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NEPHROLOGY FORUM

Dialysis-related amyloidosis

Principal discussant: KARL M. KOCH

Medizinische Hochschule Hannover, Hannover, Germany

Case presentation

A 38-year-old male required the initiation of regular hemodialysis treatment 19 years ago because of end-stage renal disease due to mesangioproliferative glomerulonephritis.

After an initial 3 months of center dialysis, during which an arteriovenous fistula was created in the left forearm, the patient was trained for home dialysis. At home, he was dialyzed overnight 3 × 10 hours/week at a blood flow of 250 ml/min and a dialysate flow of 500 ml/min using the Kiil dialyzer with cellulosic membranes and a 1 m^2 surface area; a given dialyzer was used three times before being rebuilt. After 5 years, "reuse" Kiil dialysis was replaced by single-use dialysis employing a 1 m^2 commercial plate dialyzer with cellulosic membranes; dialysis time was reduced to 3 × 8 hours/week but blood and dialysate flow rates were unchanged. At that time the patient was totally anuric. His predialysis (first dialysis of the week) serum urea and creatinine concentrations with the new dialysis regime ranged from 21–25 mmol/liter (120–150 mg/dl) and 1000–1100 mol/liter (11–12 mg/dl), respectively. The patient was unemployed but physically well; he refused to be placed on the waiting list for cadaveric transplantation.

Ten years after the start of hemodialysis, radiologic examination of the skeleton showed general osteoporosis without specific lesions attributable to renal osteodystrophy. The serum levels of C-terminal PTH were moderately elevated. The patient was normocalcemic and had intermittent mild hyperphosphatemia (< 6 mg/dl). Bone histology from an iliac crest biopsy revealed only slight osteitis fibrosa, as well as minor evidence of defective mineralization. The dose of oral phosphate binders and oral calcium carbonate was increased, and treatment with calcitriol (0.25 μg/day) was initiated.

In the following years, clinical and neurophysiologic signs of carpal-tunnel syndrome developed and increased in intensity; 13 years after the start of hemodialysis, bilateral carpal-tunnel release procedures were required for relief of symptoms. Light and electron microscopy examination of the excised tissue showed synovial amyloid deposits with positive immunoperoxidase staining for β_2 microglobulin in the right carpal tunnel. Histologic search for amyloid in tissue obtained from the left carpal tunnel was negative. In both carpal tunnels, synovial collagen was massively increased. Skeletal radiography performed at the time of the operation showed small cystic lesions in both radial heads, a single cyst in the left navicular bone, several cystic translucencies with a maximal diameter of 1.5 cm in both femoral heads, and one juxtaarticular cyst in the right os acetabulum.

Subsequently, increasing pain and limitation of movement in both hip joints developed; 16 years after the start of hemodialysis, the patient could walk only with the help of crutches because of severe pain in the left hip. Radiography revealed fractures of both femoral necks (left side, complete; right side, incomplete) in areas of cystic transformation. Bilateral total hip replacement was performed. Histologic examination disclosed masses of β_2 microglobulin-related amyloid within the synovia as well as in cystic bone lesions. In comparison with the histologic findings on an iliac crest biopsy obtained 6 years earlier, the extent of osteitis fibrosa had increased; staining for aluminum was negative.

Postoperatively, the daily calcitriol dose was increased to 0.5 μg. Dialysis was performed with a 1.2 m^2 polysulfone capillary membrane at a weekly dialysis prescription of 3 × 6 hours. The β_2-microglobulin concentrations decreased from an average predialysis level of 45 mg/liter (during dialysis with the cellulosic membrane) to an average of 30 mg/liter.

One year later, the patient developed hypercalcemia, which persisted after calcitriol therapy was stopped. At the same time, serum alkaline phosphatase activity started to rise and C-terminal PTH was found to be extremely high; subtotal parathyroidectomy was performed.

During his 19th year of hemodialysis, the patient began to experience severe pain in the upper cervical vertebrae, which was induced by movements of the neck and which spread to both shoulders and upper arms. Radiography showed massive destruction of the arcus of the second cervical vertebra and cystic lesions of the third cervical vertebra (Fig. 1). A nuclear magnetic resonance scan revealed nodular masses with reduced signal intensity overlying destroyed areas of the right lateral parts of the second and third cervical vertebrae (Fig. 2). These findings were interpreted as consistent with amyloid deposits. There was no indication of spinal cord compression.

The risks of cord damage from neurosurgical intervention were unacceptable to the patient so that no operative procedure to stabilize the cervical vertebrae was performed. He was provided with an external stabilizing cravatte to prevent painful and dangerous movements. Now in his 19th year after hemodialysis, the patient has expressed willingness to receive a renal transplant and has been placed on a waiting list for a cadaveric graft.

Discussion

PROF. DR. KARL M. KOCH (*Professor of Medicine, Head, Division of Nephrology, Hannover Medical School, Hannover, Germany*): This case, with its succession of osteoarticular lesions such as carpal-tunnel syndrome (CTS) and multiple fractures due to cystic bone transformation, unfortunately

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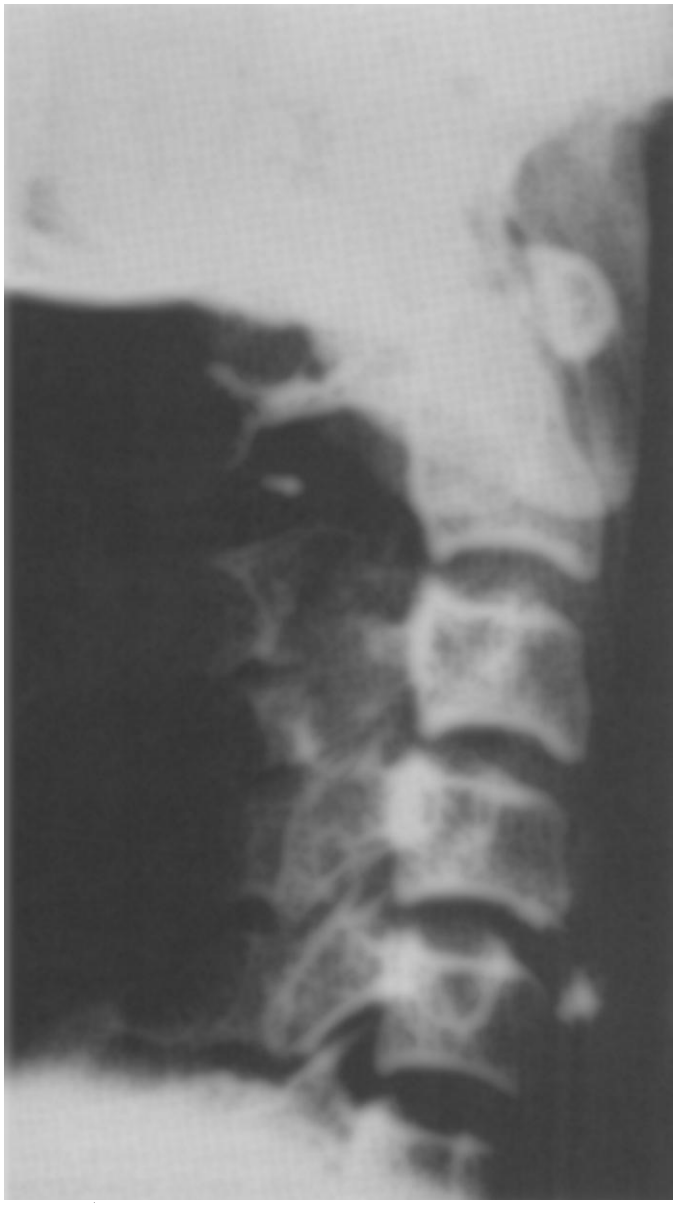

Fig. 1. *The arcus of the 2nd cervical vertebra is nearly destroyed by a large cyst* (arrow). The arcus of the 3rd vertebra shows a number of small cysts. In addition the dorsal part of the atlas is significantly narrowed.

exemplifies a common clinical syndrome in patients with end-stage renal disease (ESRD) who survive for 10 or more years with regular dialysis treatment (RDT). This syndrome—dialysis-related amyloidosis—with its crippling consequences, is now recognized as a very serious complication of RDT, one that in fact may limit the duration of long-term RDT.

