Kidney International, Vol. 35 (1989), pp. 567–575

EDITORIAL REVIEW

Amyloid syndromes associated with hemodialysis

The introduction of regional hemodialysis centers has precipitated a tremendous increase in the number of patients with end-stage renal disease undergoing treatment with regular hemodialysis throughout the world. Although most of the clinical manifestations of uremia are effectively managed by this technical advance, renal clinicians have encountered a number of syndromes that are unique to the dialysis patient. Acquired metabolic bone diseases, that is, osteitis fibrosa and aluminum-related osteodystrophy, were among the complications to be encountered in patients as early, but relatively short-term experience was gained with the use of dialytic techniques to support patients with kidney failure. Systematic clinical study of these patients and intensive investigation at the biochemical level have led to effective therapeutic regimens and improved patient well being.

Long term survival of patients with chronic renal diseases treated with regular hemodialysis therapy for as long as two decades is not uncommon, but such long-term dialysis appears to be associated with unique problems. The observations of astute clinicians have uncovered an association between the carpal tunnel syndrome, bone cysts, pathologic fractures, and scapulohumeral periarthritis among long-term dialysis patients [1–4]. These musculoskeletal syndromes are not caused by hyperparathyroidism or by aluminum accumulation, two recognized major causes of musculoskeletal disease; rather, there is reason to believe that these syndromes have a similar etiology. A unique variety of amyloid deposit has been recovered from tissues in a substantial number of these cases [3–12], and the term "hemodialysis-related amyloidosis" (HRA), has been used to describe the constellation of signs and symptoms [13].

The rapidly expanding literature in this field, along with the likelihood that the renal clinician will encounter patients with these syndromes, make a review of this subject timely. This discussion reviews the clinical and biochemical data, pathologic findings and potential mechanisms to account for HRA. Preliminary data are examined regarding methods which attempt to prevent or minimize the accumulation of amyloid precursors. Potential modifications of dialytic techniques that might be employed to manage the affected patient are also considered. This will hopefully allow the clinician to critically evaluate dialysis manufacturers claims about dealing with this condition.

Background and historical perspective

Amyloid is the term used to describe a group of relatively-insoluble proteinaceous materials with unique biochemical and structural properties. A characteristic repetitive polymer of fibrils and the property of insolubility in physiological solutions are common to all amyloid proteins. However, the formation of a stable suspension in distilled water has allowed the elucidation of the chemical structures of these proteins [14]. The beta-pleated sheet and repetitive fibrillar pattern of amyloid materials, as revealed by x-ray crystallagraphic and infrared analysis of the tertiary structure [15], account for the term, beta fibrilloses, to describe the diseases of amyloid accumulation. Although histologically identical, biochemical analysis of these proteins reveals heterogeneity. There is both a large and small molecular weight fraction. The latter peak varies in molecular weight, ranging 4,200 to 31,000 daltons, permitting the chemical and immunological characterization of the amyloid proteins [14].

When amyloid proteins become insoluble and deposit within the physiological environments of the cell, thereby replacing normal tissue constituents, the function of the affected organs can be disturbed. Biopsy of the involved tissue may reveal amyloid proteins when the specific histologic staining methods and electron microscopic techniques, described below, are employed.

The diseases characterized by the accumulation of amyloid proteins were initially categorized as *primary* or *secondary*. The former, which is also called idiopathic amyloidosis, occurs in association with multiple myeloma and other plasma cell dyscrasias that are characterized by the unregulated production of light chain immunoglobulins. The amyloid protein of *primary amyloidosis* is derived from light chain immunoglobulins and is designated AL. *Secondary amyloidosis* is often associated with chronic inflammatory or infectious conditions, and the amyloid protein is designated AA. In the United States and Europe, where chronic granulomatous infections have been largely eradicated by the use of chemotherapeutic agents, secondary or reactive amyloidosis most commonly occurs in association with chronic rheumatic diseases. The protein fibrils of AA are comprised of degradation products of the hepatically synthesized acute phase reactant protein, SAA. Amyloidosis has also been reported with the amyloid protein derived from precalcitonin arising from medullary carcinoma of the thyroid gland [16], from prealbumin associated with the familial amyloid cardiomyopathy [17], and from beta protein [18] in Alzheimers disease. The protein in HRA is derived from beta$_2$-microglobulin (β_2-M), a specific immunoglobulin that is described below.

