Systemic amyloidosis of β_2 -microglobulin type: a complication of long term haemodialysis

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SUMMARY A patient receiving long term haemodialysis developed systemic amyloidosis, which was shown immunohistochemically to be of β_2 -microglobulin type, a previously unrecognised form of systemic amyloidosis. Histologically, the amyloid deposits were closely associated with foci of acute and granulomatous inflammation and vasculitis, although it was not clear if the amyloid deposits directly caused the inflammatory process, or if amyloid was deposited preferentially in areas of inflammation of uncertain aetiology.

Patients on long term haemodialysis seem to have a high risk of developing the carpal tunnel syndrome or other synovial and joint problems.¹⁻⁵ Histological examination of tissue removed from such patients at carpal tunnel release or by synovial biopsy has shown a high incidence of amyloid deposition.^{2,4,5} Interestingly, the major component identified from these deposits is β_2 -microglobulin, a previously unrecognised type of amyloid protein.^{6,7}

Although small deposits of amyloid have been detected in rectal and skin biopsy specimens from patients receiving haemodialysis with the carpal tunnel syndrome,^{2,4,5} there have been no reports of widespread systemic amyloidosis of β_2 -microglobulin type.

Case report

A married nurse of West Indian origin presented in end stage renal failure in 1972 at the age of 31. The aetiology was unknown, but she had had acute glomerulonephritis when aged 12. She was anaemic but not hypertensive. An intravenous pyelogram showed small kidneys. Immunoglobulins and C₃ were within the normal range, and protein electrophoresis yielded normal results. A sickling test was negative.

She started maintenance haemodialysis in 1973 and at this time developed cervical tuberculous lymphadenitis, which was successfully treated with rifampicin and isoniazid. In 1975 biochemical evidence of osteomalacia became apparent, confirmed on bone

biopsy, and this was treated with calcium supplements.

In 1979 she developed bilateral carpal tunnel syndrome, confirmed by electrophysiological studies, and later that year bilateral carpal tunnel decompression was carried out. Nine months later she presented again with malaise, generalised arthralgia and myalgia, and slight hepatomegaly. Abnormal liver function tests were found, with an aspartate transaminase activity of 63 IU/ml, alkaline phosphatase of 1041 IU/ml, and raised 5-nucleotidase activity. Albumin and bilirubin concentrations were 36 g/l and 7 μ mol, respectively. A liver biopsy specimen showed microgranulomata within hepatic sinusoids, with some macrophages containing refractile material, in keeping with hepatic deposition of silicone from intravenous lines.

In 1981 uterine curettage was carried out for menorrhagia. A further right carpal tunnel decompression was also performed for recurrent symptoms. In 1982 she underwent a cadaver renal transplantation, but her postoperative course was complicated by three episodes of severe acute rejection and graft nephrectomy was carried out after five weeks. One month later she developed symptoms of fever, myalgia, arthralgia, and she had abnormal liver function tests. Reactivated cytomegalovirus infection (CMV) was diagnosed, with an increase in titre from 1/8 preoperatively to 1/256.

She presented again in 1983 with bilateral small vulval masses, one of which was biopsied. She remained relatively well until early 1984 when she developed an illness characterised by the acute onset

of fever, confusion, and leucocytosis. She was admitted for investigation of probable infection. Shortly after admission she vomited and suffered aspiration, with a subsequent cardiac arrest from which she could not be resuscitated.

Pathology

The vulval nodule consisted of amorphous eosinophilic material, with scattered foreign body type giant cells. This material stained positively with Congo red and showed dichroism on polarised microscopy. The Congo red staining was resistant to treatment by permanganate. The features were those of an "amyloid tumour".

At necropsy aspiration of stomach contents was performed. The kidneys were symmetrically grossly reduced in size with finely granular surfaces but without calyceal distortion. No other macroscopic abnormalities were recognised.

There was widespread amyloid deposition, especially within the wall of the blood vessels. This was particularly extensive in the lungs and the broad

ligament, and to a lesser degree in the kidneys, heart, and liver. Large parenchymal deposits were restricted to the broad ligament, with smaller ones in the kidney and lymph nodes.