During the last decade, clinical investigators gradually realized that the syndrome might have no direct relationship with established pathogenetic mechanisms of osteoarticular disease in RDT such as secondary hyperparathyroidism or aluminum accumulation, but that it could have a specific pathogenesis of its own. This hypothesis was confirmed when amyloid deposits were found in the carpal synovia and perineural tissue in long-term, regular hemodialysis patients with CTS [1–4], and in 1985 when Gejyo et al demonstrated that the lesions contained a new type of amyloid with beta-2-microglobulin (β_2m) as the constituent protein [5, 6]. Because β_2m amyloidosis associated with osteoarticular pathology appeared to be limited to the dialysis population, the term dialysis-related amyloidosis (DRA) was introduced.

The specific histologic diagnosis of β_2m amyloid depends on immunohistochemistry, because, like all types of amyloid, it exhibits nonspecific Congo-red staining with green birefringence under polarized light; β_2m amyloid deposits stain specifically with anti-β_2m antibodies [5, 6]. Also like other types of amyloid [7], β_2m amyloid deposits contain serum amyloid P (SAP) component [8] and therefore also stain with anti-SAP-antibodies [9]. Electron microscopy reveals, in contrast to the straight, longer, and thinner fibrils seen in other types of amyloid, shorter and thicker curvilinear fibrils aligned in parallel and aggregated in bundles [10–13].

Clinical observations

Carpal-tunnel syndrome and destructive arthropathy associated with cystic bone lesions are the major clinical manifestations of DRA [1–4, 6, 13–23]. Although some clinical studies [1–4, 12, 14, 15, 18, 19] of the then-putative entity were performed before Gejyo demonstrated that β_2m was the amyloid precursor protein [5, 6], it seems justified to attribute the reported cases also to β_2m amyloidosis because the radiologic and clinical findings were indistinguishable from those reported in well-established cases of β_2m amyloidosis and because none of the established causes of other types of amyloidosis was found.

The main clinical symptoms and electrophysiologic findings of carpal-tunnel syndrome in dialysis patients are identical to those of idiopathic CTS and CTS accompanying other diseases such as rheumatoid arthritis, old fractures, and diabetes mellitus; these symptoms and findings include hypesthesia, hypalgesia as well as pain (especially at night), thenar weakness and wasting, decreased motor nerve conduction velocity, and prolongation or absence of distal motor latency. In hemodialysis patients, simultaneous or successive bilateral occurrence of CTS is very common. In severe cases of CTS in regular hemodialysis patients, contraction of the hand and atrophy of the digital muscles—the so-called "amyloid hand"—can develop.

The occurrence of CTS in patients undergoing RDT was known before the association between CTS and β_2m amyloid deposits in the carpal tissue was recognized [24, 25], but the reported incidence of CTS increased in the years following the discovery of β_2m amyloidosis. This increase might have been due to stimulated interest and better diagnosis, but it is also possible that it reflected the increasing number of patients with longer duration of RDT. In a recent study in a large population of RDT patients, the percentage of patients who required operation for CTS increased steeply from almost zero before 8 years of regular hemodialysis to 50% at 14 years and 100% at 20 years [26]. In one multicenter retrospective study, age at the onset of dialysis correlated significantly with the development of CTS [27].

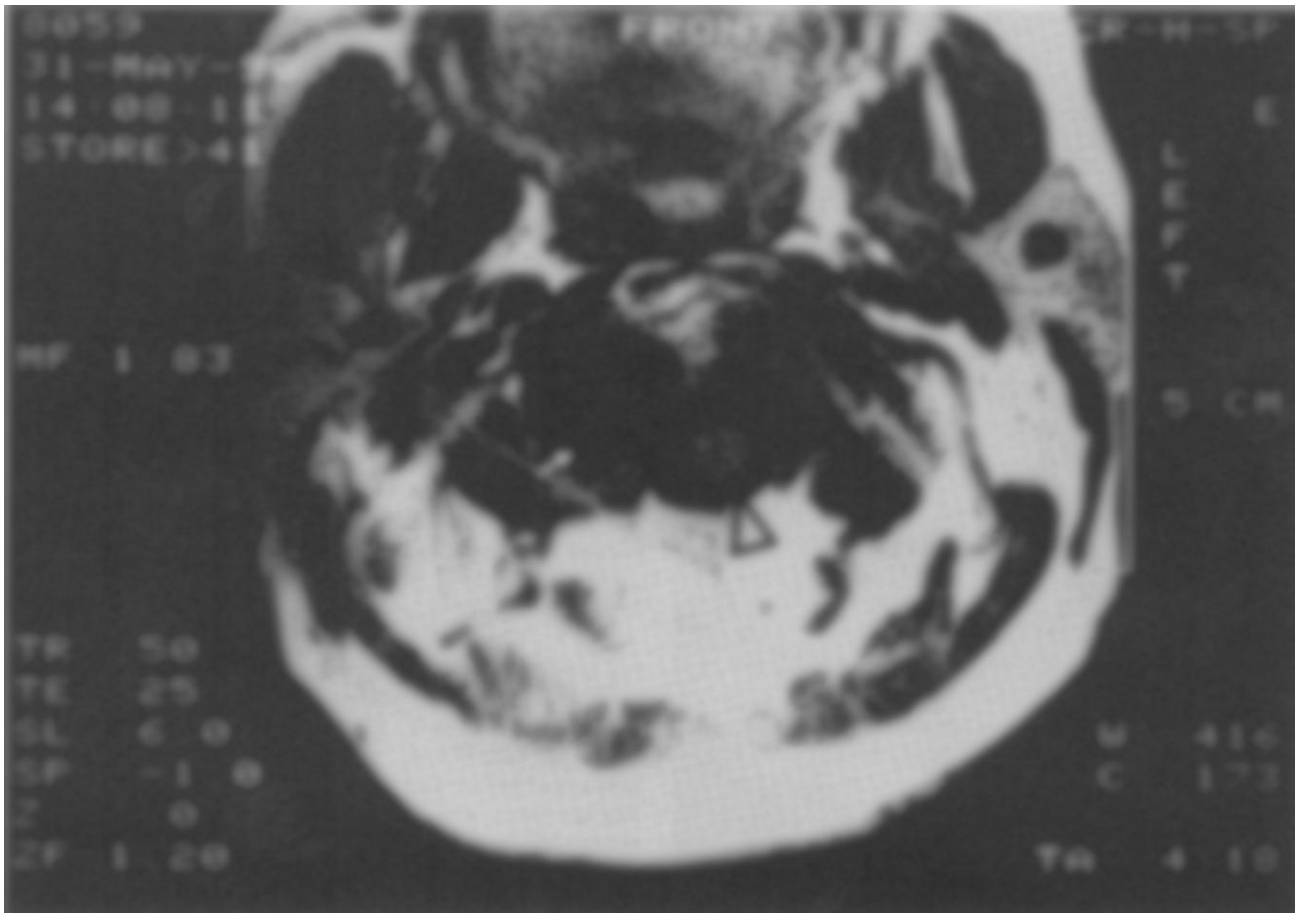

Fig. 2. *T1 weighted axial magnetic resonance image.* Right-sided destruction of the lateral parts of the second and third cervical vertebra is apparent. The low-signal area (arrow) in place of the destructive lesion is compatible with an amyloid deposit (Δ marks spinal channel with myelon).

