [21], diffuse demineralization [22], and avascular necrosis due to amyloid deposition in marrow vessels [23], may all contribute to bone weakness.

We also attempted to assess whether $\beta_2$m accumulation was associated with any particular histologic type of renal osteodystrophy. There was a suggestion from this analysis of an increase in high turnover (osteitis fibrosa) and decrease in low turnover lesions (aplastic, osteomalacic) in the $\beta_2$m positive patients. Since $\beta_2$m has been reported to be mitogenic for osteoblast-like cells in culture [15], it is possible that it participates with other osteoblast mitogens such as insulin-like growth factor 1 [24, 25] and PTH [26] in stimulating osteoblastic proliferation. On the other hand $\beta_2$m has also been reported to cause a mineralization defect (a feature of low turnover lesions) in vitro [16]. Thus, what effect $\beta_2$m might have on bone cells in vivo is hard to predict.

Circulating interleukins (IL) may have a role in the pathogenesis of dialysis-related $\beta_2$m deposition. Levels of serum IL-1 are elevated in patients who have received hemodialysis for longer than 10 years [27]. More recently, it was shown that serum concentrations of the soluble IL-2 receptor, a 55 kD IL-2 binding protein produced by activated T lymphocytes, are elevated in hemodialysis patients [28]. $\beta_2$m and IL-2 receptor levels in serum rise during hemodialysis, suggesting that the dialytic procedures itself may cause shedding of these cell-surface proteins. Moreover, patients with the carpal tunnel syndrome have higher levels of IL-2 receptor in serum when compared to patients without clinically evident amyloidosis [28]. Whether elevated levels of the interleukins are associated with $\beta_2$m deposition in bone is unknown. However, it has been shown that IL-1 stimulates cellular proliferation when added to cultures of osteoblast-like cells [29]. Thus, circulating IL-1 when elevated could enhance bone turnover by increasing the number of active osteoblasts that are capable of forming new bone.

It has been suggested that aluminum or iron may act to promote the polymerization of $\beta_2$m into amyloid fibrils [30–32]. In our study, aluminum in bone and iron in the bone marrow were found in most of the bone specimens examined. Neither metal was found in the same location as the $\beta_2$m and both metals were present in patients regardless of whether they had $\beta_2$m deposition or not. These data, therefore, suggest that aluminum and iron deposition are complications of long-term hemodialysis, similar to $\beta_2$m deposition, and are probably not important in the pathogenesis of $\beta_2$m deposition bone.

Plasma $\beta_2$m levels have been reported to be lower in patients treated with high flux dialysis when compared to patients using cuprophane membranes. The larger sized membrane cut-off (> 12,000 daltons) and higher flow rates achieved by high flux dialysis are likely responsible for the increased clearance of $\beta_2$m [33–37]. Although $\beta_2$m levels have not predicted the occurrence of clinically relevant amyloid deposition [38], long-term dialysis with polyacrylonitrile membranes appears to be associated with a lower incidence of amyloid-related problems when compared to long-term use of cuprophane membranes [39]. Long-term trials comparing conventional and other high-flux dialysis membranes should help determine the optimal dialytic regimen which will prevent $\beta_2$m amyloidosis.

In conclusion, our results show that $\beta_2$m deposition in bone is common in uremic patients who have received hemodialysis longer than 10 years. The increased prevalence of femoral neck fracture in patients with $\beta_2$m in the iliac periosteum suggests that this site may be helpful in predicting femoral fracture in high risk patients. While the mechanisms responsible for $\beta_2$m deposition are unknown, the presence of aluminum or iron appears to be unimportant in the bone deposition of $\beta_2$m. Because of the possible association of bone $\beta_2$m and osteitis fibrosa in our study and the known proliferative effect of $\beta_2$m in cells of the osteoblast lineage, it may be that chronically elevated plasma levels of $\beta_2$m are involved in promoting high bone turnover in chronic renal failure. Alternatively, $\beta_2$m deposition and PTH elevation may both be consequences of long-term dialysis and just be coincidentally related.

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