Despite the description of systemic amyloidosis in 1886 [19], involvement of the skeleton with this proteinaceous substance was not reported until 1922 [20] when diffuse deposition of amyloid in the tendons, ligaments, and joint capsules was observed in a patient with massive amyloid infiltration of the heart. In 1939, Koletsky and Stetcher [21] reported the rheumatic complaints resulting from amyloidosis. These symptoms included swelling and stiffness of the hand associated with tingling and burning sensations (carpal tunnel syndrome). After many years, the patient developed a pathologic fracture of the

Received for publication October 9, 1987
and in revised form June 6, 1988

femur, and widespread amyloid involvement of joints and bones was found at autopsy. The shoulders of the patient were so distorted by amyloid infiltration that the authors described them as "padded." More recently, the term, "shoulder pad sign" has been applied to patients with amyloid deposition within the shoulder joint because of the resemblance to a football player's shoulder pads [22]. Although the biochemical type of amyloid differs in patients affected by HRA, the similarity in the clinical findings—especially the rheumatic complaints—with these earlier historical descriptions will become apparent.

Amyloid-related syndromes in dialysis patients

In HRA, amyloid fibrils are predominantly deposited in the perineural and periarticular structures, joints, bone, skin and subcutaneous tissue [1–4, 5, 6, 9–12, 23–31]. Far less commonly amyloid tissue has been found in the rectal mucosa, liver, spleen, kidney, prostate [30] and blood vessels [30, 32, 33]. There is a single report of a dialysis patient with kidney stones in which HRA was the predominant proteinaceous material [34]. Since the organs systems from which HRA has been recovered is diverse, it would not be a surprise if more systemic involvement with this type of amyloid is observed and reported from long-term dialysis patients.

A variety of arthritic and neuromuscular syndromes, unrelated to crystalline-induced arthritis, were described with the wide use of dialysis to treat end-stage renal failure [2, 4, 35]. A recent survey indicated that the tissues from long-term hemodialysis patients most consistently found to contain amyloid material included bone, joint and the synovium [31]; thus, it is no surprise that the carpal tunnel syndrome, arthritic complaints and bone disease are commonly observed clinical manifestations. For ease of discussion, HRA is divided into these different clinical presentations. It should be understood, however, that there is considerable overlap in symptoms referable to the musculoskeletal system and that individual patients will differ in their clinical presentation. The degree to which β_2-M can be attributed as the only factor in the pathogenesis of these syndromes is not yet apparent, and thus, reference to specific reports will be noted in which β_2-M has been recovered from anatomical sites.

Carpal tunnel syndrome (CTS)

The carpal tunnel syndrome (CTS) results from entrapment of the median nerve at the wrist. It is accompanied by discomfort and occasionally sensory loss of palmar surfaces of the thumb, index, and middle fingers and the radial aspect of the fourth finger. Measures to elicit the symptoms include light tapping over the median nerve (Tinel's sign) and either forced flexion (Phalan's sign) or forced extension of the wrist. Pain is often most severe at night, and it may waken the patient from sleep. As the CTS progresses, there may be weakened opposition and abduction of the thumb with atrophy of the thenar eminence.

The conditions associated with the CTS in non-dialyzed patients include pregnancy, diabetes mellitus, hypothyroidism, rheumatoid arthritis, old fractures and amyloidosis. Also, trauma to the wrist, such as occurs with scrubbing, with repeated and prolonged extension and pressure on the wrist, is associated with the CTS.

Even though the diagnosis is most often made by a history and physical examination, objective studies can be helpful in confirming the diagnosis. Nerve conduction studies often demonstrate a decrease in sensory conduction, manifested by decreased amplitude and distal latency. Motor nerve conduction is less frequently abnormal.

Table 1. Reports of hemodialysis patients with carpal tunnel syndrome and hemodialysis-related amyloidosis

Reference *No.*	Dialysis patient *No.*	Patient with CTS *No.*	Duration of dialysis *Yrs*	Amyloidosis Bx-positive *No.*	Total Bx *No.*
2	312	7	8.6	7	7
4	1000	31	10.3	17	24
5	230	9	10	9	—
6	236	17	8–13	13	15
7	110	—	0.9–12	38	52
23	100	12	0.3–12	7	12

Since the first description of CTS in hemodialysis patients in 1975 [36], its incidence in large groups of dialysis patients has ranged from 2 to 31%. The symptomatology experienced by dialysis patients with CTS does not differ from the non-uremic patient afflicted with this disorder. In a questionnaire survey of hemodialysis patients [34], two-thirds of the patients reported symptoms of the CTS; confirmatory nerve conduction studies were not performed. Among afflicted patients, 31% described pain, 25% swelling, and 61% had parasthesias localized to the first four digits. Symptoms occurred both during the dialysis procedure and on non-dialysis days. A minority of patients had a decrease in light touch sensation on physical examination; Tinel's sign was present in one-half of their patients, but this has rarely been reported by others [5, 24]. Symptoms are the most sensitive early feature of the CTS, and nerve conduction tests may be abnormal only when the degree of compression is severe.