There is considerable variation in the number of amyloid-positive cases of CTS and in the mass of amyloid found in the carpal tissue. Whereas in some studies 70% to 100% of the operated cases were amyloid positive [3, 4, 21], other studies reported a lower incidence of amyloid-positive cases [14, 15, 18]. These differences might be explained in part by differences in the methods or thoroughness of investigation, but they also could be interpreted as indicating pathogenetic mechanisms other than amyloidosis. This latter interpretation is supported by the observation that the amyloid deposits in some patients with CTS are scattered, patchy, and minute islets within hyperplastic connective tissue [16, 17]. Nevertheless, in other studies the amyloid deposits were so massive that they alone could account for median nerve entrapment [6, 28, 29].

In CTS, amyloid usually is deposited in the carpal synovia, finger flexor tendon sheaths, transverse carpal ligament, and perineural tissue [29, 30]. Histologically one finds a pericollagenous distribution of the deposits [12, 17] associated with hyperplasia of the synovial tissue [28, 31]. When the deposition in the tendons and tendon sheaths of the fingers is excessive, these lesions cause "amyloid hand."

The main target of β_2m amyloid-related destructive arthropathy is large and medium-sized joints. The pathology ranges from synovitis and effusions to erosions and destruction [11, 20–23, 32] accompanied by stiffening and pain of the involved joints.

As in CTS, the lesions can start unilaterally but in many cases the contralateral joint soon becomes affected. Symptoms due to involvement of shoulders and knees usually arise approximately 8 to 10 years after the start of RDT and very often coincide with the manifestation of CTS. As a rule, the hips subsequently become affected (that is, 12 to 15 years after the start of RDT), culminating in pathologic fractures of the femoral neck.

Spinal symptoms—especially destructive spondyloarthropathy—manifest at variable times. The spine can be affected early, but more often it begins to deteriorate many years after the start of RDT. Whether the β_2m amyloid found in affected locations [23, 33–36] is a primary factor in the pathogenesis of spondyloarthropathy in regular dialysis patients is still controversial [37]. Kuntz et al have suggested that the deposition of hydroxyapatite crystals in the intervertebral discs plays a pathogenetic role in dialysis-related spondyloarthropathy [38]. There is no doubt, however, that β_2m amyloid deposits were responsible for the development of spinal neurologic complications in 3 reported cases [36, 39, 40]. Finally, in many cases, β_2-microglobulin amyloidosis also affects the sternoclavicular

joint. The deposits can be asymptomatic [41] or accompanied by painful joint swelling [42].

Radiologic examination in β_2m-amyloid-associated arthropathy demonstrates erosions and marginal defects of the affected bones, mainly at synovial insertion sites. A characteristic finding is periarticular cystic bone lesions, which grow in number and size with the continuation of RDT. Commonly affected sites include the carpal bones, femoral and humoral heads, acetabulum, tibial plateau, and distal radius [20–23, 29, 32]. Subchondral cysts in the knee, hip, and shoulder can precede articular narrowing by 2 to 4 years [21]. Progression of cystic bone defects in the femoral neck results in pathologic fractures [11, 21, 32]. Extraosseous radiologic findings, which include swelling of articular and periarticular soft tissue mainly involving joint capsules, synovia, and tendons, can be visualized and quantified by ultrasonography [43].

Distinguishing β_2m-amyloid-related bone cysts from brown tumors of secondary hyperparathyroidism is not always easy. If radiologic signs of subperiosteal bone erosion are missing, and bone cysts increase in size despite parathyroidectomy, one can probably assume that DRA is present. Of further differential diagnostic value is that hyperparathyroid cysts often appear in the metaphysis and epiphysis of tubular bones, jaws, and ribs, whereas the amyloid-related cysts are restricted to the vicinity of synovial joints [20, 21, 23, 44]. One also should take into account that cysts attributable to secondary hyperparathyroidism rarely are located in carpal bones [45]. Finally, subchondral bone cysts appear to be very specific for β_2m amyloidosis. Zingraaf and coworkers pointed out a close correlation between the occurrence of subchondral bone cysts and histologic evidence of β_2m amyloidosis in the sternoclavicular joint [41].

Immunohistologic evaluation of biopsy and autopsy specimens discloses β_2m amloid deposits localized mainly in the synovia of the affected joints. Amyloid also is detected in material obtained from the bone cysts, either by cyst puncture or during surgery for repair of pathologic fractures from cystic bone lesions. In these patients with cystic bone lesions, the amyloid deposits were found to be especially massive [11, 21, 32].

Using the radiologic and clinical criteria I have outlined, one can conclude that the prevalence of osteoarticular lesions attributable to β_2m amyloid, like the prevalance of CTS, increases with the duration of RDT; beyond 15 years, virtually 100% of hemodialysis patients have osteoarticular lesions [32]. Older patients are at a higher risk of being affected [27].

The salient radiologic and clinical feature of DRA is the appearance of deposits in osteoarticular tissue. Deposition of β_2m amyloid outside these sites, in locations such as abdominal fat, the gastrointestinal tract, lung, liver, heart, and endocrine tissue, has been demonstrated in a limited number of patients [28, 46–56]. Although the visceral amyloid deposits generally are small and mainly perivascular, in a few patients, these deposits can have significant clinical consequences, such as cardiac or intestinal malfunction [47, 51–55]. Because of the limitations in deposit size and distribution of extraskeletal β_2m amyloid, easily accessible regions such as abdominal subcutaneous fat and the rectum are not reliable biopsy target areas for diagnosing β_2m amyloidosis [22, 28, 48, 55–57].

Pathophysiology

Beta$_2$-microglobulin, an 11,800 dalton globular protein, consists of 100 amino acids arranged in a single polypeptide chain. As part of the human class-I major histocompatibility complex, β_2m is expressed on the surface of all nucleated cells [58–60]. Also found as an intragranular protein within neutrophil granulocytes, it is released from these cells during degranulation [61]. Experimental studies in hepatocytes showed that in these cells β_2m is a secretory protein; its synthesis and release are modulated by alpha- and gamma-interferon [62].

After shedding from cellular surfaces or release from within cells, β_2m can be detected in the plasma. Plasma β_2m is not bound to other protein, and more than 90% circulates in monomeric form [63]. Approximately 95% of β_2m is eliminated via glomerular filtration [64], thereafter undergoing almost complete tubular absorption and metabolic degradation [65]; the remaining 5% is cleared at unknown extrarenal sites. Not surprisingly, a close negative correlation between glomerular filtration rate and the serum level of β_2m has been demonstrated [66]. The average daily synthesis of β_2m in normal subjects is approximately 200 mg. Measurements in 3 patients with various degrees of severe renal insufficiency showed values of the same order of magnitude [64].

Increased synthesis and release of β_2m into the extracellular space, as indicated by a rise in serum β_2m levels, occurs in inflammatory and malignant lymphoproliferative disease [67]. In vitro and in vivo studies indicate that increased synthesis and release of β_2m are mediated by cytokines: stimulatory effects on synthesis and release in cultures of various cell types could be ascribed to tumor necrosis factor (TNF), interleukin-2 (IL-2), and interferon (INF) alpha and gamma [62, 68–70]. In vivo application of recombinant INF alpha and gamma as well as of TNF increased β_2m serum levels [70, 71]. Findings regarding an in vitro stimulatory effect of interleukin-1 are controversial [62, 69, 70, 72]. In vivo, however, a stimulatory effect of IL-1 via induction of release of other cytokines such as INF and IL-2 is predictable [73].

