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Diagnosis and immunohistochemical classification of systemic amyloidoses

Report of 43 cases in an unselected autopsy series

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Abstract Fourty-three cases of systemic amyloidosis were identified in an unselected autopsy series from our institute (6305 autopsies between 1979 and 1993) and classified immunohistochemically by means of a panel of antisera directed against five major amyloid fibril proteins. Amyloid A (AA) amyloidosis was the most common type, being found in 21 cases (48.8%). Transthyretin-derived (ATTR) amyloidosis was present in 11 cases (25.6%), and immunoglobulin light chain-derived (AL) amyloidosis in 10 cases (23.3%). A single case (2.3%) contained deposits of more than one type of systemic amyloid. AA amyoloidosis was associated with chronic inflammatory or infectious diseases (81%), malignant tumours (19%) or both (9.5%). Immunoglobulin light chain-derived amyloidoses were associated with myeloma (50%) or primary (idiopathic; 50%). In AA and AL amyloidosis the kidney was the organ most frequently involved. ATTR amyloid affecting mostly the heart and lungs presented as senile systemic amyloidosis. Systemic amyloidosis was the cause of death in 5 cases (12%) and caused symptoms in 17 cases (39%). Our results suggest that most cases can be classified by using a panel of sensitive and specific antibodies against five major amyloid fibril proteins. This technique may make amyloid type-specific therapy possible for AL amyloid patients who do not have evidence of an underlying plasma cell dyscrasia.

Key words Systemic amyloidosis · Postmortem study · Immunohistochemistry · Classification · Histomorphological pattern

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Introduction

The term systemic amyloidosis is applied to a variety of disease entities with a wide morphological and clinical spectrum. It is characterized by the extracellular deposition of amyloid proteins in various tissues and organs. Failure of the heart or the kidney is a common complications and sometimes even fatal. Though all amyloid proteins have biophysically comparable features (Congo red binding, green colour in polarized light, fibrillar appearance on electron microscopy, β-sheet structure), there are numerous distinctive types of amyloid classified by their diverse biochemistry [2, 16, 18, 27].

Cases of systemic amyloidosis can be classified in tissue sections by using well-defined antibodies against the various amyloid fibril proteins. Single antibodies have already been tested on biopsy or autopsy collections [10, 25, 48], but few systematic studies of the immunohistochemical classification of systemic amyloidosis in autopsy material have been conducted [5, 10, 21, 25]. Only one study, by Baretton et al. [1], reviewed an *unselected* autopsy series where cases of systemic amyloidosis were classified by immunohistochemistry.

The present study was performed to evaluate the quality of various antibodies used for the immunohistochemical classification of systemic amyloidoses in a large unselected autopsy series. These antibodies were directed not against the precursor, but against the amyloid fibril proteins AA, Aλ, Aκ, ATTR and Aβ2M. We also examined the clinical and morphological features of systemic amyloidoses.

Materials and methods

The study group was made up of 6305 autopsy cases dissected at our Institute between January 1979 and December 1993, which were reviewed to identify cases of systemic amyloidosis. Histological sections of several organs (heart, lung, liver, kidney, spleen and pancreas) were routinely available. In some cases, various numbers of additional organs or tissues were investigated. All cases in which amyloid was detected macroscopically or microscopically in the routine autopsies were selected. The criterion for

Table 1 Antibodies applied for immunohistochemical classification of systemic amyloid (*AA* amyloid A protein, *Aλ* amyloid of immunoglobulin lambda-light chain origin, *Aκ* amyloid of immunoglobulin kappa-light chain origin, *ATTR* amyloid of transthyretin origin, *Aβ2M* amyloid of β2-microglobulin origin, *PAP* peroxidase-antiperoxidase, *ABC* avidin-biotin peroxidase complex)

Antibody	Animal	Monoclonal/ polyclonal	Dilution	Method	Reference
Anti-AA (mcl)[a]	Mouse	m	1:10	PAP	[30, 36][c]
Anti-Aλ (HAR)[b]	Rabbit	p	1:1200–1:4000	ABC	[31, 36]
Anti-Aκ (SIN)[b]	Rabbit	p	1:1000	ABC	[31, 36]
Anti-ATTR (TIE)[b]	Rabbit	p	1:600–1:1500	ABC	[29, 36]
Anti-Aβ2M (WOE)[b]	Rabbit	p	1:200	ABC	[43][c]

[a] Code of monoclonal antibody
[b] HAR, SIN, TIE, WOE: initials of the patients from whom the amyloid fibrils used as immunogen were isolated
[c] Possible alternative source: Dakopatts, Hamburg

systemic amyloidosis was the involvement of at least two organs. Clearly organ-limited or local amyloid deposition (e.g. pancreatic insular amyloid) was not further examined.

In each case, tissue specimens were formalin fixed (4% buffered formaldehyde) and paraffin embedded. All available tissue sections of cases with amyloid deposits were stained with alkaline Congo red [42] and inspected with a Leitz microscope in bright and polarized light with tension-free optics. Green birefringence under polarized light proved existing amyloid. In all cases with amyloid deposits in two or more organs, sections containing amyloid were used for immunostaining.

