Virchows Arch. A Path. Anat. and Histol. 379, 113–118 (1978)

Virchows Archiv A

Study of Light-, Electron- and Immunofluorescence Microscopy of Urinary Sediment in Amyloidosis

C. Orfila*, P. de Graeve, A. Guilhem, and J.M. Suc
Groupe INSERM U133, Laboratoire de Cytologie, CHU Rangueil, and
Laboratoire d'Optique Electronique du CNRS, F-31054 Toulouse Cedex France

Summary. Urinary sediment of 11 patients with amyloidosis and 12 without (with proteinuria or in good health) have been studied by different morphological techniques.

By light microscopy, an amyloid-related substance was occasionally demonstrated both in patients with amyloidosis and in control subjects. Immunofluorescence (IF) showed substance A (amyloid component) to be present in some cases of amyloidosis and in controls. On electron-microscopy, fibrils with characteristic appearance of amyloid substance were found in some cases of amyloidosis (4 out of 11), but were also found in controls.

It therefore seems difficult to establish the diagnosis of amyloidosis by microscopic studies of the urinary sediment.

Key words: Amyloidosis – Urinary sediment – Light-microscopy – Immunofluorescence – Electron-microscopy.

The diagnosis of amyloidosis by electron-microscopic and immunologic studies of urinary sediment has been recently proposed by some authors (Derosena et al., 1975; Neale, 1976; Nimoytin et al., 1976). In contrast, other studies have commented on the "uncertain value of urinary sediment in the diagnosis of amyloidosis (Shirahama, 1977). We have attempted to detect amyloid-like substance in urinary sediments by morphological and immunological studies.

Material and Methods

We studied urine specimens from 11 patients with biopsy-proven amyloidosis (Table 1), 4 patients with proteinuria caused by renal-biopsy-proven nephritis without amyloidosis and from healthy subjects.

* Attachée de recherche INSERM

For offprints contact: C. Orfila

0340-1227/78/0379/0113/$01.20

Table 1. Biological data in cases of amyloidosis

No.	Type of biopsy	Type of amyloidosis	Proteinuria (g/24 h)	Serum creatinine µmol/l
1	Renal	Primary	6	877.3
2	Renal	Secondary	13	88.5
3	Neural	Familial	1	159.3
4	Renal	Secondary	3.50	840.7
5	Renal	Secondary	0.16	619.5
6	Renal	Secondary	0.88	115
7	Rectal	Secondary	1.53	105.2
8	Synovial	Secondary	1	132.7
9	Renal	Secondary	3	654.9
10	Rectal	Secondary	0.16	752.2
11	Renal	Secondary	3	132.7

For light-microscopic examination, 120 ml of urine were centrifuged (cytocentrifuge Shandon Southern) at 2500 rpm for 15 min. Urinary sediment was fixed in 50% ethanol and stained with Papanicolaou, May-Grunwald-Giemsa, periodic acid Schiff, Crystal violet and Congo Red (Heptinstall, 1974) stainings, and examined under conventional and polarized light microscopy.

For immunofluorescent studies, the urine specimen was centrifuged at 12,000 g for 60 min at 4° C in a Beckman J 21 Centrifuge. The sediment was resuspended in 2 ml of urine and one drop was transferred onto slides. It was then processed routinely for direct IF technique, using monospecific fluorescein conjugated antisera to human IgG, Fibrinogen (Behring Laboratories), IgM, IgA, C3, Fibrin (Hyland Laboratories), Kappa light-chain, Lambda light-chain (Meloy Laboratories), A component and P component of amyloid-substance (Atlantic Antibodies). The monospecificity of these antisera was tested by immunoelectrophoresis against normal human plasma and normal human serum.

For electron-microscopic examination, aliquots of urinary sediment (obtained after centrifugation at 12,000 g) were fixed in 4 per cent glutaraldehyde for one hour, washed in Hcl-cacodylate buffer, postfixed in 2 per cent osmium tetroxyde for one hour and embedded in Epon 812. Thin sections were stained with uranyl-acetate and lead-citrate and examined in a OPL electron-microscope.

Semi-thin sections of Epon-embedded material were stained with Congo Red and examined with a light microscope.

Results

The essential findings are summarized in Table 2.

By light-microscopy, the urine of 6 patients with amyloidosis showed metachromatic masses stained with Crystal violet. 4, stained with Congo Red, demonstrated classic deep-green yellow birefringence under polarized light. The urine of 2 patients with amyloidosis showed metachromatic masses without birefringence. The urine of one proteinuric patient without amyloidosis demonstrated metachromatic Congo Red spots and birefringence, one showed irregular staining.