Retention and synthesis of β_2m in patients on RDT. Serum concentrations of β_2m, normally ranging from 1 to 3 mg/liter, can be elevated as high as 60-fold in patients receiving regular hemodialysis who have no residual renal function [63]. The retention of the precursor protein in terminal renal failure is an absolute prerequisite for the development of β_2m amyloidosis. In view of the known effects of cytokines on β_2m production and with the growing evidence that hemodialysis induces an inflammatory response, it is conceivable that the process of hemodialysis itself might increase β_2m synthesis and release, thereby contributing to the accumulation of the amyloid precursor protein. Activation of cells and the release of inflammatory mediators during or following hemodialysis have been postulated since the early 1980s as part of the "interleukin hypothesis" [74]; the theory has been widely documented since. During hemodialysis, blood cells can release several mediators including IL-1 [75–77], TNF [77], reactive oxygen species [78], and prostaglandins [79]. Furthermore, complement activation occurs on some dialysis membranes [80], which in turn induces cellular monokine mRNA production, thereby preconditioning

cells to an increased monokine release upon subsequent stimulation [81]. Besides the dialysis membrane, acetate (as dialysate buffer) and especially endotoxin in the dialysate induce cellular monokine release [82, 83]. Some of these mechanisms also might be operative in CAPD patients. Two reports suggest that peritoneal dialysis itself is sufficient stimulus to induce peritoneal activation of cells and cytokine release in asymptomatic patients treated with continous ambulatory peritoneal dialysis (CAPD) [84, 85].

Direct, experimental in-vitro data indicate that the inflammatory response induced by hemodialysis could cause an increased production of β_2m. Recent studies demonstrated that isolated peripheral blood lymphocytes (PBL) and peripheral blood mononuclear cells (PBMC) harvested at the end of hemodialysis with cellulosic membranes showed, respectively, an increased expression of β_2m RNA in culture [86] or higher β_2m concentrations in the culture supernatant [87] when compared with control cells harvested before dialysis. When polymethylmethacrylate (PMNA) membranes were used, the β_2m concentration in PBMC supernatants did not increase. In addition to the release of inflammatory mediators, hemodialysis can induce degranulation of neutrophil polymorphonuclear leukocytes (PMNL) and release of proteases, depending on the type of membrane used [88, 89]. It is conceivable but not yet proved that the degranulation of neutrophil PMNL also involves β_2m-containing granules [61] and thereby also results in cellular release of β_2m.

Definitive in-vivo evidence for an increased synthesis of β_2m induced by hemodialysis is not available. Increases of β_2m serum levels ranging from 5% to 40% observed during hemodialysis with cellulosic membranes impermeable for β_2m [90–92] were interpreted as an indication of increased production of β_2m [90]. Subsequently, however, 2 separate studies, one using inulin space measurements [93] and the other using comparative kinetics of myoglobin as a marker molecule of similar size [94], showed that in hemodialysis with cellulosic membranes, the intradialytic changes of serum concentrations of β_2m are mainly consequences of concomitant changes of extracellular volume. Further support for this concept comes from the finding that sham dialysis (without dialysate and hence without weight loss) using cellulosic membranes for 2 to 4 hours does not change β_2m serum levels [94].

We determined overall β_2m synthesis in 11 anuric chronic hemodialysis patients and in 5 healthy volunteers in a kinetic study using ^{131}I-β_2m [95]. The mean synthesis rate of 3.10 ± 0.79 (SD) mg/kg/day of the patient group was higher than that of 2.4 ± 0.67 mg/kg/day in healthy controls; the difference was not significant, however. Within the patient group, those treated with β_2m-impermeable, "less biocompatible" cuprophane membranes (n = 5) had a higher mean synthetic rate than did those (n = 5) treated with β_2m-permeable, "more biocompatible" polysulfone membranes, 3.26 ± 0.80 (SD) mg/kg/day versus 2.66 ± 0.46 mg/kg/day. The limited data preclude any meaningful statistical analysis of the subgroups. More information regarding the effect of dialyzer membrane would be gained by comparing rates of synthesis of β_2m before and after the use of various membranes in the same individuals.

Processing of β_2m and amyloidogenesis. In addition to retention and possibly increased synthesis of the precursor protein, further mechanisms appear to be involved in the pathogenesis of β_2m amyloidosis. Although amyloid fibril-like structures can be created in vitro from intact β_2m [96], several observations argue against simple precipitation as the sole mechanism of amyloidogenesis in DRA: (*1*) the β_2m serum level does not correlate with the presence or absence of disease [96, 97]; (*2*) fragmented and polymerized β_2m next to monomeric-intact β_2m was found to be a major constituent of the amyloid [8, 98, 99]; (*3*) the predilection of β_2m amyloid for osteoarticular tissue with relative sparing of parenchymal organs, which generally are involved in other types of amyloidosis, suggests specific local mechanisms predisposing to amyloid deposition; (*4*) the increased prevalence of DRA with age [27], and our own observation that occasional patients are spared from DRA even though they have had hemodialysis treatment with cellulosic membranes for more than 15 years, suggests that additional pathogenetic mechanisms of a more general nature exist.

In contrast to the initial findings of Gejyo [5, 6], Linke's analysis of the amino-acid sequence of β_2m-amyloid fibrils revealed that these fibrils not only consisted of intact β_2m, but to a significant degree, also of β_2m fragments, most of them with lysine-specific cleavage [98, 99]. Ogawa also showed by two-dimensional electrophoresis that in addition to intact β_2m, a "novel β_2m" with a reduced molecular weight occurred in solubilized amyloid fibrils [100]. This "novel β_2m" also was detected in the plasma of long-term hemodialysis patients with clinical manifestations of β_2m-amyloid. These findings suggest that alterations of the precursor molecule, such as limited proteolysis, are involved in β_2m amyloidogenesis; this hypothesis is consistent with current concepts of the pathogenesis of several other types of amyloid [101]. The cellular activation with subsequent release of inflammatory mediators and proteases induced by hemodialysis might be involved. Such cellular activation preceding the limited proteolysis of β_2m could take place on the dialyzer membrane itself. Alternatively, resident or blood-derived cells in the tissue (especially macrophages) activated by mediators released from circulating cells during dialysis might participate in β_2m proteolysis.

An intriguing modification of the proteolysis hypothesis was suggested recently by Argilés and colleagues [102]. They posit that incompleteness of proteolysis of β_2m is the prerequisite for amyloid fibril formation and that complete proteolysis is prevented by "amyloid-enhancing factors" such as the anti-protease α_2-macroglobulin (α_2m); further, they demonstrated the presence of α_2m in β_2m amyloid deposits. If this hypothesis is correct, the modulation of proteolysis by α_2m-macroglobulin is twofold; in addition to the proteolytic activity of enzymes already synthesized, α_2m also inhibits the protease production of macrophages following specific receptor binding [104, 105]. Adding credence to Argilés' concept is the observation that, in patients receiving RDT who have CTS, serum levels of α_2m were higher than they were in patients without amyloidosis who had had RDT for just as long [106].

The predilection of β_2m amyloid deposits for osteoarticular tissues, which are rich in collagen, and the close ultrastructural association between β_2m amyloid and collagen within these tissues [12, 17, 31] suggest a high affinity of the precursor β_2m for collagen. Indeed, Homma et al showed that β_2m binds to various collagens in a dose-dependent manner [107]. Whether this affinity for collagen is increased in hemodialysis patients because of alterations in collagen composition is unknown.

According to experimental findings by Kisilevsky and Snow, the glycosaminoglycans (GAG) composition of the interstitial matrix of the periarticular tissue also might be a local factor favoring amyloid formation [108]. A relation between amyloid deposition and GAGs also was suggested by ultrastructural and immunohistochemical studies of β_2m-amyloid deposits in hemodialysis patients [13]. Nishi et al demonstrated that the amyloid fiber bundles were surrounded by GAGs and concluded that GAGs regulate the distribution and arrangement of fibrils.