Immunohistochemical analysis was performed using a panel of antibodies reactive with five major amyloid fibril proteins as listed in Table 1: anti-AA (mc1) against amyloid A [30, 36], anti-Aλ and anti-Aκ for reaction with immunoglobulin light chain-derived amyloid [31, 36], anti-ATTR against transthyretin-derived amyloid [29, 36], and anti-Aβ2M for identification of β2-microglobulin-derived amyloid [43]. The monoclonal antibody directed against amyloid A was applied according to the peroxidase-antiperoxidase (PAP) method of Sternberger [47] (pretreatment with normal swine serum, dilution 1:50, secondary antibody goat-anti-mouse immunoglobulin, dilution 1:10, PAP complex, dilution 1:100). Anti-Aλ, anti-Aκ, anti-ATTR, and anti-Aß2M were used according to the avidin-biotin-peroxidase complex (ABC) method of Hsu et al. [22] (pretreatment with normal swine serum, dilution 1:50, biotinylated secondary antibody goat anti-rabbit immunoglobulin, dilution 1:200, ABC, dilution 1:100). Immunoreactions were visualized by using 3,3-diamino-benzidine-tetra-hydrochloride (DAB). The counterstain was haematoxylin. Immunostains were reported as negative, weakly positive or strongly positive. In all cases positive and negative controls were included, with different specimens containing known types of amyloid or amyloid-free specimens, and with omission of the first antibody. Additional immunoreactions were performed on all available tissue sections of bone marrow by conventional antibodies directed against immunoglobulin light chains (anti-λ, anti-κ). Finally, all data from previous performed immunoelectrophoreses were reviewed.

Each case was classified immunohistochemically by the corresponding antibody with the strongest staining reaction. A weak reaction with noncorresponding anti-Aλ antibody in some AA amyloids was considered to be nonspecific and due to immunoglobulin trapped within the amyloid deposits, particularly in inflammatory disease, as previously observed [1, 5, 12, 25, 36]. Since both senile and familial amyloid had revealed common antigenic determinants and a similar reaction pattern with anti-ATTR (TIE) antibody in a previous study [29], the differentiation of both was based on clinical symptoms, the age of the patient and the presence or absence of a family history of amyloidosis.

Histological sections were examined for the severity and morphological pattern of amyloid deposits without prior knowledge of clinical history and chemical types of amyloid. The degree of amyloid deposition was assessed according to established standards: a score of 1+ indicates replacement of <10% of tissue with amyloid; a score of 2+, replacement of 10–25% of tissue; a score of 3+, replacement of 26–50% of tissue; and a score of 4+, replacement of more than 50% of tissue [24]. An additional score of 0.5+ (trace) was used to identify barely detectable deposits. The pattern of deposits was classified as vascular, interstitial, or mixed type (vascular and interstitial involvement).

Fisher's exact test and the Fisher-Freeman-Halton test (modifications of the Chi-square test) were used for statistical evaluation. The correlation between amyloid types and age was analysed by the Fisher-Freeman-Halton test. The prevalences of amyloid types were correlated with the frequencies of basic diseases by Fisher's exact test. Included in the calculations were the number of patients having basic diseases recognized to be partially associated with AA or AL amyloidosis and documented as basic diseases in the autopsy reports. The correlation was graded as significant at $P<0.05$ or highly significant at $P<0.001$.

Results

Systemic amyloidosis was revealed in 43 of the 6305 unselected autopsy cases (0.7%). The immunohistochemical classification showed that there were 21 cases (48.8%) of amyloid A (AA) amyloidosis, 11 cases (25.6%) of transthyretin-related (ATTR) amyloidosis, 10 cases (23.3%) of immunoglobulin light chain-related (AL) amyloidosis, and a single case (2.3%) with coexistence of different types of amyloid (Table 2).

AA Amyloidosis

Amyloid deposits showed the strongest immunoreaction with anti-AA in 21 cases (48.8%). Furthermore, a weaker immunostaining with anti-Aλ occurred inconsistently in some specimens of 9 AA amyloid patients. The mean age of this group, consisting of 12 women and 9 men was 70 (48–104) years (Table 3, Fig. 1).

Associated diseases (inflammatory processes in 62%, chronic infectious diseases in 19%, malignant tumours in 19%) are summarized in Table 4. Statistical analysis of diseases showed a significant correlation ($P<0.05$) between rheumatoid arthritis, ankylosing spondylitis (Bechterew's disease), and renal cell carcinoma and the immunohistochemically classified AA amyloidosis. The other diseases found in association with AA amyloid as listed in Table 4 were not significantly correlated ($P>0.05$; Table 5). Amyloidosis was diagnosed before death in only 2 cases (9.5%). The other 19 cases were incidental findings.