The urine of 2 healthy persons showed a faint Congo Red birefringence.

Thus, by light-microscopy it seems difficult to define specific differences between the urinary sediments of amyloid affected and control-subjects.

By the immunofluorescent technique, urinary casts of amyloidotic patients stained with IgG (5 cases), IgA, Fibrin (3 cases), C3 (2 cases), Kappa light

Table 2a. Light-, immunofluorescence electron-microscopic results of urinary sediment of amyloidotic patients

No.	Light-microscopy		Immunofluorescence		Electron microscopy
	Crystal violet	Congo red	A component	P component	
1	Metachromasia	+	–	–	–
2	Metachromasia	+	–	–	–
3	–	–	–	–	–
4	Metachromasia	+	–	–	–
5	Inconstant metachromasia	–	±	++	–
6	–	–	–	–	+
7	–	–	±	–	–
8	Metachromasia	–	++	–	–
9	Metachromasia	–	+	–	+
10	–	+	–	–	+
11	–	–	–	–	+

Table 2b. Light, immunofluorescence electron-microscopic results of urinary sediment of control-subjects

No.	Diagnosis	Light-microscopy		Immunofluorescence		Electron micros-copy
		Crystal violet	Congo red	A com-ponent	P com-ponent	
12	Normal	Metachromasia	+	++	–	–
13	Normal	–	–	–	–	–
14	Normal	–	–	–	–	–
15	Normal	Metachromasia	–	–	–	–
16	Normal	Metachromasia	–	–	–	+
17	Normal	–	–	–	–	+
18	Normal	–	–	–	–	–
19	Normal	Metachromasia	+	–	–	+
20	"Minimal changes" nephropathy	–	–	±	–	–
21	Membranoproliferative glomerulonephritis	–	±	–	–	–
22	Cirrhotic glomerulo-nephritis	Metachromasia	+	–	–	+
23	Interstitial nephritis	–	–	+	–	–

chain (3 cases). A component of amyloid-substance was observed in casts and masses in 4 cases, P component in one case.

In proteinuric patients without amyloidosis, IgG, IgA were found in 4 cases of urinary sediment, C3, Fibrin in one case, Kappa light-chain in 2 cases, Lambda-light chain in one case, A component in 2 cases. P component was never found.

In healthy persons, A component was demonstrated in one case. By IF, it is also difficult to differentiate urine from amyloidotic patient from the urine of proteinuric or normal subjects, using A protein.

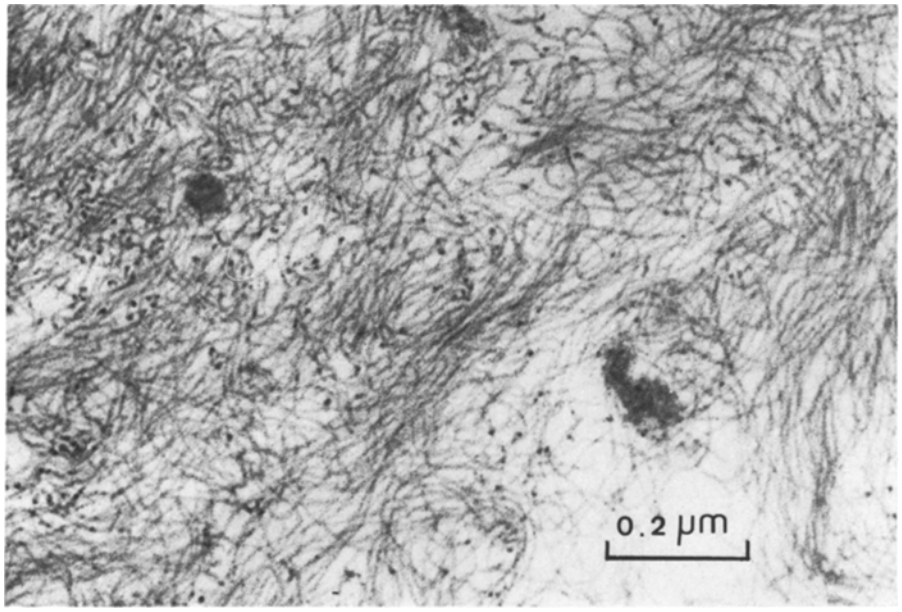

Fig. 1. Electron-micrograph of urinary sediment showing (type I) fibrillar material with wavy appearance (uranyl acetate and lead citrate)

