Dialysis-related amyloidosis (DRA) is a serious complication of chronic dialysis therapy. The protein deposited has been identified as a modified form of beta-2 microglobulin (β2m), levels of which are universally elevated in dialysis patients. However, other factors must also be important, as the serum β2m level is not predictive of development of DRA. The development of improved and more sensitive radiological screening tests may facilitate better understanding of the etiologic factors and management of DRA. Currently, there is no specific treatment for DRA. Although serum β2m levels fall rapidly with successful transplantation and patients experience a remarkable resolution in their osteoarticular symptoms, the bony lesions do not regress.

Case Report

ML, a 61-yr-old woman, developed ESRD in 1984 as a result of diabetic nephropathy. She was treated with thrice-weekly hemodialysis, 3 h each session, using a CA210 (cellulose triacetate, KOA = 930; Baxter HealthCare Co., McGaw Park, IL) membrane. She was never transplanted. In 1993, she developed a painful, swollen left knee. This knee was tapped and revealed a white blood cell count of 10,400, a red blood cell count of 53,000, with a β2m level of 27.8 mg/L. A magnetic resonance imaging scan of the knee revealed synovial nodularity, but was otherwise unremarkable. She continued to have intermittent joint effusions over the next 18 months. In March 1994, she noted pain in her left hand, which was worse at nights and during dialysis. She also had tingling in her left thumb and index finger. Nerve-conduction studies confirmed the clinical diagnosis of carpal tunnel syndrome (CTS) and a release operation was performed. Hematoxylin and eosin (H & E) staining of the synovial tissue obtained intraoperatively revealed the deposition of an amorphous eosinophilic material just below the synovial lining. Congo red staining of the amorphous material, viewed under polarized light, showed the classic apple-green birefringence consistent with amyloid presence. Immunohistochemical staining using appropriate antisera confirmed the amyloid to be β2m. Serum β2m level was 120.7 mg/L (normal, <2.7 mg/L). The patient's dialysis membrane was changed to an F80 (Polysulfone, KOA = 945; Fresenius D61343, Bad Hamburg, Germany) for 1 yr and then to a CT190 (cellulose triacetate, KOA = 920; Baxter HealthCare Co.), which was used until her death on July 1994 after a massive stroke. With the more biocompatible membranes, her β2m levels had markedly reduced to approximately 35 mg/L on the F80 and approximately 45 mg/L on the CT190 membranes.

Immunohistochemical Staining

Synovial and tendon sheet tissue obtained intraoperatively during carpal tunnel release surgery was fixed in 10% buffered formaldehyde. Four-micrometer sections of paraffin-blocked tissue were used for immunohistochemical staining with rabbit polyclonal antiserum (Zymed Laboratories, Inc., San Francisco, CA) to β2m, using the labeled streptavidine-biotin peroxidase-antiperoxidase technique according to the manufacturer's protocol (DAKO Laboratories Corp., Carpiteria, CA). On the negative control slides, in which the primary antibody was replaced with a blocking solution, there was no staining throughout the specimen (Figure 1A): the sections stained with the antibody showed areas with significant aggregates of β2m between the collagen fibers (Figure 1B). These aggregates

Received January 17, 1996. Accepted May 8, 1996.

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1046-6673/0803-0509$03.00/0

Journal of the American Society of Nephrology

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Figure 1. Immunohistochemical staining of the patient's carpal tunnel tissue obtained intraoperatively using rabbit polysera against human beta-2-microglobulin (β2m) and labeled with the streptavidine-biotin peroxidase-antiperoxidase technique, as described in the text. In Panel A (negative control), the anti-human or β2m antibody was replaced by a blocking solution, and there was no staining throughout the specimen. In Panel B, staining with anti-human β2m rabbit antiserum shows aggregates of β2m staining between the collagenous tissue. These aggregates correlated with the positive Congo red staining (data not shown).

correlated with the positive Congo red staining (data not shown).