Table 2 Clinicopathological data and immunohistochemical findings in 43 patients with systemic amyloidosis (*m* male, *f* female, *IFE* immunofixation electrophoresis)

Case no.	Type of amyloid	Age/sex	Clinicopathological diagnosis	Amyloid positive/ examined organs	Anti-AA	Anti-Aλ	Anti-Aκ	Anti-ATTR	Anti-Aβ2M	Not classifiable	Anti-λ	Anti-κ	IFE
1	AA	54/m	Lung carcinoma	7/7	7	0	0	0	0	0			
2	AA	104/m	Bronchiectasis	5/5	5	0	0	0	0	0			
3	AA	53/m	Renal cell carcinoma	2/7	2	0	0	0	0	0			
4	AA	48/m	Ankylosing spondylitis	16/17	15	0	0	0	0	1[a]	0	0	
5	AA	68/f	Bronchiectasis	7/8	7	0	0	0	0	0			Normal
6	AA	73/f	Rheumatoid arthritis	9/9	9	0	0	0	0	0	0	0	
7	AA	71/f	Tuberculosis	6/6	6	(2)	0	0	0	0			
8	AA	84/f	Bronchiectasis[b]	6/9	6	(1)	0	0	0	0			
9	AA	79/f	Bronchiectasis[b]	6/8	6	(1)	0	0	0	0			
10	AA	70/f	Tuberculosis	7/7	7	0	0	0	0	0			
11	AA	65/m	Ankylosing spondylitis	11/11	11	(6)	0	0	0	0	0	0	
12	AA	62/m	Tuberculosis	6/6	5	(2)	0	0	0	1[a]			
13	AA	75/m	Bronchiectasis	2/6	2	0	0	0	0	0			
14	AA	75/m	Gallbladder carcinoma	10/11	10	(8)	0	0	0	0			Normal
15	AA	69/f	Renal cell carcinoma[c]	11/13	11	(1)	0	0	0	0	0	0	
16	AA	73/m	Bronchiectasis	4/7	4	0	0	0	0	0			
17	AA	69/f	Rheumatoid arthritis	9/13	8	(4)	0	0	0	1[a]	0	0	
18	AA	74/m	Tuberculosis	5/6	5	0	0	0	0	0			
19	AA	66/m	Rheumatoid arthritis	17/19	17	0	0	0	0	0			
20	AA	81/f	Decubitus ulcer	2/8	1	0	0	0	0	1[a]			
21	AA	61/f	Rheumatoid arthritis	3/10	3	3	(1)	0	0	0			
22	Aλ	82/m	Multiple myeloma	5/8	0	5	0	0	0	0	1/1	0	λ
23	Aλ	62/m	Waldenström's disease	21/22	0	18	0	0	0	1[a]+2	1/1	0	λ
24	Aλ	64/m	Multiple myeloma	8/10	0	7	0	0	0	1			λ
25	Aλ	86/f	–	7/7	0	7	0	0	0	0			
26	Aλ	76/m	–	9/9	0	9	0	0	0	0			
27	Aλ	47/m	–	20/20	0	20	0	0	0	0			Normal
28	Aλ	71/f	Multiple myeloma	2/8	0	2	(1)	0	0	0	1/1	0	
29	Aλ	75/m	–	16/19	0	0	15	0	0	1[a]			
30	Aκ	77/m	Multiple myeloma	3/11	0	(3)	3	0	0	0	0	0	κ
31	Aκ	82/f	–	9/14	0	0	9	0	0	0			
32	ATTR	99/f	–	6/11	0	0	0	6	0	0			
33	ATTR	84/f	–	2/7	0	0	0	2	0	0			
34	ATTR	78/m	–	2/8	0	0	0	1	0	1			
35	ATTR	95/m	–	3/7	0	0	0	3	0	0			
36	ATTR	90/f	–	3/7	0	0	0	2	0	1			
37	ATTR	87/f	–	2/12	0	0	0	2	0	0			
38	ATTR	83/m	–	3/7	0	0	0	1	0	2			
39	ATTR	92/m	–	7/12	0	0	0	6	0	1			
40	ATTR	88/m	–	6/7	0	0	0	6	0	0			
41	ATTR	89/m	–	6/9	0	0	0	6	0	0	0	0	
42	ATTR	80/m	–	5/6	0	0	0	5	0	0			
43	Mixed	93/f	–	24/29	6	4	0	24	0	0			Normal

[a] Sample size too small for further immunohistochemical evaluation
[b] With additive colon carcinoma
[c] With additive ovarian carcinoma

Table 3 Amyloid types: relative frequency and clinical features

Type	No. (%) of 43 systemic amyloidoses	No. (%) of 6305 autopsies	Mean age (years)	Median (years)	Range of age (years)	Deviation standard (years)	Male No. (%)	Female No. (%)
AA	21 (48.8)	21 (0.33)	70	70	48–104	11.6	9 (43)	12 (57)
AL	10 (23.3)	10 (0.16)	72	75.5	47–86	11.1	6 (60)	4 (40)
ATTR	11 (24.4)	11 (0.17)	88	88	78–99	6	7 (64)	4 (36)
ATTR+AL+AA	1 (2.2)	1 (0.01)	93	–	–	–	–	1
Aβ2M	0	0	–	–	–	–	–	–