The CTS associated with HRA usually develops after an average of eight to nine years but rarely before four to five years of initiation of hemodialysis therapy (Table 1). In the careful epidemiological studies of Kachel and associates [6], severe symptoms of CTS that were confirmed by electrophysiological tests and treated by decompression of the median nerve occurred only if the total duration of dialysis was four years or more. Four percent of those undergoing dialysis for five to nine years had the syndrome, but the prevalence was 30% among patients on regular hemodialysis for longer than nine years. The frequency was more closely related to the duration of treatment with hemodialysis than the duration of renal disease.

Management of the CTS has not been altogether satisfactory. The application of an extension splint to the wrist, particularly at night is often useful for mild symptoms. The response to injection with local anesthetic agents and corticosteroids is only transient, while surgical decompression of the median nerve has been of the greatest benefit to patients with marked disability and pain from the CTS.

Arthropathy

The rheumatic disorders that have been described in dialysis patients included infectious and crystal-induced arthritis and peri-articular calcifications [37]. An increasing number of pa-

tients with arthritic complaints, many of which are idiopathic in nature, have been reported among long-term hemodialysis patients [2, 4, 9, 27, 35, 38–40]. In some reports, there was the histologic identification of amyloid material, and in many of these, further characterization revealed β_2-M [30, 41].

Although not mutually exclusive, three relatively distinct arthropathic syndromes have been described in hemodialysis patients with HRA. These include: generalized arthritis (frequently with erosions of the joints), scapulohumeral periarthritis, and an arthropathy with joint effusions [2, 4, 6, 7, 27, 35, 38–40]. The association between HRA and the latter two syndromes has been more convincing than that between HRA and generalized arthritis.

Generalized arthritis. Generalized arthritic complaints are probably most commonly encountered by the physician caring for long-term hemodialysis patients. The arthritis may be generalized or local in distribution and is often but not always destructive in nature. The most commonly affected joints include the shoulders, knees, wrists, hip and occasionally the intervertebral discs of the spine; however, any joint may be affected. In addition to symptoms of pain and stiffness, objective findings on examination include decreased joint motion, effusion, crepitance or even deformity [9, 27, 35].

In one study, eight of 11 patients hemodialyzed for greater than 10 years complained of pain and stiffness in the joints, unrelated to prior septic or traumatic events. More than one-half had multiple joints affected, and one-half complained of knee pain. Limitation in the range of motion was mostly noted in the shoulder, whereas effusions and crepitance more frequently affected the knee. Camptodactyly, the irreducible flexion of fingers, Swan neck deformities and early Heberden's nodes affected the hands of these patients [35].

Where a prolonged duration of hemodialysis therapy is the primary risk factor for the development of arthritis, other risk factors include previous parathyroidectomy and, surprisingly, a younger age [27]. There is no predilection towards one sex. The serum levels of immunoreactive parathyroid hormone and the plasma aluminum level have not been helpful in distinguishing afflicted patients from those without joint complaints [27, 42], but these determinations have not been consistently reported.

Radiographic evidence of joint erosions, not typical of renal osteodystrophy, increases in frequency with the duration of peritoneal or hemodialysis [40]. The erosive changes on X-rays most commonly involve the metacarpalphalyngeal joint, predominantly on the ulnar side, followed in frequency by the proximal and distal interphalyngeal joints, shoulder, wrist and knee [40]. Occasionally erosions are noted in the intervertebral areas [9, 43]. Many patients have clinical manifestations in the absence of radiographic erosions, and other patients who are asymptomatic have radiographic lesions. Symptoms were most commonly absent with abnormalities of the symphysis pubis and sacroiliac joints [40]. The longer cumulative duration of dialysis and higher serum alkaline phosphatase level were the only differentiating features observed patients whose radiographic changes included both erosive arthritis and renal osteodystrophy in comparison to those dialysis patients in whom only the latter were seen. Notably, one-third of the patients received peritoneal dialysis as the primary modality of treatment for renal failure. The significance of this in relation to these arthritic changes is not yet apparent [40].

Since the cumulative length of dialysis is the most consistent finding in these patients, it is tempting to attribute the signs, symptoms and radiographic features of this generalized and sometimes erosive arthritis to β_2-M. β_2-M has been recovered from many of the tissues [9, 43], but it also has been found incidentally in the sternoclavicular joint of asymptomatic patients undergoing parathyroidectomy. Because of this and the finding of hemosiderin deposits in affected joints, it has been suggested that deposits of iron, occurring either from hemarthrosis or iron overload, rather than amyloid may be the cause of arthropathy in long-term dialysis patients [42]. There is precedent for amyloidosis causing erosive arthritis, albeit less severe, from observations of articular AL amyloidosis in patients with multiple myeloma [44, 45].

Scapulohumeral periarthritis. The shoulder is the most commonly affected joint in long–term hemodialysis patients with arthritic complaints. Amyloid deposits are often recovered from the carpal tunnel tissue in patients with shoulder pain [6, 7]. One group of investigators reported positive amyloid staining in CTS deposits in 11 of 13 patients with concomitant shoulder pain [6].