If proteolytic processing of the precursor molecule is a prerequisite for fibril formation, one can reasonably assume that the proteolytic processing takes place where the precursor accumulates. Within the synovia, the predominant location of articular β_2m amyloid deposits, amyloid is found with and without synovial infiltration by inflammatory cells [31]. The prime suspects involved in β_2m proteolysis therefore are the synoviocytes. Their number in patients receiving long-term hemodialysis treatment is significantly increased [31], and a subtype (type A) morphologically and functionally displays macrophage-like features [109]. This potential involvement of the synoviocytes in fibril pathogenesis could be a further predisposing factor for the preferential articular disposition of β_2m amyloid. Whether the synoviocytes are activated and proliferate because of the mere presence of β_2m, or whether inflammatory processes induced by hemodialysis are also involved is not known.

Aluminum [110] and iron [111] also have been proposed as predisposing factors to the intraarticular accumulation of β_2m. The role of iron is controversial, and contradictory observations exist [23, 32]. The issue is complicated by the possibility that the simultaneous increase of aluminum and iron deposits, on the one hand, and of amyloid deposits, on the other, might be a fortuitous association of two independent, time-related complications of long-term hemodialysis therapy.

Therapeutic considerations

The experimental investigation of dialysis-related amyloidosis continues to pose many intriguing questions to the scientist. But how does our current knowledge aid the clinician who has to care for these patients? Let us consider some important points that can be useful in clinical decision-making.

(*1*) Dialysis-related amyloid would not exist without accumulation of the precursor.

(*2*) The possibility that the dialytic process promotes the pathogenesis of DRA via regular induction of intermittent inflammatory reactions is too great to be disregarded.

(*3*) A large, retrospective multicenter study showed that long-term hemodialysis with a highly permeable acrylonitrile membrane was superior to long-term hemodialysis with a cuprophane membrane, in that the former was associated with a lesser prevalence of clinical and radiologic manifestations of DRA [27]. Although this study is otherwise very thorough and critical, it is limited because it relied mainly on clinical and radiologic criteria, and the number of patients at risk for 15 years was small. Nevertheless, the study results do support points (*1*) and (*2*) for the following reasons: In contrast to cuprophane, which is practically impermeable to β_2m, acrylonitrile permits a weekly removal of 400–600 mg of β_2m [112]. Even though this quantity is far less than the weekly synthesis of approximately 1500 mg [95], its removal probably at least slows the continous increase of the β_2m burden. Furthermore, in comparison to cuprophane, acrylonitrile is more "biocompatible," as it has a high adsorptive capacity for cytokines [113] as well as endotoxins [114], and its use is accompanied by little release of proteases from blood cells [88], low complement activation [115], and little induction of phagocytic oxidative metabolism [78].

(*4*) The multicenter study I just mentioned showed that age at onset of RDT also affected the prevalence of chronic manifestations of DRA [27]. Patients over the age of 40 at the initiation of regular hemodialysis treatment carried a higher risk for the development of bone amyloidosis than did younger patients.

(*5*) Successful, early kidney transplantation certainly prevents DRA. Furthermore, in patients with already established DRA, renal transplantation arrests the progression of radiologic signs of DRA [116] and produces an almost immediate abatement of osteoarticular pain. The latter effect probably is mainly due to the antiinflammatory action of steroids, but it also might be caused partly by the termination of the induction of inflammatory activity by hemodialysis and the return of normal or nearly normal renal function.

Let me suggest a cautious but, I believe, justified set of policies based on these five points. Patients older than fifty, whether or not they are candidates for transplantation, should be dialyzed with the acrylonitrile membrane or a membrane with comparable qualities, such as polysulfone or polyamide. The same approach applies for younger patients who have little chance of receiving a transplant. Patients with clinically established DRA should be given a high priority for transplantation so they won't become disabled. In view of the potential of bacterial products to induce inflammatory processes during hemodialysis, the dialysate should be sterile and pyrogen-free. This measure especially applies when membranes with less resistance to the passage of bacterial products from dialysate to blood are used [117].

Should one use hemofiltration or continuous ambulatory peritoneal dialysis (CAPD) to prevent or delay the manifestations of DRA? Even though hemofilters with "biocompatible" membranes enable the weekly removal of approximately 1000 mg of β_2m [118], wide use of hemofiltration will be prevented by its prohibitive cost as long as commercial substitution fluid has to be used. The weekly removal of approximately 300 mg of β_2m in CAPD patients is lower than that achieved with hemodialysis with high-flux membranes [112]. Like hemodialysis, CAPD can induce cellular activation and release inflammatory mediators [84, 85]. Clinical reports of continuous progression of DRA bone lesions after a change from hemodialysis to CAPD [32] and of the occurrence of amyloid deposits in patients treated exclusively by CAPD [119, 120] suggest that CAPD might not be superior to hemodialysis in preventing DRA or in delaying its onset. A conclusive comparative evaluation of the prevalence of DRA in CAPD patients and regular hemodialysis patients does not exist because of the small number of patients with long-term exposure to CAPD.

The rationale for my recommendations certainly would be strengthened and additional preventive measures could be evaluated by prospective studies. So far, the prospective evaluation of strategies for preventing DRA has been hampered by

the non-availability of an early and sensitive diagnostic procedure that would be acceptable to patients and that would indicate the presence of β_2m amyloid anywhere in the body. Amyloid-related clinical symptoms or radiologic findings are late occurrences in the development of DRA, and biopsies of non-selected, easily accessible tissues at best yield only chance findings of amyloid [22, 28, 48, 55–57]. The two procedures that most often yield positive results, synovial biopsies and the aspiration of joint effusions [19, 22, 26], do not have the potential of becoming routine diagnostic procedures in prospective trials. However, two recent reports are very promising. They describe the diagnostic radionuclide tracing and imaging of β_2m-amyloid deposits by injections of either ^{123}I-labeled serum amyloid P component (SAP) [121] or ^{131}I-labeled β_2m [122]. A minor constituent of β_2m-amyloid, SAP accounts for about 5% to 15% of the total amyloid mass [123]. Injection of the radiolabeled molecule allowed the noninvasive detection of amyloid deposits of the carpal-tunnel region and metacarpophalangeal joints of two long-term hemodialysis patients [121]. It is not known, however, whether the scan also will detect minor, asymptomatic, early stages of amyloid deposition. An alternative to scanning with radiolabled SAP is offered by the injection of the radiolabeled precursor molecule β_2m [122]. Using this technique, we found localized tracer accumulation in the shoulders, hands, knees, pelvis, and vertebral column in 23 of 30 patients on regular hemodialysis treatment longer than 5 years [122]. Correlation of scan findings with available immunohistology showed a very high sensitivity and specificity of the scan for the detection of β_2m-amyloid deposits. With the scan it is possible to detect amyloid deposits before their presence is indicated by clinical symptoms and/or radiologic alterations of bones. This is shown in Figure 3, which depicts our results to date in 49 patients.

Currently, this radiodiagnostic method offers a sensitive and noninvasive way of demonstrating β_2m deposits. It might not only permit a more complete description of the prevalence of β_2m-amyloidosis but, by repeated scanning, it also might allow one to assess the influence of different therapeutic strategies on the appearance of amyloid deposits or on the fate of existing deposits.

Questions and answers

DR. F. JOHN GENNARI (*Professor of Medicine and Director, Nephrology Division, University of Vermont College of Medicine, Burlington, Vermont*): It seems to me that CAPD patients would be the appropriate controls for chronic hemodialysis patients in studies on DRA. Are there comparisons? What are the β_2-microglobulin levels in CAPD patients? I am surprised that this population has not been studied more completely.