Closely associated with the amyloid deposits in the broad ligament, kidney, and lymph node were areas of acute and granulomatous inflammation (fig 1). Occasional necrotising granulomata were seen, sometimes with central deposits of amyloid (fig 2). No organisms were recognised on Gram, periodic acid-Schiff, Giemsa, Ziehl-Neelson, Dieterle or Warthin-Starry stains, or by electron microscopy. Some of the vessels with heavy amyloid deposition showed evidence of vasculitis, with both acute and granulomatous inflammation (fig 3). Although the foci of inflammation were always closely associated with areas of amyloid deposition, not all foci of amyloidosis, especially those in the lungs, showed surrounding inflammation.

Refractile material was seen in macrophages in the liver, in keeping with silicone deposition, and a single fragment of birefringent foreign material was seen in an obliterated pulmonary artery, which also showed

Fig 1 Area of acute and granulomatous inflammation (top) from broad ligament, with central deposit of amyloid (arrow). (Haematoxylin and eosin.)

Fig 2 Necrotising granuloma from renal parenchyma, with central deposit of amyloid (arrow). Note atrophic renal tubules at the bottom. (Haematoxylin and eosin.)

amyloid in its wall. This foreign material was not present in deeper sections from the tissue block and was, therefore, not analysed further. In general, however, no foreign material was seen in association with the amyloid deposits, especially those associated with inflammation.

The kidneys were end stage, with features consistent with chronic glomerulonephritis. Amyloid was seen in vessel walls and as small parenchymal deposits, but not within the sclerosed glomeruli. The lungs, liver, and bone marrow showed no evidence of a widespread granulomatous inflammatory process, and no evidence of a plasma cell dyscrasia was present.

Review of the endometrial biopsy specimen taken three years before death showed a small deposit of amyloid within a myometrial vessel wall.

Tissue from the broad ligament was examined by electron microscopy and this showed the typical fibrillar structure of amyloid. The fibrils were often arranged in parallel bundles (fig 4), an unusual fea-

ture for amyloid fibrils, but one which has been noted previously in amyloid deposits from patients^{4,7} on haemodialysis.

One of the amyloid deposits was examined by the Oxford scanning proton microprobe analyser,⁸ but no unusual extraneous or unexpected elements were detected.

Material and methods

In an attempt to characterise the amyloid further, formalin fixed paraffin embedded sections from the broad ligament, vulval nodule, and lung of this patient were immunostained with the antibodies detailed in the table, using a standard indirect immunoperoxidase technique.⁹ As controls, formalin fixed paraffin embedded sections containing amyloid of presumed light chain type (patient with myeloma), amyloid A type (patient with rheumatoid arthritis), and prealbumin type (senile cardiac amyloidosis) were also immunostained by all these antisera.

Fig 3 Vessel wall from broad ligament, showing heavy amyloid deposition (arrows), with associated acute and granulomatous inflammation. (Congo red.)

Fig 4 Ultrastructure of amyloid deposits from broad ligament showing prominent parallel arrangement of fibrils.

Table Primary polyclonal antibodies used in this study

Antibody	Dilution	Source
β_2 -microglobulin	1/200	Dako
λ light chains	1/800	Dako
κ light chains	1/800	Dako
Amyloid A	1/100	Behring Diagnostics
Amyloid P	1/100	Dako
Prealbumin	1/100	Dako

Results

There was strong specific staining of the amyloid deposits in the vulva broad ligament and lung using the anti- β_2 -microglobulin (fig 5). Weak staining for amyloid P was noted, but no staining was detected with any of the other antisera. The anti- β_2 -microglobulin studied failed to stain the amyloid deposits of presumed amyloid light chain, amyloid A, or amyloid prealbumin type, although inflammatory cells in all sections stained acting as an internal control for β_2 -microglobulin.

Fig 5 Amyloid deposit in centre of area of acute and granulomatous inflammation, immunostained using anti- β_2 -microglobulin. (Peroxidase.)