Discussion

In 1975, Warren et al. were the first to report a high incidence of CTS in patients on chronic hemodialysis (1). Five yr later, it was discovered that the CTS was secondary to amyloid deposition, and in 1985 Gejyo et al. demonstrated that β2m was the predominant protein deposited in cases of DRA (2). Although originally believed to affect only hemodialysis patients, it has since been found in patients treated solely with peritoneal dialysis, and occasionally in patients with chronic renal impairment before the initiation of dialysis. Although it is known that elevated serum β2m levels are necessary for the development of DRA, these levels do not correlate with disease activity. Factors other than β2m accumulation must therefore be involved, leading to β2m modification with subsequent amyloid formation.

Clinical Presentation and Risk Factors

DRA may initially present with many different symptoms. Patients with a higher risk of developing DRA include older patients and those patients who have been on dialysis for more than 8 yr (Table 1). The role that dialysis-membrane bioincompatibility plays both in the possible pathogenesis and the treatment of DRA remains controversial (3). DRA should always be considered in any chronic dialysis patient with CTS, large-joint arthropathy, or cystic bone disease (Table 2). CTS arises from entrapment of the median nerve by β2m-amyloid

Table 1. Potential risk factors for dialysis-related amyloidosis

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<th>Factor</th>
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<td>Age &gt;40 yr at the outset of dialysis</td>
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<td>Length of time on renal replacement therapy</td>
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<td>Bioincompatibility of dialysis membrane</td>
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deposition at the wrist, and is one of the more common initial manifestations of DRA. There is no difference between the symptom complex of CTS secondary to DRA and idiopathic or other secondary forms of CTS. The patient complains of hand pain (often nocturnal or during dialysis) associated with thenar weakness, muscle wasting, and sensory loss over the palmar surfaces of the thumb, index, and middle fingers. The condition is frequently bilateral, and in severe cases, contraction of the hands atrophy of the digital muscles may occur, a condition referred to as “amyloid hand.”

Destructive arthropathy secondary to DRA predominantly affects large- and medium-sized joints. The usual presenting symptoms are arthralgia and decreased joint mobility. A joint effusion may be present. The synovial fluid is serous, sterile, and noninflammatory. Amyloid material may be observed if the fluid sediment is stained with Congo red. Although it often initially occurs as a unilateral problem, the contralateral joint will almost inevitably become involved. Spondyloarthropathy, typically involving the cervical spine, may also occur. β2m-amyloid deposits have been found both in the intervertebral discs and paravertebral ligaments. Although it is frequently mild, cervical spine instability and spinal cord compression may occur. Bone cysts, predominantly affecting large bones and bones in proximity to synovial joints, are commonly seen on the x-rays of patients with DRA. These cysts contain β2m-amyloid deposits, increase in number and size with time, and do not regress after transplantation (4). They may lead to bone weakening and, if situated near a bearing joint, may play a role in the development of pathological fractures. Systemic involvement occurs at a later stage of the disease and fortunately only rarely gives rise to major clinical problems. Nevertheless, intestinal obstruction, spontaneous tendon rupture, renal stones composed of β2m (5), and myocardial dysfunction secondary to β2m-amyloid deposition have all been described, emphasizing that DRA can potentially become life-threatening (6).

**Radiological Findings**

Because of difficulties in obtaining involved tissue for histopathological examination, radiographic evidence of bone cysts in a patient with clinical symptoms that suggest DRA may be helpful. In certain cases, computed tomographic scans and magnetic resonance imaging scans of bone will provide additional information to assist in the differentiation of amyloid bone cysts from other cystic bone diseases encountered in hemodialysis patients. Narrowing of the intervertebral disc space with destructive changes involving the adjacent vertebral bodies may be seen in patients with spinal involvement. The development of iodine-125-labeled β2m, and iodine-123 serum amyloid P component scans may prove useful by allowing diagnosis of DRA at an earlier stage, with improved estimation of the extent and distribution of amyloidosis present in the body (7). These scans may enable us to evaluate the effectiveness of various interventions more accurately, e.g., biocompatible versus bioincompatible membranes, transplantation, etc., on the development and course of DRA, and allow the formulation of more efficient preventive measures (8). Ultrasound tests of the shoulder to measure the thickness of the rotator-cuff tendons may also be a useful, noninvasive method to select patients at risk of developing DRA before bone lesions occur (9).