DR. KOCH: The populations with long-term exposure to CAPD, that is, longer than 10 years, are very small, so no conclusive comparison of the prevalence of DRA in CAPD patients and regular hemodialysis patients is available. Nevertheless, the number of case reports with β_2m-amyloid being found in CAPD patients are increasing. Actually there exists one study by Benz et al comparing the incidence of CTS in two large populations of regular hemodialysis and CAPD patients [124]. There were no significant differences, but no histologic examinations for amyloid were performed. Regarding β_2m plasma levels in CAPD patients, plasma β_2m is in the range of that in hemodialysis patients provided renal function is comparable.

DR. CHARLES VAN YPERSELE (*Professor of Medicine, Renal Unit, Cliniques Universitaires, St. Luc Université de Louvain, Louvain, Belgium*): In our hands, adequate radiologic examination of the wrists and, more recently, ultrasonic quantitative assessment of tendon thickness in the shoulders and the hips provide early specific evidence for the diagnosis of DRA. Under the circumstances, what is the place of isotopic imaging techniques?

DR. KOCH: I totally agree regarding the usefulness of x-rays and ultrasonography in the clinical situation. The isotopic imaging techniques, since they are highly sensitive and specific and in our hands enable us to prove the existence of amyloid before characteristic skeletal alterations are visible, are excellent research tools. This especially applies for their use in prospective trials comparing the effect of different dialytic treatment modalities on the development and progression of DRA.

DR. VAN YPERSELE: ^{131}I-β_2-microglobulin entails a 2 to 3 mCi load that has radiation hazards unacceptable to Belgian ethics committees. We should not forget that β_2-microglobulin amyloid was demonstrated prior to the onset of dialysis [125]. It is worth pointing out that the synovial deposition of amyloid is not limited to β_2-microglobulin amyloidosis but is a characteristic of amyloidosis due to AL amyloid as well. Further, spleen deposits have been identified by Pepys and his colleagues, who relied on ^{123}I serum amyloid P component imaging techniques [126].

DR. KOCH: At present the radiation dose of the ^{131}I-β_2m scan technique is comparable to that of a barium enema x-ray investigation of the colon. We therefore apply this technique very restrictively but hope to be able to reduce the radiation exposure by employing other isotopes. The serum amyloid P (SAP) component scan developed by Pepys and coworkers imparts less radiation exposure because the imaging of the amyloid deposits takes place after 24 hours instead of 72 hours with the ^{131}I-β_2m scan.

To my knowledge, there exists only a single reported patient with proven β_2m-amyloid prior to the onset of dialysis [125]. The patient was 69 years old and had long-standing, severe renal insufficiency due to cortical necrosis. Histologically proven splenic deposition of β_2m-amyloid so far has been reported in only 4 patients. In view of this very rare incidence, the frequent positive splenic imaging with the SAP scan (12/35 RDT patients) reported by Pepys and coworkers is very surprising [126].

DR. ANDREW REES (*Professor of Nephrology, Royal Postgraduate Medical School, London, United Kingdom*): I would like to focus on the specificity of the β_2-microglobulin scans. The SAP scan has been shown to be highly specific for amyloid both by autoradiography and by the use of appropriate controls. Is the same true for the β_2m on scans? My second question relates to the increase in RNA for IL-1β. It appeared to be transient, as there appears to be no increase in cells tested after 3 hours of dialysis. Was the increase in IL-1β message associated with synthesis of the protein, or were you just showing changes in mRNA?

DR. KOCH: The ^{131}I-β_2m scan also is highly specific regarding the imaging of β_2m amyloid deposits. In all cases we have so far investigated histologically, we have found β_2m amyloid in sites

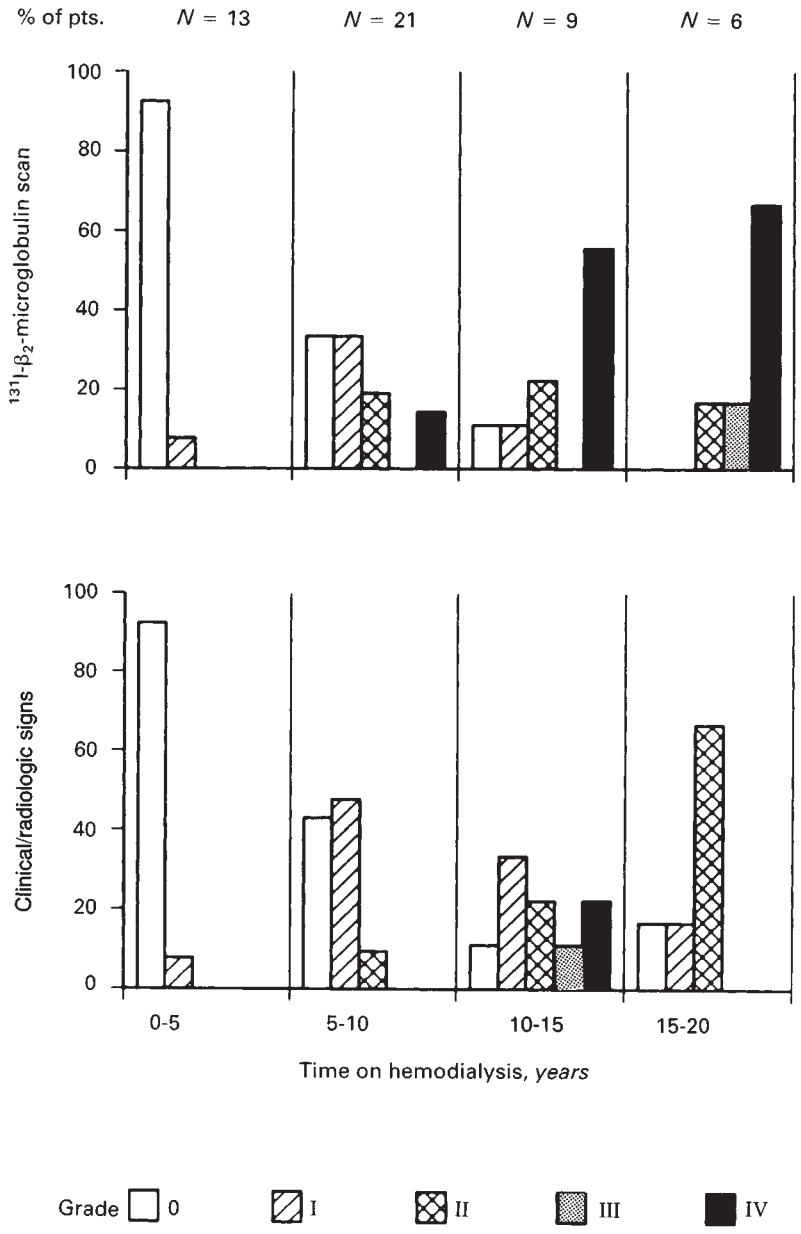

Fig. 3. *^{131}I-β_2m accumulation in hemodialysis patients versus clinical and/or radiologic findings compatible with the diagnosis of β_2m-amyloidosis.* Tracer accumulation and clinical/radiologic symptoms were graded according to the number of sites involved: O, none; I, one or two sites; II, three or four sites; III, five or six sites; and IV, more than six sites.

with prior positive imaging. Inflammatory lesions of other causes, such as active chronic polyarthritis and abscesses, when they were present in the investigated hemodialysis patients, showed no positive imaging.