The histologic diagnosis of HRA can be made by an excisional biopsy of tissue from the shoulder joint. Surgical exploration reveals swelling and thickening of the subacromial bursa. Predominant areas of amyloid infiltration included the synovium in the interstitial region and the tendon sheath. Yellowish brown granular deposits and rupture of the biceps tendon may be noted. The bursal lumen can contain massive amounts of bloody fluid. These findings are similar to those found in patients with idiopathic or secondary amyloidosis involving the shoulder space, except that in HRA, immunofluorescent staining with antiserum to β_2-M is strongly positive [41, 46].

Effusive arthropathy. A third clinical presentation for rheumatic involvement with HRA is joint effusions. The frequency of joint effusions ranges from 2% to 8% in reported series [2, 40], but the incidence reaches nearly 50% in patients receiving dialysis for more than 10 years [35]. The usual clinical presentation is the persistent swelling and effusion of a joint in a patient who has undergone hemodialysis for longer than eight years. The swelling may persist for up to two years [2]. Involvement is frequently bilateral and accompanied by mild discomfort. Occasionally, symptoms of acute pain are severe and frank arthritis is noted upon physical examination.

Aspiration of the joint space yields a serous, sterile fluid that is characteristically non-inflammatory. The cell count has ranged from 50 to 5,000 cells/mm^3 [2, 40]. The glucose level is normal and the protein content low. Crystals are not present when polarized light or electron-microscopic methods are utilized to visualize the fluid [40]. In two of seven patients with effusions, an acute arthritis was superimposed upon a chronic effusion and the synovial fluid leukocyte count increased from low baseline levels to 8,000 and 100,000 cells/mm^3. Sediments of centrifuged joint fluid that are fixed in paraffin and stained with Congo red sometimes reveal the typical green birefrigence of amyloid material, and this may serve as a relatively easy means of diagnosis when amyloid is present in the synovial fluid; the amyloid can more often be found on the synovial surface of the involved joint or in the tendon sheath during an arthroscopic procedure. Synovial biopsies reveal mesothelial hyperplasia or nonspecific chronic synovitis. Four of the seven

patients with joint effusions and HRA also had the CTS [2]. When the latter was present, amyloid deposits were uniformly found in the tissue fragments removed from carpal tunnel release procedures. One patient with positive staining for amyloid in the CTS also had amyloid in a fat pad biopsy, although the experience of others in diagnosing amyloid by abdominal fat pad aspiration in long-term dialysis patients has been disappointing [47]. All the patients of this series [2] with effusions had radiographic evidence of severe renal osteodystrophy, defined as changes of severe hyperparathyroidism; serum PTH and aluminum values, however, were not reported. The relevance of excess PTH or aluminum toxicity to the development of effusion and synovitis is unknown, although high aluminum levels have been found in the joint tissues of patients receiving regular treatment with hemodialysis [48].

Skeletal manifestations

Bone can also be affected by amyloidosis with β_2-M deposits in patients on hemodialysis; usually it manifests clinically as a cystic change or pathologic fracture within the bone most often at the site of a tendinous insertion. Radiographic evidence of cystic changes within juxta-articular bone, often resembling brown tumors, has been noted in the femoral heads [47], acetabula [3], humerus, radius [26], carpal bones [1], tibial plateaus [4], pubic symphysis, patella [2], and tarsal bones [47, personal observations]. These cystic and destructive lesions reveal typical amyloid material when aspirated or when pathologic fractures were repaired surgically [28, 47].

The natural history of these bony abnormalities is one of progressive enlargement of the cystic area (revealed by serial radiographs), and subsequent replacement by the amyloid material [49]. This can cause pathologic fractures within bone, especially when in proximity to a weight bearing joint [28, 47, 49]. Thus, HRA can cause fractures that are unrelated to the presence of renal osteodystrophy, and this condition becomes part of the differential diagnosis of such lesions in patients receiving regular hemodialysis.

The bony involvement with HRA should not be completely surprising since lytic lesions of the skeleton have been reported as the clinical presentation of both primary and secondary amyloidosis [46, 50]. As with other syndromes of HRA, the cumulative duration of dialytic therapy is long and appears to be the greatest risk factor for the development of cystic roentgenographic features and pathologic fractures of long bones.

In addition to conventional radiographic techniques, amyloid involvement of the skeleton has been evaluated by various scintigraphic techniques. The uptake of gallium 67 citrate [51], Indium 111 bleomycin [52], technectium-99 sulfur colloid, technectium 99 pertechnetate [53], technectium-99 pyrophosphate [50, 54–56] and technectium-99-methylene diphosphonate [54, 56] have been utilized to detect amyloid involvement of the heart, liver, bone, muscle and kidney. Of these compounds, technectium 99 diphosphonate and methylene diphosphonate were the most sensitive and specific for the detection of amyloid deposits in skeletal and soft tissues [50, 54–57], although Tc 99 pyrophosphate was better than methylene diphosphonate in a prospective study to detect AA and AL amyloid in soft tissue [56].