**Pathogenesis**

**Proposed mechanisms of β2m-amyloid formation.** β2m is an 11.8-kd glycosylated polypeptide consisting of 100 amino acids aligned in a single chain. It is an integral part of the human leukocyte antigen (HLA) Class 1 antigen complex, but may also be found in non-HLA-associated forms. The body produces 50 to 200 mg of β2m daily, and the normal serum level for β2m is 0.5 to 2.0 mg/L. Lymphocytes, and T cells in particular, have the highest rate of synthesis. Once released from the cell surface, β2m circulates predominantly in the monomeric form and is not bound to plasma proteins. Increased β2m synthesis occurs in inflammatory and malignant conditions. The kidney is the only known major elimination pathway for β2m, which explains the accumulation of β2m in patients with increased duration of uremia and dialysis.

In DRA, unlike other forms of amyloidosis, the substance deposited is biochemically very similar to the circulating molecule, and spontaneous generation of β2m amyloid fibrils may occur in salt-free solution in vitro (10). However, the pathogenesis of DRA cannot be fully explained solely on the basis of simple precipitation of intact β2m in body tissues. Many other polypeptides, all of which originate from β2m, have also been isolated from affected tissue samples. Linke et al. detected β2m that had been cleaved at lysine residues, from synovial amyloid and amyloid urinary stones (5). Ogawa et al. described a “novel” β2m isolated from amyloid deposits and serum, which had a more acidic isoelectric point and lower molecular weight because of the substitution of aspartic acid for asparagine at the 17th residue (11). In 1993, Miyata et al. demonstrated the modification of β2m in amyloid fibrils with advanced glycation end products (AGE) (12). Amyloid deposits are usually considered to be acellular, but histologically, β2m amyloid fibrils are often surrounded by macrophages. It is known that AGE-modified proteins are chemotactic for monocytes, and that macrophages possess receptors for the endocytic uptake of these modified proteins (13). The presence of these AGE-modified proteins may therefore explain the unusual inflammatory nature of the amyloid deposits in DRA (14). Although this evidence strongly suggests that AGE-modified β2m is a major constituent of DRA (12,14), another possibility that needs to be ruled out is that the deposited β2m amyloid undergoes secondary AGE modification.

**Table 2. Clinical presentations of dialysis-related amyloidosis**

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<td>Carpal tunnel syndrome</td>
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<td>Large joint arthropathy/spondyloarthropathy</td>
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<tr>
<td>Cystic bone disease</td>
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<td>Rarely systemic amyloid disease</td>
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Direct effects of β2m on bone resorption. DRA predominantly affects osteoarticular structures, including bone and synovium. There are probably several reasons for this. AGE-modified β2m has been shown to stimulate macrophages to secrete tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β) (14). Both of these cytokines, levels of which are also elevated in chronic hemodialysis patients, have been shown to cause bone resorption, which may lead to exposure of osteoarticular structures to β2m (15,16). In addition, Miyata et al. further demonstrated that AGE-modified β2m caused sufficient secretion of TNF-α and IL-1β from macrophages to stimulate collagenase synthesis in cultured synovial cells (14). This may subsequently lead to collagen degradation and connective tissue breakdown. β2m itself appears to have a predilection for osteoarticular structures, and Homma et al. found a preferential collagen-binding affinity for β2m that is dependent on the concentration of both β2m and collagen (17). It is also likely that β2m plays an active role in bone resorption. Neonatal mice injected subcutaneously with β2m show histologic evidence of bone resorption with osteoclast-mediated bone mineral dissolution (18). Sprague et al. further demonstrated that this β2m-induced calcium efflux is dependent on IL-1, a substance whose levels are elevated in dialysis patients (19).