Fig. 1 Different types of systemic amyloid by age

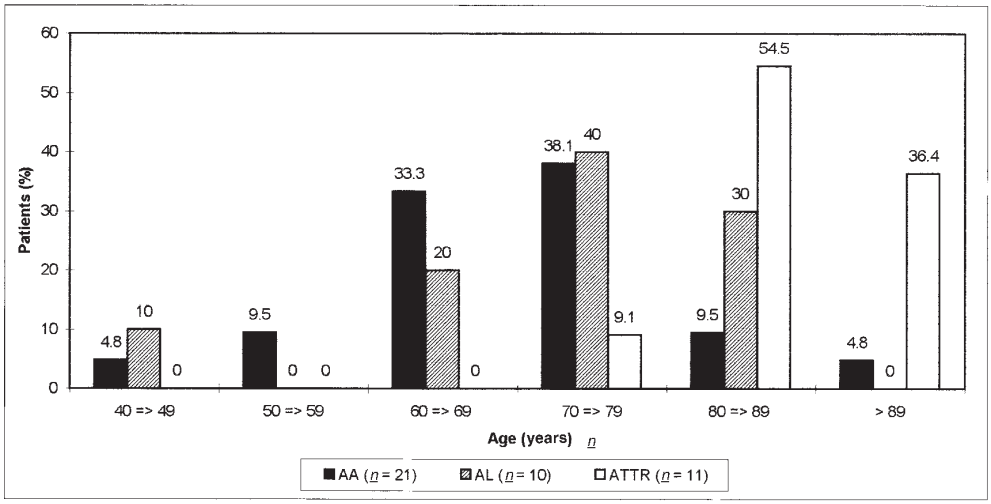
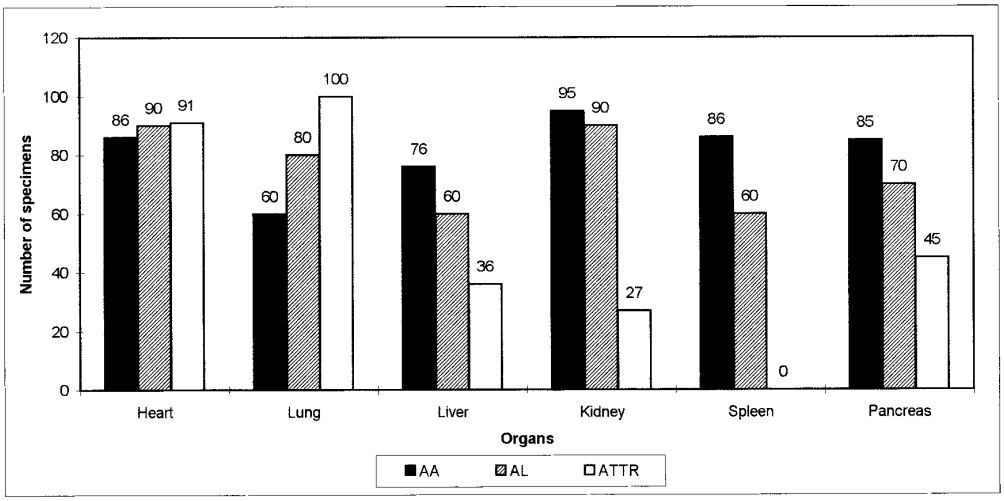


Table 4 Amyloid type and associated disease

Type of amyloid	Associated clinical disease/diagnosis	Number
AA	Bronchiectasis[a]	6
	Rheumatoid arthritis	4
	Tuberculosis	4
	Ankylosing spondylitis	2
	Renal cell carcinoma[b]	2
	Lung carcinoma	1
	Gallbladder carcinoma	1
	Decubitus ulcer	1
Aλ	Primary (idiopathic) amyloidosis	3
	Multiple myeloma	3
	Waldenström's macroglobulinemia	1
Aκ	Primary (idiopathic) amyloidosis	2
	Multiple myeloma	1
ATTR	Senile systemic amyloidosis	11
ATTR+Aλ+AA	Senile systemic amyloidosis	1
Total		43

[a] Two with additive colon carcinoma
[b] One with additive ovarian carcinoma

Fig. 2 Distribution of the different types of systemic amyloid by organ



The average amount of amyloid was estimated semiquantitatively in 8 patients as grade 1+ (38%) and in 13 as grade 2+ (62%). The pattern of organ distribution showed the kidneys were most frequently involved, being affected in 20 of 21 cases, or 95%, (Fig. 2). Heart (18/21=86%), spleen (18/21=86%), liver (16/21=76%), pancreas (vessel amyloid, not islet amyloid: 17/20=85%) and adrenal gland (5/5) were other major sites of deposition.