Although these studies were performed on patients without HRA, the preliminary application of nuclear bone scanning techniques with technectium-99-methylene diphosphonate to hemodialysis patients afflicted with HRA syndromes has recently been reported [58]. Four of five patients undergoing hemodialysis for greater than 12 years with proven osteoarticular β_2-M had abnormal articular and periarticular uptake of Tc-99 methylene diphosphanate, several in multiple areas. The increased tracer uptake was generally focal in distribution. Three of the five patients had hyperparathyroidism (as demonstrated by elevated plasma PTH levels and radiographic findings of subperiosteal erosions) and all were noted to have evidence of aluminum overload, as revealed by positive deferoxamine challenge. Diffuse tracer uptake is seen with hyperparathyroidism and osteitis fibrosa [59], while a generalized decrease in Tc-99 methylene diphosphonate uptake is often observed in patients with aluminum related osteomalacia [60]. Thus, proper interpretation of these nuclear scans in a dialysis patient with rheumatic symptoms would require a consideration of the effect of these metabolic bone diseases [59–63]. However, if a long-term hemodialysis patient was noted to have an increased focal uptake of Tc-99 methylene diphosphanate in the presence of an otherwise normal bone scan, and had neither septic, crystalline induced nor aluminum or hyperparathyroid related osteomalacia, the possibility of HRA should be entertained. Further studies in larger numbers of patients will help to establish the role of these non-invasive nuclear scanning techniques in the diagnosis of the patient afflicted with HRA and the usefulness of screening asymptomatic long-term hemodialysis patients for evidence of HRA.

The mechanism(s) of how these isotopes are taken up by amyloid tissue is presently unknown. Since the lytic lesions in bone are devoid of osteoblasts, isotope uptake by this cell seems unlikely [50]. Amyloid tissue has been noted by some investigators to have an increased calcium content, possibly explaining the increased isotope binding [54, 64–66]. Further studies are needed to elucidate the mechanisms by which amyloid binds these isotopes and specifically whether isotopes are also bound to β_2-M.

Biochemistry of hemodialysis-related amyloid

The amyloid isolated from the synovium and bone of hemodialysis patients includes features common to other types of amyloid. On histologic examination, the deposits stain positive for Congo red and exhibit an apple-green birefringence under polarized light. Characteristic curvilinear fibrils are seen on electron microscopy. The twisted beta-pleated sheet configuration by x-ray crystallography, essential for Congo red affinity, is present and explains the resistence to normal degradative processes in vivo.

Permanganate treatment of tissue sections will dissipate the birefringence of amyloid AA and HRA [11]. This finding initially led investigators to believe that the amyloid of dialysis patients was the AA type. However, neither anti-human AA nor anti-human prealbumin reacted significantly with histochemical tissue sections from tissue obtained during surgical decompression of dialysis patients with the CTS [11, 12, 41]. Immunoblot analysis of tissue sections with antisera to β_2-M was strongly positive, providing the first clue that HRA was biochemically distinct from previously described types of amyloid.

Chemical analysis confirmed the HRA to be comprised of

β_2-M [13, 67]. After solubilization of these amyloid fibrils, amino acid sequencing of the major protein fraction obtained by the various gel filtration techniques showed a protein with a structure identical to that of β_2-M. Two-dimensional gel filtration revealed 12 and 24 kilodalton (kD) proteins that correspond to human β_2-M. This heterogeneity of the β_2-M proteins isolated by gel electrophoresis occurs because of the presence of dimers, tetramers and polymers of the basic 12 kD β_2-M protein. More than 95% of the β_2-M in the plasma of dialysis patients is monomeric and consists of the 12 kD protein. These elegant biochemical studies have given persuasive evidence that a unique amyloid protein is deposited in the synovium and bone of many long-term dialysis patients. Different biochemical methodologies used to isolate HRA demonstrated that globin chains may also be a major constituent of HRA [68].

Physiology and metabolism of beta$_2$-microglobulin

β_2-M is a globular protein with a molecular weight of 11,800 daltons, first isolated from the urine of patients with Wilson's disease and cadmium poisoning [69]. It is composed of a single polypeptide chain of 100 amino acid residues with an intrachain disulfide bridge between positions 25 and 81. The protein is normally present on all cell membranes other than erythrocytes and trophoblastic cells [70]. Although not identical, there is striking homology between the amino acid sequence of β_2-M and the constant domains of the heavy and light chain immunoglobulins [71]. β_2-M comprises the beta chain of the HLA class I molecule [72] that is necessary for cell-cell recognition. The gene coding for the structure of β_2-M has been isolated on chromosome 15 [73].