It would appear therefore that the etiology of DRA is probably multifactorial (Figure 2). Chronic uremia and inadequate removal of β2m by current dialysis techniques leads to high serum levels of β2m, which upon further modification serves as a potential amyloidogenic substance that has a predilection for bone and collagen. The importance of other factors in the etiology of DRA has not yet been determined and requires further study. Patients with DRA often have concomitant hyperparathyroidism and iron or aluminum overload. These may lead to renal bone disease and the exposure of collagen-rich tissues, with subsequent β2m deposition. Additional local and systemic factors present in some patients will result in β-pleated sheet formation, which is required for acquisition of the fibrillar ultrastructure and amyloid formation. It should be emphasized that not all β2m deposits represent amyloid, and one may find significant histochemical staining for β2m in tendons and subcutaneous collagen with negative Congo red staining.

Diagnosis and Treatment

No case of DRA has been reported in a patient with a serum β2m level of less than 10 mg/L. However, once elevated above this level, serum β2m levels are not useful as a diagnostic test. As stated above, radiological studies may be useful for confirming clinical suspicions of DRA. However, a definite diagnosis of DRA can only be made by demonstrating the presence of typical apple-green birefringent material in Congo red staining of the involved tissue. The nature of the amyloid deposit may subsequently be confirmed by immunohistochemical techniques using appropriate antisera. β2m-amyloid fibril deposition has been demonstrated in bony cysts, carpal tunnels, intervertebral discs, and synovium. Unlike other types of amyloidosis, abdominal fat-pad and rectal-mucosa biopsies are of little value.

To date, there is no specific treatment for DRA. It is therefore important to concentrate on prevention of DRA and to control symptoms if they occur (Table 3). Every attempt should be made to identify those patients who have a higher risk of developing the condition (Table 1).

Role of Dialysis. The clearance of β2m in hemodialysis patients appears to follow a modified three-compartment model (20). In all cases, the initial apparent rise in serum β2m levels during hemodialysis is secondary to extracellular volume loss. Two dialysis-membrane properties are particularly important with regard to β2m clearance on dialysis—the permeability of the membrane to β2m and the membrane’s bio-compatibility. High-flux dialysis membranes, e.g., polyacrylonitrile (PAN), are not only more permeable to β2m, but also actively bind β2m, neither of which occurs with the older cellulosic membrane. The role that membrane bioincompatibility plays in intradialytic β2m generation is more controversial. Incubation of cellulosic or PAN membranes with peripheral

![Figure 2. Proposed mechanism for β2m-amyloid formation in dialysis patients.](image)
blood monocytes in vitro will lead to an inhibition of $\beta_2m$ synthesis by these cells. This would suggest that any $\beta_2m$ synthesis observed during dialysis is not caused by a direct blood/dialysis membrane interaction (21). Moreover, DRA has also been reported in continuous ambulatory peritoneal dialysis patients, who possess the ultimate biocompatible membrane. One possibility is that the in vivo interaction of blood with the bioincompatible cellulose membranes leads to complement activation, with IL-1 and TNF release. This may lead to lymphocyte activation in the pulmonary vascular bed, with subsequent increased $\beta_2m$ synthesis.

Although many studies do suggest delayed development of DRA and a decrease in DRA symptomatology with the use of biocompatible membranes, it has been difficult to demonstrate a clear unequivocal benefit. Chanard et al. showed that the routine use of PAN membranes reduced the annual positive balance of $\beta_2m$ in the body by 50%. They also showed a lower incidence of CTS in patients dialyzed with long-term PAN membranes, as compared with cellulose membranes (22). Other groups have noted a similar decrease in the incidence of cystic bone lesions in PAN-dialyzed patients (3). In a European Dialysis Transplantation Association (EDTA) registry study, however, Brunner et al. were unable to show a statistically significant difference in the incidence of bone cysts in patients dialyzed with AN69 versus cellulose membranes. Many of their patients were not exclusively dialyzed with AN69 membranes, and some had been switched from cellulose membranes to AN69. Despite this, there was a trend toward fewer bone cysts in the AN69 group, compared with the cellulose group (23% versus 38%) (23). Although it is more permeable to $\beta_2m$, the PAN membrane will not normalize serum $\beta_2m$ levels. Indeed, Canaud et al. were only able to reduce $\beta_2m$ levels to 20 mg/L with daily hemofiltration with PAN membranes, a value still ten times normal (24). However, in patients who are deemed unsuitable for transplantation, and particularly in high-risk groups such as the elderly, high-flux biocompatible membranes should be considered for dialysis therapy in an attempt to slow $\beta_2m$ accumulation and disease development (3).