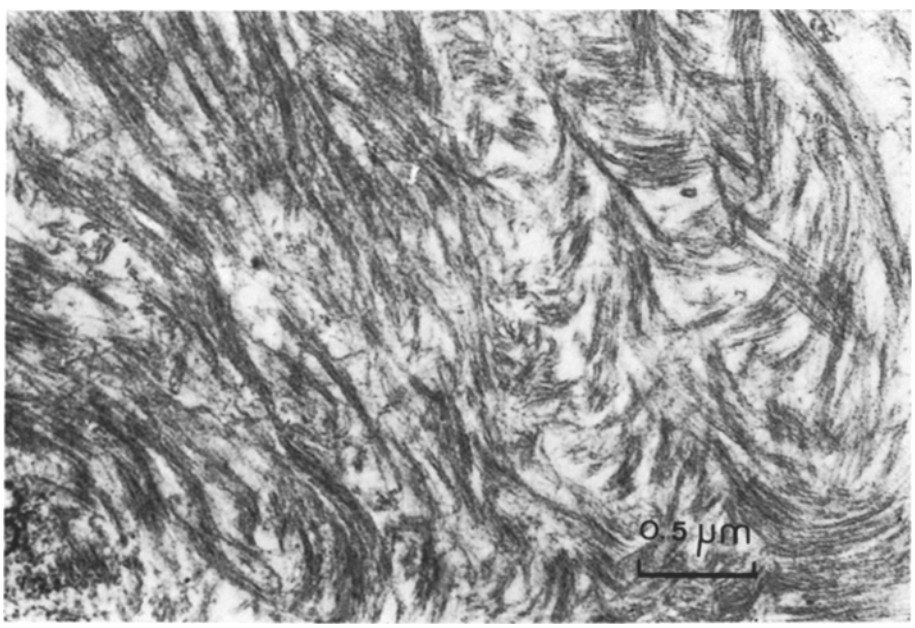

Fig. 2. Electron-micrograph of urinary sediment showing (type II) fibrillar material with bundle-appearance (uranyl acetate and lead citrate)

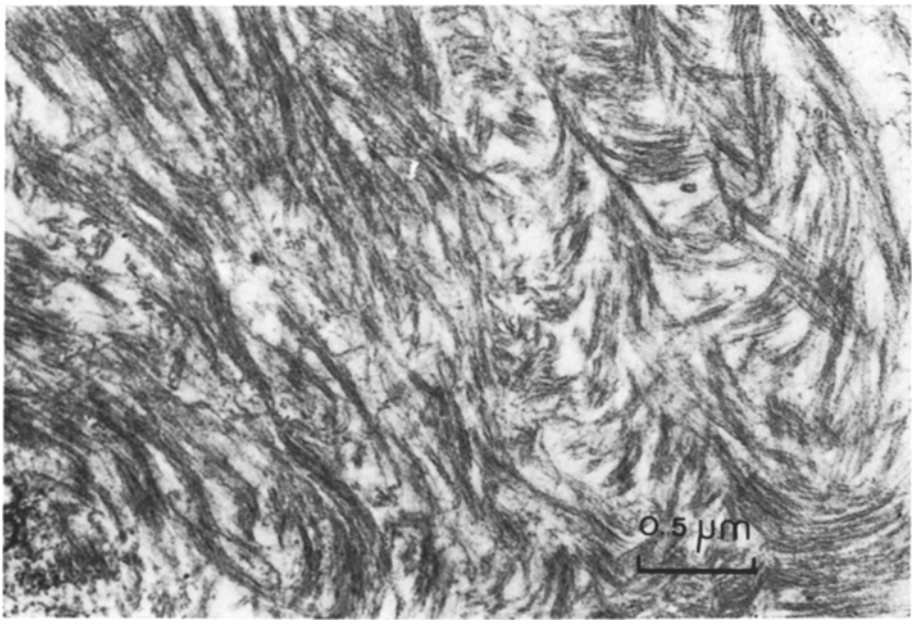

Fig. 3. Electron-micrograph of urinary sediment showing (type III) fibrillar material having size and periodicity of amyloid-fibrils (uranyl acetate and lead citrate)

By electron-microscopy, different types of fibrils were observed among cells and casts. The most common (Type I) were wavy fibrils (about 80 Å in diameter) observed inside casts (Fig. 1) and might represent tonofibrils (Shirahama et al., 1977). They were seen in 5 out of 11 amyloidotic patients, 5 out of 8 healthy persons and in one proteinuric patient.

Other fibrillar structures (Type II) had the appearance of longer fibrils of 130 Å in diameter, in groups of three or more, forming some bundles (Fig. 2). They were observed in 6 cases of amyloidosis, 3 cases of proteinuria and one normal subject.