The appearance of β_2-M in tissue fluids most likely arises from the high turnover of cell membranes. The lymphoid system is quantitatively a large producer of β_2-M in both the unperturbed state and after stimulation with mitogens and paracrine factors in vitro [74, 75]. Although it is likely that the lymphoid system predominates in the production of β_2-M in vivo, direct proof of this is lacking [70].

The normal levels of β_2-M in serum and synovium are less than 3 μg/ml [76]. At least 95% of the β_2-M recovered from urine and serum is the free monomer [77]. Like many low molecular weight proteins, β_2-M is freely filtered at the glomerulus and reabsorbed in the proximal tubule. Significant renal catabolism of this protein has been demonstrated by turnover studies using I-125 β_2-M in both subjects with normal renal function and patients with varying degrees of renal insufficiency [70, 78, 79]. The disappearance of the molecule after intravenous injection of the radiolabel is bimodal; the first half-life (9 to 20 min) is independent of GFR and corresponds to diffusion of the protein into the extracellular space; the major subsequent fall in serum level is dependent on GFR and renal catabolism. Non-renal catabolism contributes to less than 3.5% of the total breakdown of β_2-M. The normal catabolic rate of the β_2-M is approximately 150 mg/day [80].

With chronic inflammatory diseases and malignancies, an increase of its production can elevate the β_2-M serum levels when renal function is normal [81]; otherwise the most important cause of high serum levels is renal failure. In humans, the serum β_2-M levels correlate positively with serum creatinine and inversely with renal function until end-stage renal failure ensues [70, 78].

Pathogenesis of HRA

Despite a formidable number of clinical reports attributing HRA to β_2-M, the potential mechanisms responsible for the formation and deposition of this amyloid in long-term hemodialysis patients are presently unknown. Several hypotheses have been forwarded to explain why HRA might develop. The factors that may contribute to the amyloidogenic potential of β_2-M include: the persistently elevated plasma concentrations of β_2-M, iron overload, aluminum intoxication, local effects of the arterio-venous fistula, and long-term and repeated stimulation of the immune system with the intermittent production of interleukin 1 by the contact between the patients blood and the hemodialysis membranes.

Early reports suggested that CTS arose from local pressure caused by the arteriovenous fistula. Edema, engorgement and ischemia in the region of the angioaccess were thought to contribute to the CTS in dialysis patients [82–84]. However, Charra and others found no relationship between the location of the arteriovenous fistula and the limb affected by the CTS [7]. The recovery of amyloid from the synovium of joints that are not in anatomic proximity to the angioaccess, bilateral involvement of CTS, and its presence in an extremity without a fistula, provided further evidence against this theory [4].

The CTS has been reported in patients both with and without previous parathyroidectomy, suggesting that parathyroid hormone levels or parathyroidectomy do not affect its occurrence; however, the levels of parathyroid hormone have not been reported systematically. Ectopic calcification occurs no more frequently in dialysis patients with CTS than in those without it; therefore, it has been implied that alterations of calcium and phosphorous metabolism play little or no role in the pathogenesis of the CTS [27].

Increased deposition of aluminum in the joint spaces has been reported in hemodialysis patients receiving aluminum-containing phosphate-binding agents [48]. Aluminum can cross the synovial barriers, and the aluminum concentrations in synovial fluid are two- to tenfold higher in dialysis patients ingesting aluminum containing gels than in patients not taking aluminum gels. Although plasma aluminum levels have not been reported in patients with HRA, the suggestion of potential synergism between aluminum and β_2-M fragments causing amyloid deposits to form joint and bony abnormalities [85] merits further investigation.

Iron may be deleterious to joints or joint spaces as it accumulates in dialysis patients. In one report, dialysis patients with the most severe arthropathy had the highest serum ferritin levels, an observation leading to the speculation that iron overload may have a pathogenetic role in the arthritis of dialysis patients [27, 42]; also, hemosiderin has been recovered from affected joints [42]. Iron in a low valency state can promote the production of free radicals which can lead to peroxidation and inflammation of the synovium in patients with rheumatoid arthritis [86]. Once again, the potential interaction between iron and the β_2-M protein is unknown.

An engaging theory of relevance to the pathogenesis of HRA is related to the immunologic changes that occur when a patient's blood contacts the membrane of the artificial kidney, leading to the release of cytokines and lymphokines. In vivo and in vitro studies have both demonstrated that the comple-

ment cascade is activated following contact between human plasma and a dialysis membrane composed of cupraamonium sulfate (Cuprophan^R) [87]. Both C5a and the adherence of macrophages to this membrane cause the release of interleukin 1 from mononuclear cells. Interleukin 1 (IL-1), previously termed endogenous pyrogen, is a family of polypeptide hormones with molecular weights ranging from 15,000 to 17,000 daltons. The systemic effects of interleukin 1 include increased hepatic synthesis of several acute phase proteins, including SAA protein, C reactive protein, fibrinogen and ferritin. Moreover, the plasma levels of zinc and iron fall, and serum copper and ceruloplasmin increase. The interleukin 1 produced by macrophages causes leukocytosis and activates lymphocytes, which in turn lead to the febrile response to acute injury, infection, or other inflammation [88, 89].