Amyloid deposits affected the arterioles and small arteries of parenchymal organs, but hardly any veins and

Table 5 Correlation between amyloid type and associated disease

Basic disease	Autopsy cases (*n*=6305)	Non-AA cases (*n*=6284)	AA cases (*n*=21)	Non-AL cases (*n*=6295)	AL cases (*n*=10)	*P* value
Rheumatoid arthritis	34	30	4	34	0	*P*<0.001
Ankylosing spondylitis	3	1	2	2	0	*P*<0.001
Tuberculosis[a]	183	179	4	183	0	*P*<0.05
Renal cell carcinoma	33	31	2	33	0	*P*<0.05
Decubitus ulcer[a]	14	13	1	14	0	*P*=0.05
Colon carcinoma[b]	191	189	2	191	0	*P*>0.05
Lung carcinoma[c]	192	191	1	191	1	*P*>0.05
Ovarian carcinoma[b]	64	63	1	0	0	*P*>0.05
Gallbladder carcinoma[b]	75	74	1	75	0	*P*>0.05
Bronchiectasis	About 2500	About 2500	6	About 2500	0	*P*>0.05
Multiple myeloma	35	0	0	31	4	*P*<0.05
Waldenström's macroglobulinaemia	2	0	0	1	1	*P*<0.05

[a] Minimal number of cases scored as basic diseases
[b] Adenocarcinoma
[c] Squamous cell carcinoma

interstitial tissues only to a lesser extent (Fig. 3). Amyloid was found in the glomeruli as vascular and interstitial (especially mesangia) deposits (Fig. 4). The spleen displayed the most abundant amyloid masses. In 6 of the 9 cases examined, intestinal tissue specimens had a mild to moderate amyloid (score 1+ to 2+), which was vascular and interstitial in all layers, especially in mucosal and submucosal arterioles. In 9 of 21 cases amyloid deposits were found in all tissue specimens examined.

About one third of the patients (6/21=29%) suffered from kidney involvement resulting in clinical symptoms ranging from renal dysfunction to failure. Cardiac amyloid probably resulted in heart failure in 4 cases (19%) in which the amount of vascular amyloid clearly exceeded the grade of atherosclerosis.

AL Amyloidosis

Six men and 4 women with a mean age of 72 years (47–86) were involved (Table 3, Fig. 1): 7 cases were classified as Aλ type and 3 as Aκ type.

Underlying clinical diseases occurring at a statistically significant frequency ($P<0.05$) were multiple myeloma and Waldenström's macroglobulinaemia in one half of the cases, whereas the other half were classified as primary (idiopathic) (Tables 4, 5). Only 5 (50%) of the 10 cases of AL amyloidosis were recognized clinically during the patients' lifetime.

Assessment of the extent of average amyloid deposition revealed mild to moderate extent (score 1+) in 1 case; moderate (score 2+), the most frequent extent, in 7, and moderate to severe (score 3+) in 1 case. The heart and the kidney contained amyloid most frequently (9/10=90%) (Fig. 2), the extent mostly being mild to moderate. The heart nearly always showed the strongest amyloid involvement, with moderate to severe extent. Lung (8/10=80%), pancreas (7/10=70%), liver and spleen (6/10=60%) were less frequently and less extensively involved. Moreover, AL was apparent in many other different organs or tissues of predominantly mesenchymal origin (tongue) (4/4), skeletal muscles (3/3), thyroid gland (4/4), skin (2/2), peripheral nerves (3/3) and intestinal tissue (6/6). In contrast to amyloid A, AL amyloid was obviously more interstitial than vascular (Figs. 3, 5).