Fibrillar material (Type III) with the characteristic appearance of amyloid (90 Å to 120 Å in diameter) was observed in 4 patients with amyloidosis (Fig. 3) but also in one proteinuric patient and in 3 healthy persons.

Discussion

In previous studies (Jao and Pirani, 1972; Limas et al., 1973) amyloid fibrils have been demonstrated in Bowman's spaces and tubular casts in renal biopsies from amyloidotic patients. It is reasonable to expect to find these fibrils in the urine and this finding could be of investigative value. Some authors have described these fibrils in urinary sediment of patients with amyloidosis (Jao and Pirani, 1972; Derosena et al., 1975; Neale, 1976; Nimoityn et al., 1976) but in another recent study (Shirahama, 1977) an attempt to detect amyloid-related substances in urine by different techniques failed.

It is established that in secondary amyloidosis the major constituent of

fibrils forming amyloid deposits is AA protein (Watanabe et al., 1977), immunologically similar to a particular plasma protein SAA which is also found in both normal and secondary amyloidosis sera (Franklin et al., 1975) and in increased amounts in the sera of patients with chronic infections (Rosenthal et al., 1976). AA protein might thus be present in the urine of patients with secondary amyloidosis, as Shirahama expected, but he failed to find AA protein in the urine specimen studied, using I.F. techniques.

In our cases the A component of amyloid substance was occasionally found in cases of amyloidosis, but was also demonstrated in some normal subjects and in proteinuric patients. Light-microscopic studies of Congo red-stained urinary sediment from amyloidotic patients have failed to established the presence of amyloid-substance. The material was sometimes identified in amyloid affected patients but was also found in proteinuric patients and in one normal control.

By electron-microscopy, fibrillar material with the characteristic appearance of amyloid was noted in some but not all cases of amyloidosis and in some control patients without amyloidosis. These findings are in agreement with the previous results (Shirahama et al., 1977). The nature of these fibrils has not yet been demonstrated; there is no apparent correlation between electron-light-microscopic and IF observations in this respect. Thus it seems difficult at present to diagnose amyloidosis by the detection of amyloid related substance in the urinary sediment using morphological or immuno-morphological studies.

Acknowledgments: We are indebted to Mrs. J. Quercy for excellent technical assistance and to Miss C. Sabardu and Mr. C. Mora for the preparation of the manuscript.

References

Derosena, R., Koss, M.N., Pirani, C.L.: Demonstration of amyloid fibrils in urinary sediment. N. Engl. J. Med. **293**, 1131–1133 (1975)

Franklin, E.C., Rosenthal, C.J., Pras, M.: Etude de la protéine amyloide A (AA protein) et d'un composant serique apparenté. Purification, caractérisation partielle, origine et distribution tissulaire au cours des différents types d'amylose. In: Actual. Nephrol. Hôp. Necker, Paris: Flammarion 279–285 (1975)

Heptinstall, R.H.: Amyloidosis, multiple myeloma, Waldenström's macroglobulinemia, mixed IgG-IgM cryoglobulinemia and benign monoclonal gammopathy. In: Pathology of the kidney (Heptinstall, R.H., ed.) 2nd ed, pp. 737–780. Boston: Little Brown and Co., 1974

Jao, W., Pirani, C.: Renal amyloidosis: electron-microscopic observations. Acta Pathol. Microbiol. Scand. **80**, supp. 233, 217–227 (1972)

Limas, C., Wright, J.R., Matsuzaki, M., Calkins, E.: Amyloidosis and multiple myeloma: a reevaluation using a control population. Am. J. Med. **54**, 166–173 (1973)

Neale, T.J.: Amyloid fibrils in urinary sediment. N. Engl. J. Med. **294**, 444–445 (1976)

Nimoityn, P., Lasker, N., Soriano, R.Z.: Detection of urinary amyloid in familial Mediterranean fever. Br. Med. J. **2**, 284 (1976)

Rosenthal, C.J., Franklin, E.C., Frangione, B.: SAA the serum precursor of the AA protein (abstr.) Clin. Res. **24**, 449A (1976)

Shirahama, T., Skinner, M., Cohen, A.S., Benson, M.D.: Uncertain value of urinary sediments in the diagnosis of amyloidosis. N. Engl. J. Med. **297**, 821–823 (1977)

Watanabe, S., Jaffe, E., Pollock, S., Sipe, J., Glenner, G.: Amyloid AA protein. Cellular distribution and appearance. Am. J. Clin. Pathol. **67**, 540–544 (1977)

Received March 31, 1978