Regarding the demonstration of mRNA for IL-1β in mononuclear cells of samples taken at the venous blood line of a cellulosic dialyzer, this indeed was a transient phenomenon. The disappearance of mRNA in samples taken during the later course of hemodialysis suggests that the induction of mRNA for IL-1β is due to C5a, which preferably is generated during the early part of hemodialysis, when the membrane is not yet coated by protein. So far we only have looked for mRNA in mononuclear cells lyzed after they had been kept for 30 minutes at room temperature following harvesting. We are in the process of studying whether these cells also synthesize and release IL-1β.

DR. MICHEL GOLDMAN (*Head, Department of Immunology, Hôpital Erasme, Université Libre de Bruxelles, Brussels, Belgium*): If we consider the clinical data of Charles van Ypersele together with your own laboratory data, it appears that the incidence of amyloidosis is lower with the use of synthetic membranes that are permeable to cytokine-inducing substances. The important factor therefore could be the complement activation induced by cellulosic membranes. Since this

phenomenon can be prevented by reusing the membranes, does the reuse procedure have any influence on the development of dialysis-related amyloidosis?

DR. KOCH: I do not know of any study of the effect of dialyzer reuse on the development of dialysis-related amyloidosis. Whether complement is activated in a reused dialyzer depends on the membrane-cleansing procedure applied before reuse. Of course we have to realize that cell-membrane contact and complement activation might not be the only mechanisms involved in cytokine induction. We also have to consider that bacteria-derived substances contained in the dialysate might permeate the dialyzer membrane and induce cytokines on the blood side.

DR. MICHAEL J. MIHATSCH (*Chairman, Department of Pathology, University of Basel, Basel, Switzerland*): What is known about the turnover and disappearance rate of β_2m amyloid in transplant patients?

DR. KOCH: So far there exists no direct evidence that β_2m amyloid deposits are reduced or disappear after successful transplantation in RDT patients. The immediate and impressive reduction in amyloid-related symptoms after transplantation probably is due to the antiinflammatory effects of steroids. Studies by Dr. van Ypersele also showed that there is no further progression of the radiologic manifestations of DRA but also no regression after successful transplantation. Pepys and coworkers reported negative SAP scans in patients after transplantation despite histologically proven DRA before transplantation [126]. This observation indeed suggests reduction of amyloid mass after transplantation. An alternative explanation could be a lack of newly formed amyloid fibrils with free ligands for SAP binding.

DR. CHARLES M. MION (*Professor of Nephrology and Head, Division of Nephrology, University of Montpellier, School of Medicine, Montpellier, France*): I believe that the residual renal function in CAPD patients probably is extremely important in retarding the development of β_2m amyloidosis. Second, I have seen carpal-tunnel syndrome with equal frequency in patients treated with and without cuprophane membranes. Would you comment?

DR. KOCH: I agree; residual renal function is generally better preserved during the first years of CAPD, so the accumulation of β_2m, the amyloid precursor protein, might be reduced and the development of β_2m amyloidosis might be retarded.

Your observation of an equal frequency of CTS in patients treated with cellulosic membranes and those treated with noncellulosic membranes does not disagree with the results of the European multicenter study: Whereas there was a significant effect of the choice of membrane on bone amyloidosis, radiologically determined, there was no effect on the prevalence of CTS sufficiently severe to require surgery. A cautious interpretation of these findings is that, in hemodialysis patients, β_2m amyloid might not be the only cause of CTS.

DR. ARTURO BORSATTI (*Professor of Nephrology, Institute of Internal Medicine, University of Padova, Padova, Italy*): Can a steady state of β_2-microglobulin be achieved in dialysis patients, or is there a constant increase of β_2-microglobulin in the blood? Can one remove the β_2-microglobulin synthesized?

DR. KOCH: If you define a constant β_2m plasma level as indicative of a steady state, then chronic hemodialysis patients are in a steady state as long as residual renal function and β_2m removal by dialytic treatment do not change. Accordingly, a continuous increase of plasma β_2m is seen during the early period of regular hemodialysis treatment when residual function shows pronounced changes. In patients with very long times spent on regular hemodialysis treatment, plasma levels tend to decrease again, as shown by Charra et al [26] and supported by our own experience. This finding has been interpreted as a possible indication of increased β_2m amyloid formation. Regarding your question whether β_2m synthesis can be balanced by removal, our own data suggest that this is not possible even with high-flux hemodialysis or hemofiltration [95]. On the other hand, it is conceivable that even a moderate reduction of β_2m accumulation might retard the development of DRA.

DR. CHRISTOPHER G. WINEARLS (*Consultant Nephrologist, Renal Unit, Churchill Hospital, Oxford, United Kingdom*): You showed intriguing data on IL-1β in RNA expression in cells harvested from the venous return of the dialyzer. Could you comment on the synthesis rates, and on any differences pre- and post-membrane?

DR. KOCH: We did not quantify the synthesis rate for IL-1β mRNA. The cells harvested from the arterial line contained no or negligible amounts of mRNA, whereas there was a very strong signal for mRNA in the cells taken from the venous blood line at the same time.

DR. WINEARLS: Can you reassure us that the cell populations were the same in the arterial and venous lines?

DR. KOCH: The assayed cell populations of the venous and arterial sample were different. The mononuclear cells harvested from the venous sample by Ficoll/Hypaque centrifugation separation were contaminated by as much as 40% with granulocytes, whereas by the same technique we gained almost pure mononuclear cells from the arterial sample. We attribute this difference to a change of specific gravity of the granulocytes after they pass the dialyzer. However, since granulocytes can only express very small amounts of IL-1β, we are very sure that we measured the mRNA of mononuclear cells in the venous sample.

DR. WINEARLS: What explains the speed with which the RNA was expressed?

DR. KOCH: Before lysis of the cells for mRNA measurement, the samples were kept for 30 minutes at room temperature. So there was some time allotted for mRNA synthesis. The speed of synthesis might not have been exceptionally high.

DR. ALEX M. DAVISON (*Consultant and Renal Physician, Department of Renal Medicine, St. James's University Hospital, Leeds, United Kingdom*): You mentioned some of the possible reasons for the distribution of β_2m amyloid. Would you expand your thoughts on this?

DR. KOCH: The predilection of β_2m amyloid for synovial tissue is very striking. This affinity might be due to a high affinity of β_2m for collagen. When one studies synovial β_2m amyloid deposits with the electron microscope, one almost always finds the amyloid fibrils very closely arranged to collagen fibrils. In this context it may be of importance that Homma recently showed that β_2m binds to various collagens in a dose-dependent manner [107]. Some cells within the synovia display macrophage-like functions and therefore might be involved in proteolytic processing of β_2m, which could be a prerequisite for local amyloid fibril formation.

Another local predisposing factor might be glycosoaminoglycans (GAGs) which, according to experimental findings of Kisilevsky and Snow, are operative in amyloid fibril formation [108]. Using tissue obtained from hemodialysis patients with DRA, Nishi et al showed that β_2m amyloid fibril bundles often are surrounded by GAGs [13]. These researchers concluded that GAGs exert a regulatory effect on the distribution and arrangement of amyloid fibrils.

DR. JORDAN J. COHEN (*Dean, School of Medicine, State University of New York at Stony Brook, Stony Brook, New York*): You noted that there is a poor correlation between the amount of β_2-microglobulin amyloid and the amount of collagen seen histologically. In fact, even in the patient presented today, carpal-tunnel tissue from one side contained β_2-microglobulin amyloid, but on the other side β_2m-amyloid was undetectable. This discrepancy raises the possibility that the presence of β_2-microglobulin is an epiphenomenon. Is it possible that β_2-microglobulin is not involved in the pathogenesis but is somehow being captured by the collagen, and that something else triggers the mass of collagen that produces the symptoms of carpal-tunnel syndrome?