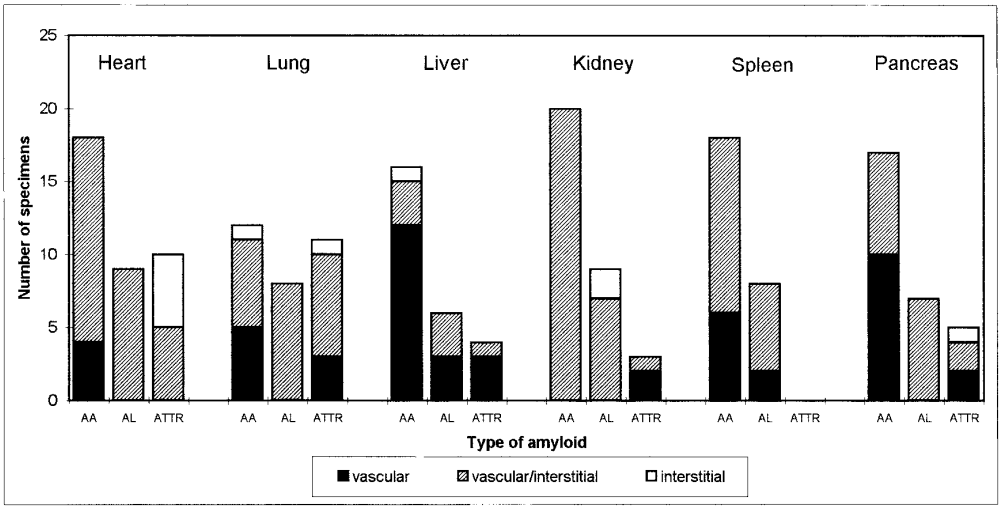
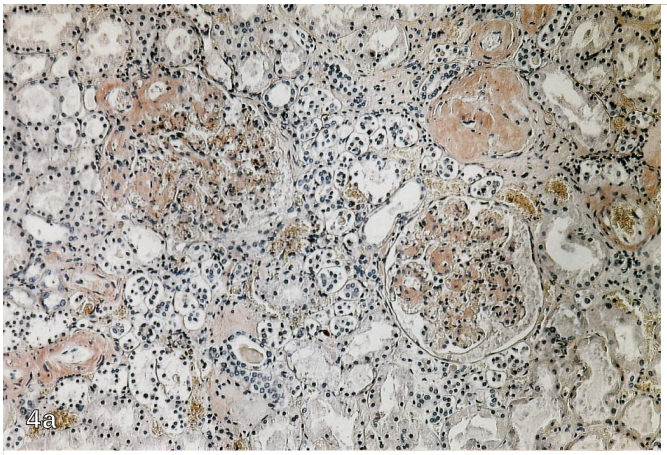
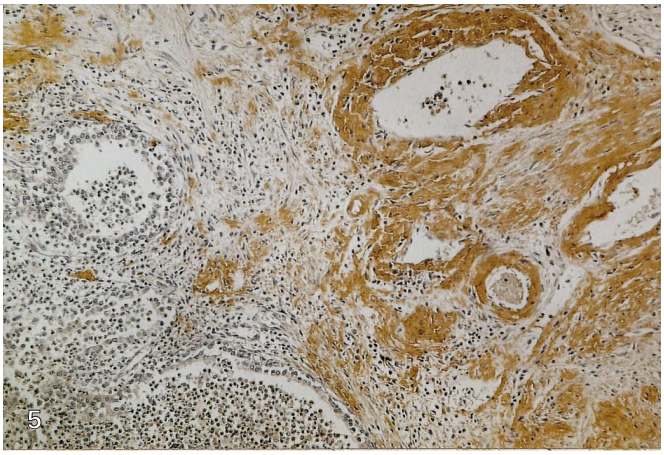
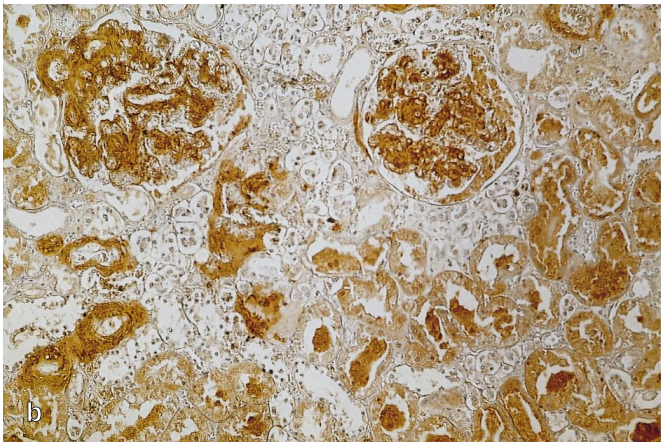


Fig. 3 Deposition pattern of the different types of systemic amyloid in six organs







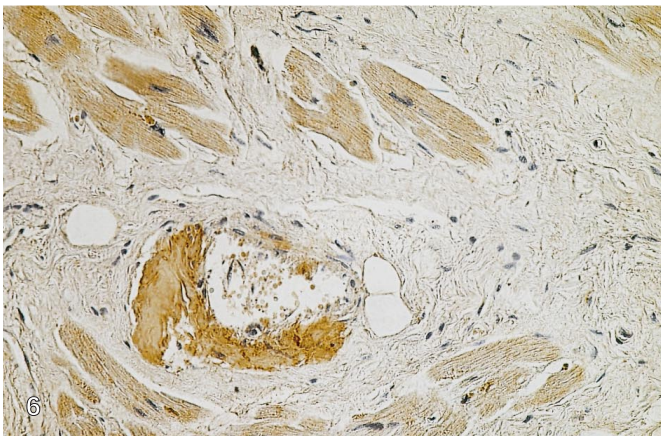


Fig. 4a, b Amyloid A deposits in the glomeruli of the kidney. **a** Congo red **b** Parallel section. Anti-AA, PAP, hematoxylin, ×90

Fig. 5 Aλ deposits in the vessel of the prostate. Anti-Aλ, ABC, haematoxylin, ×170

Fig. 6 ATTR deposits in a vessel wall of heart. Anti-ATTR, ABC, haematoxylin, ×170

In 9 of 10 cases (90%) clinical symptoms had been recorded, while in the remaining case there was no record of any symptoms. Cardiac insufficiency was seen in 7 patients (70%), three of whom died of heart failure; 2 also had macroglossia. Renal insufficiency was reported in 4 cases (40%) by the clinicians.

Senile systemic amyloidosis

All ATTR cases were diagnosed as senile systemic amyloidosis on the basis of age and the absence of heredity. The mean age of this group, comprising 7 men and 4 women, was 88 years (78–99 years) (Table 3, Fig. 1). The prevalence of senile systemic amyloidosis among the 6305 autopsy cases increased significantly with age ($P<0.001$).