Transplantation and steroid therapy. Renal transplantation should be considered in all suitable patients before the development of established DRA. It will rapidly normalize serum $\beta_2m$ levels and, in patients who have been on dialysis for less than 8 yr, should prevent disease progression (4). Even in patients with symptomatic DRA, transplantation will successfully alleviate the bone pain associated with DRA, an effect that may be secondary to the use of steroid treatment (4). Indeed, Bardin has demonstrated some success in treating bone pain in patients with DRA who are on dialysis with the use of low-dose prednisone (4 to 8 mg), but further studies are needed to confirm his findings (25). Transplantation does not appear to lead to any decrease in the size of bone cysts, and $\beta_2m$ has been recovered from these cysts up to 10 yr after transplantation (4).

Symptomatic treatment. Symptomatic treatment with nonsteroidal anti-inflammatory drugs may help to relieve pain, but does not alter disease progression. Unlike AA amyloidosis, there is no evidence that colchicine is of any benefit in DRA. Treatment of CTS usually requires surgical release to avoid permanent neurological damage, although local steroid infiltration may transiently improve symptoms. In some cases, repeated surgery may be required. Severe large-joint disease, e.g., that of the hips, will often require total joint replacement. Endoscopic resection of the coracoacromial ligament has been moderately successful in the treatment of shoulder pain in long-term dialysis patients (26). It may be necessary to consider cervical spine stabilization if the patient has evidence of cervical spine instability as a result of erosive spondyloarthropathy of DRA.

Summary

DRA is a cause of significant morbidity and is potentially life-threatening in patients on long-term dialysis. Although it has only been described recently, much has been learned about the condition, but the exact pathogenesis continues to allude us. Because many of these patients already suffer from renal osteodystrophy, it is important for nephrologists to recognize this condition and attempt to slow its progression in susceptible groups.

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### Nephrology Training Program at St. Louis University

The nephrology training program at St. Louis University, under the direction of Dr. Kevin Martin, has ten full-time faculty members with diverse interests in basic and clinical research. The curriculum of the fellowship program is flexible and allows for two major paths. The clinical nephrology track comprises a 2-yr fellowship consisting of 18 to 20 months of clinical rotations and 4 to 6 months of clinical and/or laboratory investigation. Fellows wishing to pursue a career in academic nephrology will be assigned to the research track. The clinical activities of this path are analogous in the first year to those obtained via the clinical path; however, the second and optional third yrs of training are devoted exclusively to laboratory investigation. Clinical nephrology training occurs at St. Louis University hospital, a 365-bed tertiary-care hospital that serves as a major medical center in the metropolitan St. Louis area and at the John Cochran Veterans Administration medical center. First-year clinical fellows develop expertise in consultative nephrology, management of acute and chronic renal failure, electrolyte disorders, and all aspects of hemodialysis, including continuous dialytic therapies and continuous ambulatory peritoneal dialysis. The Nephrology Division is also actively involved in the care of renal transplant recipients. In addition, the fellow will rotate among ancillary services, including renal histopathology, chronic hemodialysis, and outpatient transplantation services. Each fellow is also assigned an outpatient nephrology clinic.

Basic research is carried out under careful supervision by the faculty. Current active research projects include the effects of polynsaturated fatty acids on cell biology, including prostaglandin metabolism and cell proliferation, the study of acid-base regulation, and calcium-regulating hormones in the kidney and bone. Collaborative projects involving the departments of biochemistry, pharmacology, and physiology are also ongoing. The Nephrology Division conducts several weekly conferences designed to cover a broad spectrum of clinical and basic pathophysiological material relevant to the practice of nephrology.