DR. KOCH: This is a very good point. Whereas in some patients the amyloid deposits are very massive and without doubt cause local damage, for instance compression of the median nerve, in other cases one finds only minute amounts of amyloid but masses of collagen within the carpal tunnel tissue. So there is the suspicion that the pathogenesis not only involves β_2m-amyloid but also alterations of collagen metabolism.

DR. NICOLAOS E. MADIAS (*Chief, Division of Nephrology, New England Medical Center, Boston, Massachusetts*): Regarding pathogenesis, an IL-1 receptor antagonist recently has been developed and is being used in animal and human investigation. Has this antagonist been used in an effort to evaluate the effects of dialysis-induced IL-1 release on β_2-microglobulin production? Also, is there an animal model for the syndrome? Recognizing the long time required for the syndrome to develop in humans, one could employ exogenous administration of β_2-microglobulin in association with dialysis to study the pathogenesis of the syndrome.

DR. KOCH: I do not know of any experiments studying the effect of IL-1 receptor antagonists on IL-1 induction during hemodialysis. So far there also do not exist animal models with β_2m amyloidosis. I assume it would be necessary to dialyze anuric animals for prolonged times. The additional infusion of grams of β_2m might speed up the development of β_2m amyloidosis.

DR. MICHEL OLMER (*Chief, Nephrology Unit, Hôpital de la Conception, Marseille, France*): I am interested in whether the use of pyrogen-free dialysate is associated with β_2-microglobulin deposition. In Marseille, we find amyloid deposits only in patients treated with nonsterile dialysate.

DR. KOCH: Bacteria-derived substances contained in the dialysate can permeate certain dialyzer membranes and thereby could induce cytokines such as IL-1β and tumor necrosis factor (TNF) on the blood side. The involvement of such a chain of events in the pathogenesis of DRA is conceivable but not proven. The only clinical report supporting this concept is your own work, which showed that the use of a dialysate with a very low endotoxin content was accompanied by a lower incidence of CTS [127]. However, as we discussed before, CTS is not definite proof of the presence of amyloid.

DR. JOHN T. HARRINGTON (*Chief of Medicine, Newton-Wellesley Hospital, Newton, Massachusetts*): Are there experimental data regarding the pharmacologic inhibition of β_2-microglobulin synthesis?

DR. KOCH: Considering our own studies in uremic patients, one has to assume that at least 75% of their β_2m synthesis is due to normal cell turnover. To inhibit β_2m synthesis significantly, one would have to suppress normal cell turnover. This would necessitate the application of cytostatic drugs. I do not think this is advisable in RDT patients who already suffer from a multitude of immune defects.

DR. BRUNO BAGGIO (*Associate Professor of Nephrology, Institute of Internal Medicine, Division of Nephrology, Padova, Italy*): Do you think that the plasma oxalate level and, in particular, its abrupt variation during the interdialytic period could play a role in dialysis-related amyloid syndrome?

DR. KOCH: I do not know of any data suggesting a relation between plasma or tissue oxalate levels and DRA. We used the ^{131}I-β_2m scan to study a patient being treated with regular hemodialysis who suffered from primary oxalosis. None of his massive tissue oxalate deposits appeared positive with the scan.

DR. MIHATSCH: Beta$_2$-microglobulin amyloid is always deposited in close association with collagen. The specificity of the β_2 deposition might be related to the specific type of collagen. Are there data regarding cytokines in synovial fluid and how they might influence this binding?

DR. KOCH: Your question concerning a preference of β_2m for a specific collagen certainly merits investigation. The same applies for cytokine levels in joints with synovial β_2m amyloid deposits. Elevated levels would indicate local inflammatory processes. I am sorry that I cannot be more specific.

DR. MICHAEL H. RAMBAUSEK (*Department of Nephrology, University of Heidelberg, Heidelberg, Germany*): Beta$_2$-microglobulin is part of the HLA system. This fact raises the question of whether there is any genetic predisposition for the development of dialysis-related amyloid disease.

DR. KOCH: So far no data suggest a genetic predisposition for DRA. The very high prevalence of DRA in long-term hemodialysis patients actually argues against a major role for genetic factors.

DR. JOHN F. DONOHOE (*Consultant Nephrologist, Department of Nephrology, Beaumont Hospital, Dublin, Ireland*): What is known of the relative prevalence or incidence of DRA between the United States and Europe? Are there known differences within Europe, that is, north versus south? If these data are available, what are the implications regarding the pathogenesis of DRA?

DR. KOCH: I am afraid that currently no representative data are available that permit a comparison between the incidence of DRA in the United States and Europe. The major obstacles obviously are the lack of standardization of diagnostic criteria and incomplete information concerning possibly relevant background conditions such as dialysate quality, application of reuse, etc.

DR. LEENDERT A. VAN ES (*Professor of Medicine, Department of Nephrology, Leiden University Hospital, Leiden, The Netherlands*): Is the magnitude of the renal elimination of

β_2-microglobulin based on the measurement of arteriovenous differences across the kidney in humans?

DR. KOCH: Yes; by measuring renal arteriovenous differences of β_2m concentrations in patients with normal renal function, we have found that this difference is equal to or slightly higher than that of inulin. This suggests that β_2m is freely filterable in the glomerulus. Similar findings are known from animal studies.

DR. MADIAS: Has your scanning technique with radiolabeled β_2m suggested a greater systemic (that is, non-osteoarticular) deposition of β_2-microglobulin than clinically suspected?

DR. KOCH: No, our experience with the ^{131}I-β_2m scan does not suggest a very significant systemic deposition of β_2m amyloid outside osteoarticular tissues. Histologically proven non-osteoarticular deposits in the majority of reported patients were found to be minute and therefore might not be detected by the scan.

DR. MION: Would you comment on recent observations showing early synovial deposits in dialysis patients [128]? What is the sensitivity of the various scans in the detection of these early deposits?

DR. KOCH: Obviously, our scan is not able to detect microscopic amyloid deposits. Nevertheless, it enabled us to spot amyloid deposits in patients who had been treated with dialysis for less than 6 years and who had no clinical or radiologic indications of DRA.

DR. WINEARLS: May I comment on the SAP scans? Professor Pepys' group at the Hammersmith Hospital recently completed an evaluation of this technique in hemodialysis patients [126]. The principal findings were: (*1*) there is focal localization of tracer at sites where histologic examination confirms amyloid deposition but, apart from the spleen, no evidence of visceral involvement; (*2*) the scans do not always detect β_2m-amyloid in deep sites; and (*3*) scans in four patients with a history of dialysis amyloid proven histologically, who had had successful renal transplants, were negative, suggesting that accumulation of amyloid had at least been arrested.

DR. KOCH: I agree regarding the very limited systemic accumulation of β_2m amyloid. Regarding β_2m amyloid in deep sites, the ^{131}I-β_2m scan proved to be very effective in imaging amyloid deposits located in the hip joints. We have no post-transplant experience with the scan, because administered β_2m is eliminated too rapidly by glomerular filtration when renal function is restored; thus our scan cannot be applied in this setting.

DR. COHEN: How practical is it to maintain sterile dialysate? Does it increase the cost of the procedure excessively? Would you recommend using sterile dialysate routinely for all patients?

DR. KOCH: In our experience with a well-designed and carefully serviced reverse-osmosis unit and good machine sterilization, we can produce dialysate with a very low endotoxin content, as low as 50 to 100 pg/ml.

A further improvement could be achieved by placing polysulfone or polyamide hemofilters in the dialysate line downstream from the machine. Hemofilters made of these membranes reject and adsorb bacteria-derived products and can be reused as long as the cleaning procedure is performed with sterile and pyrogen-free water and also includes back-filtration.

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Reprint requests to Prof. K. Koch, Medizinische Hochschule Hannover, Abteilung Nephrologie, Konstanty-Gutschow Str. 8, D-3000 Hannover 61, Germany

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