The most frequent disease caused by TTR-amyloid was congestive heart failure, which was found in 100% of ATTR cases. None of these cases was diagnosed while the patient was alive, despite the clinical symptoms.

The degree of amyloid involvement was scored as grade 1+ in 8 cases (73%) and as grade 2+ in 3 (27%). Systemic senile amyloid (ATTR) had affected the lung in all 11 and the heart in ten patients as far as amyloid was detectable, whereas extracardiac amyloid in smaller amounts was detected less frequently, for example, in pancreas (5/11), liver (4/11) and kidney (3/11) (Fig. 2). This ATTR pattern of organ distribution differed clearly from the AL pattern and even more markedly from the AA pattern, as demonstrated in Fig. 2.

The heart contained the most extensive deposits of amyloid. Its histomorphological pattern was relatively distinct from that of other amyloid types (Fig. 3). We found only interstitial amyloid in 45%, combined with smaller vascular deposits in 55% (Fig. 6). Perimyocytic interstitial deposits coexisted with patchy deposits in some cases. In contrast to the heart, more vascular than interstitial amyloid was found in the other organs, such as lung, liver, kidney, spleen and pancreas (Fig. 3).

On the basis of autopsy findings, congestive heart failure was considered to have been caused by TTR amyloid in 5 cases (45%), lethal myocardial infarction being seen in 1 of them. The autopsy results suggested that the severity of symptoms had corresponded to the amount of amyloid present.

Coexistence of different amyloid diseases

The coexistence of different amyloid classes in the same patient was noted only in a single case, in which general-

ized senile systemic amyloidosis with symptomatic heart failure was identified by positive immunoreaction with anti-ATTR in 23 of 28 organs and tissues immunostained. Additional deposits of both AA and Aλ immunoreactivity were found at different localizations, as published in detail elsewhere [43].

Discussion

Examination of 43 unselected autopsy cases of systemic amyloidoses enabled us to identify all cases analysed in a retrospective series, with AA amyloid in 21 cases, ATTR in 11, Aλ in 7 and Aκ in 3 cases. Aβ2M amyloid was not found at all, which can be explained by the absence of patients with uraemia treated with long-term haemodialysis [15]. An exact immunohistochemical classification of amyloid type was possible in each of the 43 cases since chemically different and thus clearly identifiable proteins can assume amyloid conformation [2, 16, 18].

Formerly used classifications based on clinical and/or morphological features [20, 40] are less precise [5, 10, 21, 28, 49] and failed to classify the cases in this study precisely. Although there were differences in the patterns of amyloid deposition, they did not allow a reliable distinction to be made between the different types in individual cases. Similarly, some basic diseases correlated significantly with certain types of systemic amyloid in our series and seemed to be causally involved in the amyloid deposition. Other syndromes of the same chemical amyloid type, however, remained without any clear relationship to associated disease or lacked any associations. These morphological and clinical findings are in good agreement with current knowledge.

The potassium permanganate method [50] was the first histochemical differentiation technique and is now considered today less differential and less precise than immunhistochemical techniques [5, 10, 21, 48, 49]. We used the immunoperoxidase technique, which shows less background staining and longer stable reaction products than the immunofluorescence method [11].

Comparison of our series with previous reports of a few comparable examinations [1, 5, 10, 21, 25, 36] indicated some interesting differences in methods and results. The methods used were – in order of increasing sensitivity – the indirect immunoperoxidase (IP) [33], peroxidase-antiperoxidase (PAP) [11, 30], avidin-biotin-peroxidase complex (ABC) [22], and streptavidin-biotin (SP) methods [45].

The varying frequency of AL and ATTR amyloids seen in the different series was striking: the numerical dominance of secondary AA was a common result seen in five out of seven studies. Fujihara et al. [10], van de Kaa et al. [25] and Hoshii et al. [21] did not find any ATTR amyloid at all. The lack of ATTR amyloid was thought to be the result of examining mainly kidney or intestine rather than heart as the major deposition site of senile systemic amyloid [21, 25] or of the unavailability of an appropriate antiserum [10]. Additional reasons for discrepancies in results may come from antigenicity loss during chemical preparation or from the presence of small amounts of ATTR amyloid that were inadequate for detection.

Hoshii et al. [21], who applied antibodies raised against all major types of systemic amyloid by the streptavidin-biotin (SP) method – the most sensitive technique [45] – had 19 cases (14%) they were not able to classify in their large series. Fujihara et al. [10] were unable to classify 23 of their 51 cases (43%), and Chastonay et al. [5], 9 out of 71 (13%). In spite of an occasional strong resemblance between AL and ATTR amyloids in clinical and morphological characteristics [28], the nonclassified cases were assumed to be AL amyloidoses or, less probably, ATTR because of the greater general difficulties with immunohistochemical identification of AL than of ATTR amyloidoses. These difficulties were assumed to be due to varying antigenic specificity of the light-chain variable regions, but this was never confirmed [16, 23]. Moreover, most of the nonclassified cases (16 of 19) in the study of Hoshii et al. [21] were clinically diagnosed primary or myeloma-associated amyloidosis.