The measurement of IL-1 in the serum of hemodialysis patients is hindered by a circulating protein inhibitor. More refined techniques that facilitate its measurement have demonstrated a twofold increase in IL-1 in patients on hemodialysis for more than 10 years when compared to normal controls [90, 91].

In vitro experiments that studied the interaction between monocytes and various dialyzer membranes, including polyaminonitrile, regenerated cellulose acetate and Cuprophan^R have demonstrated that IL-1 production is increased significantly even in the absence of complement or endotoxin. There are no data on the effect of IL-1 on levels of β_2-M of normals or patients receiving regular hemodialysis. Whether these changes could account for higher β_2-M levels immediately after dialysis is uncertain but requires further study.

A likely mechanism to account for the development of HRA is the markedly increased plasma levels of β_2-M, per se. As noted above, β_2-M is largely cleared and metabolized by the kidney, and the plasma concentrations of β_2-M are markedly elevated in dialysis patients compared to normal subjects. In addition, the plasma levels of β_2-M continue to rise slightly with the cumulative duration of dialysis treatment. Dialyzed patients with some residual renal function have lower serum levels of β_2-M than anuric or anephric patients. How serum levels of β_2-M are affected by the type of dialysis membrane is discussed below.

A unique physiochemical property of the β_2-M which characterizes HRA, is the ability of β_2-M to form polymers and adopt a fibrillar structure in vitro, either by increasing the β_2-M concentration or by altering the ionic strength of the solution [92]. Also, more than 50% of the bovine homologue of β_2-M, added in vitro, can form the beta-pleated sheet pattern that is common to amyloid protein [93, 94]. Thus, β_2-M is the only known protein precursor that can form amyloid fibrils in vitro.

The development of HRA is not simply related to or determined by the serum level of β_2-M in a dialysis patient. Gejyo et al [95] attempted to distinguish hemodialysis patients with amyloid deposits from the serum level of β_2-M. Ten of the 210 patients had amyloid deposits found with surgery for the CTS, yet the serum β_2-M level did not distinguish these patients from other dialysis patients without clinical evidence of HRA. The fact that HRA is generally not clinically apparent until eight or more years of dialysis therapy is instituted implies that the pathogenesis is complex. The predilection of HRA to involve the synovium and bone has led some to suggest the importance and presence of local tissue factors [67] or an amyloid enhancing factor [95]. β_2-M levels within synovium of hemodialysis patients have not been measured, although one would assume that the tissue levels would parallel the serum levels, as occurs in those without renal failure.

Role of hemodialysis membranes in HRA

The relationship between the chemical composition and porosity of the artificial kidney membrane, and the generation and retention of β_2-M is currently undergoing intensive investigation. However, the importance of these factors in the development of HRA is uncertain. Few data are available on the types of artificial kidneys used by long-term hemodialysis patients who developed these syndromes. The data reported are promising but still preliminary in terms of prevention and especially in the treatment of HRA. The institution of widespread changes in dialysis membranes for stable, long-term hemodialysis patients should be done only with caution at this time. The individual practitioner should read studies pertaining to this rapidly expanding area of research with caution and view some of the recommendations as speculative. Nonetheless, investigators are systematically examining different dialysis membranes for both their propensity to generate IL-1 and their ability to reduce the serum levels of β_2-M in dialysis patients.

Patients using the polyacrylonitrile and polysulfone membranes for both standard blood flow and high-flux dialysis have slightly lower serum levels of β_2-M than those using Cuprophan^R membranes for dialytic treatments [96, 97]. The higher permeability dialysis membranes, such as polyacrylonitrile (PAN) and polysulfone (PS), reduced the plasma β_2-M level by approximately 30% during a single dialysis session [98]; this difference may be related to the higher sieving coefficient of these membranes [96, 99, 100].

In a systematic evaluation of different membranes employed for high-flux dialysis, it was found that the removal of β_2-M was not equal: Cuprophan^R membranes did not remove β_2-M whereas PAN, polymethylmethacrylate (PMMA) and PS all removed this low molecular weight protein. Moreover, the PS membrane had the highest sieving coefficient and net removal of β_2-M. The PS dialysis membranes lowered the β_2-M levels in plasma by 12 to 24% during a single dialysis treatment, while the reductions were only 8 and 5% by PAN and PMMA membranes, respectively [101].