Discussion

Most current classifications of amyloidosis are based on the identity of the major protein component.^{10 11} The deposits can either be localised (such as within the skin or in endocrine tumours) or generalised and systemic. Only amyloid of prealbumin, light chain, or amyloid A type has been previously shown to be capable of widespread systemic deposition.

It is now widely known that patients receiving long term haemodialysis are at risk of developing amyloid deposits of β_2 -microglobulin type, (amyloid β_2 M), and that this particularly affects the osteo-articular system. This is therefore currently classified as a form of localised or organ limited amyloidosis.¹¹ The case reported here, however, shows that amyloid β_2 M can be deposited systemically. Occasional previous reports have described systemic amyloid deposition in patients receiving haemodialysis,^{12 13} but the amyloid was not further characterised, and furthermore, one of these patients had a coexistent monoclonal gammopathy.¹³ Post mortem examination performed on a patient with a lytic bone lesion composed of amyloid β_2 M showed an additional single small deposit within a coronary artery.¹⁴ This was not further typed, although elderly people commonly have deposits of amyloid of prealbumin type within the heart.¹¹

Beta₂ microglobulin is a protein of 11 800 daltons molecular weight, which is too large to be removed by most current haemodialysis membranes.^{6 15} As its normal route of elimination from the body is through the kidneys and it is constantly produced by the breakdown of cells with the release of HLA class I molecules, β_2 -microglobulin accumulates within the serum of patients receiving haemodialysis and reaches high concentrations.¹⁵⁻¹⁷ Furthermore, as the molecular structure of β_2 -microglobulin bears similarities to immunoglobulin light chains,^{14 18} a known precursor of amyloid fibrils, then it is, perhaps, not totally surprising that the excess β_2 -microglobulin can be deposited as amyloid in these patients. There is evidence, however, that a high serum concentration itself is not sufficient for the development of amyloidosis, as the type of dialysis membrane used seems to be important.^{16 17} Other factors, therefore, should be considered.

Amyloid is generally considered to be an inert substance, causing little inflammatory reaction other than a foreign body giant cell response. It was, therefore, of surprise to note the close physical association between the amyloid deposits and the foci of acute and granulomatous inflammation in the current case, especially as the aetiology of the inflammatory illness was not apparent on clinical, serological, or pathological examination. Particularly striking features were

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deposits of amyloid within the centre of necrotising granulomata and within the walls of vessels showing vasculitis.

Several explanations of this phenomenon are possible other than coincidence. The patient may have had a granulomatous illness of unknown aetiology, possibly an unusual infection, and the deposits of amyloid β_2 M acted as foci on which the inflammatory response was almost exclusively centred. Secondly, the amyloid may have directly caused the inflammatory response. In the light of the past experience with amyloidosis this hypothesis seems unlikely, although it must be borne in mind that amyloid β_2 M is a relatively newly recognised condition and may not behave in the same manner as other amyloid types. "Haemodialysis arthropathy," however, shows only a mild lymphoid infiltrate histologically,⁵ even in the presence of large amounts of amyloid β_2 M, and in the case reported here, the vulvar amyloid mass and pulmonary deposits were not associated with any unusual degree of inflammation. Finally, and perhaps most likely, the local conditions for amyloid deposition may have been particularly favourable in the areas of greatest inflammation. Indeed, it may be that an inflammatory condition causing an acute phase response or tissue necrosis is one of the other factors necessary for the development of amyloidosis in such patients with raised serum β_2 -microglobulin values receiving haemodialysis.

Patients receiving long term haemodialysis represent a unique population at risk of conditions not recognised in the general population. Acquired polycystic kidney disease^{19 20} and silicone granulomata of the liver²¹ have been recognised for some years, and it is now clear that amyloidosis, both localised and systemic, must be added to these. The development of current haemofiltration techniques, which use highly permeable membranes and are capable of removing β_2 -microglobulin from the body, may become an important factor in reducing the morbidity of a population already over burdened with disease.

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