Almost all systemic amyloidoses were classified by Baretton et al. [1], van de Kaa et al. [25], Linke et al. [36], and by us despite using different methods: IP [36]; PAP [1, 25]; ABC (this study) but the same antibodies [anti-Aλ(HAR), anti-Aκ(SIN)] directed against amyloid of immunoglobulin light chain origin. The results achieved by us and by Baretton et al. [1] were underlined by the fact that completely unselected cases were examined (in other studies clinical-pathologically diagnosed cases selected randomly were used).

Successful classification of each case is practicable because of the high specificity and sensitivity of the antibodies applied. These were directed against the amyloid fibril proteins and not against the precursor proteins as in the study of Chastonay et al. [5]. Using antibodies against precursor proteins (pooled Bence-Jones proteins) proved to be less sensitive [3]. About 90% of amyloid deposits were classified in three immunofluorescence studies in amyloid-proven, frozen biopsy tissue sections [12, 13, 26], and this comparatively high percentage of successfully classified cases of AL amyloid reactive with anti-light-chain antiserum raised against pooled kappa or lambda Bence-Jones proteins was attributed to the presence of sufficient constant region determinants in the amyloid.

The quality of our antibodies has been demonstrated previously. The use of a large panel of antibodies prepared against amyloid fibril proteins and the selection of the antibodies with the widest specificity finally resulted in reagents [anti-Aλ(HAR), anti-Aκ(SIN)] that were shown to detect virtually all Aλ and Aκ amyloids [1, 4–7, 14, 25, 28, 31, 32, 36–39, 44, 46] regardless of idiotype and λ-chain subgroup. The fact that Aλ amyloids of horse, dog and cat [14, 34, 38, 44] could also be specifically identified with anti-Aλ(HAR) indicates that the an-

tigenic determinants responsible are independent of the respective idiotype. Data from serum immunoelectrophoresis and bone marrow data corresponded with the immunohistochemical results in this study. Similarly, the aminoacid sequence analysis on extracted amyloid fibril proteins confirmed the immunohistological classification in all cases in which such examination was subsequently performed [38, 39].

The basis of the broad reactivity and high sensitivity of the antibodies applied, especially anti-Aλ(HAR) and anti-Aκ(SIN), is still unclear in detail. This reactivity cannot be explained by the presence of the constant region peptides in the amyloid, because parts of the constant region were found only inconstantly in a maximum of about 80–90% of AL amyloid fibril proteins [3, 8]. There must be another antigenic component common to virtually all amyloids of the same immunoglobulin light chain class. Some findings suggest that conformational changes of the tertiary structure arising from the fibrillogenesis of amyloid influence the antigenic characteristics [35]. This "switch of antigenic epitopes" was recently studied in the pathogenesis of amyloid of transthyretin origin [19], and the results indicate that previously hidden antigenic determinants of the precursor protein may become accessible due to amyloidogenesis. This may explain why antibodies raised against the amyloid fibril proteins functioned better than those raised against the precursor proteins.

Immunoglobulins were also found in AA and ATTR amyloids (perhaps as a part of the amyloid, having been trapped amid the amyloid fibrils), and so anti- Aλ(HAR) gave weakly and inconsistently positive reactions in some tissue sections of a few cases with obvious AA or ATTR amyloid. This has also been found in other series [1, 5, 12, 25, 36]. However, this reaction pattern differed qualitatively and quantitatively from the pattern seen in specific immunoreactivity, so that the weak reaction was interpreted as nonspecific. With an essential panel of antibodies raised against various amyloid fibril proteins, the strongest immunohistochemical reaction corresponded to the chemical amyloid type in all cases in this study and in other examinations [32]. Absent immunoreactivity of the antibodies applied was seen in only a few tissue amyloid deposits where the amyloid was either too small in amount for further examination or of cerebrovascular origin. We assumed that the cerebrovascular amyloid was most probably nonsystemic β-amyloid, since there different vascular manifestations of organ-limited amyloid in the brain are described [17, 41].

Immunohistochemistry with a panel of sensitive and specific antibodies directed against various major amyloid fibril proteins is very useful in the differential diagnosis and chemical classification of systemic amyloidoses. This is also true in the case of AL amyloid patients who do not have evidence of an underlying plasma cell dyscrasia and whose amyloidosis is thus difficult to diagnose correctly. The rarity and rapid progression of this disease means that diagnosis is often delayed until multiorgan involvement limits the efficacy of treatment [9]. Whereas missing the diagnosis of secondary AA in the case of a known inflammatory process or senile cardiac amyloidosis is not likely to shorten life, missing the diagnosis of AL or familial amyloidosis will shorten life, since there are effective treatments for both conditions.

Our results suggest that immunohistochemical staining techniques such as were applied in this study are suitable for diagnostic purposes when used in unfrozen, paraffin-embedded tissues. This approach enables a chemical classification of most cases of systemic amyloidoses and may help patients to obtain timely access to therapies for this otherwise fatal disease.

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