One study of 10 patients receiving hemodialysis with Cuprophan^R membranes found that the serum β_2-M level rose after a four hour hemodialysis, with the maximal level in the third and fourth hours of the procedure. In contrast, the serum level of β_2-M fell with PAN membranes [102]. Another group found the average serum β_2-M levels to rise 21% after one hemodialysis treatment with a cuprophane membranes and 16% with a cellulose acetate dialyzer [103].

Most studies that evaluate the changes in β_2-M after dialysis do not take into account the ultrafiltration and hemoconcentration that occurs during the dialysis procedure. A formula has been devised to take this into account and to "correct" the β_2-M levels before and after hemodialysis [104]. When these parameters were taken into account, the corrected values for β_2-M with the "low permeability" membranes (Cuprophan^R, cellulose acetate, polymethylmethacrylate and polycarbonate) was not markedly different from baseline, whereas significant

decreases in corrected β_2-M occur with PS (−47%), PAN (−33%), and cellulose acetate (−15%). Since bioincompatibility is potentially a reason for the evaluation in serum β_2-M levels that occurs after hemodialysis with various dialyzers, further and more detailed studies will be needed to differentiate the relative importance of bioincompatibility versus the impaired clearance of β_2-M by various artificial kidney membrane surfaces [105, 106].

Case control studies have not found the serum β_2-M level as a predictor of risk for developing the HRA syndromes [95]; however, it seems logical to believe that the lower the serum levels of β_2-M, the less the chance that HRA will develop. In a retrospective study of patients treated with either Cuprophan^R or PAN membranes, none of 11 patients treated with PAN for 7 to 10 years developed HRA; while approximately 15% of those treated with Cuprophan^R developed clinical syndromes associated with HRA. More dramatically, none of the three utilizing PAN for 11 to 14 years developed HRA compared to 6 of 10 who used Cuprophan^R dialysis membranes for a similar period [49].

HRA and peritoneal dialysis

There has been considerable growth in the use of peritoneal dialysis for the long term treatment of patients with end-stage renal diseases [107], and it has been questioned whether patients undergoing CAPD have equal predisposition to HRA [108]. The relative short-term experience with chronic ambulatory peritoneal dialysis (CAPD) compared to that for hemodialysis probably explains the small number of cases reported with HRA during CAPD, and it is likely that a critical time period has not lapsed for the clinical manifestations to appear. Careful and appropriate histochemical stains may not have been reported from tissue fragments obtained during surgical procedures for CTS or other joint abnormalities that developed in these patients. Only one study describes peritoneal dialysis patients developing HRA, but, the number of years of therapy with CAPD was not reported [40].

Data have appeared regarding β_2-M levels in patients on CAPD [100, 105]. In short term studies with CAPD for less than one and one-half years, β_2-M levels are markedly elevated, similar to those in patients treated with hemodialysis [100]. However, when the cumulative duration of peritoneal dialysis is longer, there was a significantly lower serum level of β_2-M in the CAPD patients [105, 109]. This may be explained by the relatively greater clearance of the high molecular weight proteins, such as β_2-M, with peritoneal dialysis techniques; moreover, peritoneal dialysis has been used therapeutically to reduce the elevated levels of immunoglobulins in patients with paraproteinemias [110]. The peritoneal membrane is permeable to β_2-M; however, peritoneal fluid concentration of β_2-M reach only 10 to 15% of serum levels. The 24 hour clearance using four standard exchanges per day has been estimated to be 30 to 40 mg, which is still far short of the 150 mg excreted and metabolized by the functioning kidney. Whether patients utilizing CAPD as the primary dialysis modality will be less likely to develop the syndromes associated with HRA must require further time and study.

Summary

A historical review and current clinical findings relating a new type of amyloid material to long term hemodialysis are presented, followed by a review of the biochemistry, metabolism and involvement of β_2-M and theories for the pathogenesis of HRA. The syndromes develop several years after replacement of renal function by dialysis, and seem to be progressive over time. Preliminary clinical studies utilizing more permeable artificial kidney membranes suggest their potential usefulness in the prevention of HRA syndromes, specifically those attributable to persistent elevation of serum β_2-M; however, caution in their employment is advised. The development of effective treatment for long-term hemodialysis patients afflicted with CTS, arthritic symptoms and skeletal manifestations of HRA is unfortunately constrained by deficiencies in our knowledge. Renal transplantation has been demonstrated to reduce the elevated serum β_2-M levels in hemodialysis patients to normal [111]; however, the effectiveness of this modality to treat clinical manifestations of HRA has not been reported. Thus, efficacious treatment strategies have lagged considerable behind diagnostic techniques. Intensive research is needed as the story of this new form of renal osteodystrophy unfolds.

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Acknowledgments

Dr. Kleinman was a recipient of a Research Fellowship from the National Kidney Foundation of Southern California.

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