

Formal Pathogenesis of Systemic and Localized Amyloidosis

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Received: March 20, 2019; **Published:** April 29, 2019

Abstract

The golden rules of formal pathogenesis of systemic and localized amyloidosis are summarized and documented based on autopsy and biopsy material in one institute.

The patients died or were treated at the National Institute of Rheumatology between 1963 and 1999.

Amyloidosis was diagnosed histologically according to Romhányi by a modified (more sensitive) Congo red staining. Amyloid deposits were identified in serial sections by immunohistochemical and histochemical methods.

The pertinent literature of different types of systemic and localized amyloidosis has been reviewed.

Conclusions: All forms of amyloidosis connected to the circulation are systemic, and all forms of amyloidosis not connected to the circulation are isolated (localized).

The golden rules of formal pathogenesis of systemic and localized amyloidosis syndromes are: amyloidosis is a progressive, cumulative process, involving in its early stage only a few structures in some organs, and increasingly more in the later stages of the disease, the rate and amount of systemic amyloid deposition may be linked to the differences in blood supply per unit volume amyloid protein deposition begins in the organs and tissue structures that are frequently involved and later show marked deposition of amyloid, but where deposits are infrequent or less marked, deposition starts later. Prevalence and severity of amyloid deposition are different aspects of the same phenomenon usually running parallel to each other, and the quantitative differences of amyloid deposits are accompanied by qualitative differences as well.

Keywords: Systemic and Localized Amyloidosis Syndromes; Formal Pathogenesis

Abbreviations

RA: Rheumatoid Arthritis; SLE: Systemic Lupus Erythematosus; SSc: Systemic Sclerosis; PsA: Psoriatic Arthritis; PMR: Polymyalgia Rheumatica; TA: Temporal Arteritis; sAA: Systemic AA Amyloidosis; cAA: Cardiac AA Amyloidosis; rAA: Renal AA Amyloidosis; SAA: Serum Amyloid A; L λ or L κ : Immunoglobulin Light Chain- λ or - κ ; TTRwt: Transthyretin Wild Type; TTRm: Transthyretin Mutant; β 2M: β 2Microglobulin; AL λ : Amyloid Light Chain- λ ; AL κ : Amyloid Light Chain- κ ; ATTRwt: Amyloid Transthyretin Wild Type; ATTRm: Amyloid Transthyretin Mutant; A β 2M: Amyloid β 2 Microglobulin; ACR: American College of Rheumatology; FAP: Familial Amyloid Polyneuropathy; FAM: Familial Mediterranean Fever; HE: Hematoxylin-Eosin Stain; PAS: Periodic Acid Schiff Reaction

Introduction

Amyloidosis syndromes are systemic or localized disorders characterized by the extracellular deposition of chemically heterogeneous fibrillar proteins.

To date 36 distinct precursor proteins have been identified in humans [1]. The precursor proteins can form amyloid filaments (7 - 10 nanometers in diameter) and fibrils (10 - 40 nanometers in diameter) of disparate chemical character. The amyloidosis syndromes are named according to these fibrillar proteins [1]. For example, after amyloid A protein (AA) deposits (precursor: serum amyloid A - SAA, produced by hepatocytes) the disease is named AA amyloidosis (Amyloid A protein amyloidosis - AAa), after amyloid Light chain (AL) deposits (precursor: immunoglobulin light chain- λ or light chain- κ - L λ or L κ , produced by B-cells) is designated as AL amyloidosis (Amyloid Light chain amyloidosis - ALa), after amyloid Heavy chain (AH) deposits (precursor: immunoglobulin heavy chain, produced by B-cells) is specified as AH amyloidosis (Amyloid Heavy chain amyloidosis - AHa) or after amyloid Transthyretin (ATTR) deposits (precursor: altered wild type transthyretin present in natural conditions or genetically determined mutant transthyretin variants) is called ATTRwt or ATTRm amyloidosis (Amyloid Transthyretin wild type or mutant amyloidosis - ATTRwta or ATTRma), after amyloid β 2microglobulin (A β 2M) deposits (precursor: β 2-microglobulin, produced by lymphoid-cells) is assigned (Amyloid β 2M amyloidosis - A β 2Ma), etc.

“Systemic amyloidosis is related to the cardiovascular system and the precursors becomes generalized via the bloodstream, while organ- or tissue-limited isolated amyloidosis is an extravascular phenomenon, the precursors are not directly related to the systemic circulation and the process remains localized” [2-4].

All forms of amyloidosis connected to the circulation are systemic, and all forms of amyloidosis not connected to the circulation are isolated (localized) [2-4]. This early statement was confirmed by Sipe as an important conclusion of XIth International Symposium on Amyloidosis, held in Woods Hole, Massachusetts, USA, at November 5-9, 2006 [5].

Several diseases or disorders may be complicated by systemic or localized deposition of amyloid proteins [1,6] and are subjects of amyloidosis symposia and published in the proceedings of conferences.

The main types of amyloid diseases (with *precursors* in parentheses) are summarized in table 1, including but not limited to all entities.

1.	Systemic (generalized) amyloidosis
1.1	<p>Secondary, reactive (associated with chronic infections or autoimmune diseases) AA amyloidosis (Amyloid A amyloidosis) - (<i>serum amyloid A - SAA</i>) n of autopsy patients with AAa: Rheumatoid arthritis (RA) n=56 (16.0 %) of 350, Systemic lupus erythematosus (SLE) n=2 (3.84 %) of 52, Systemic sclerosis (SSc) n=1 (8.33 %) of 12 [7], Psoriatic arthritis (PsA) n=2 (16.66 %) of 12 [8]</p>
1.2	<p>Primary (associated with myeloma or B-cell dyscrasia) AL amyloidosis (Amyloid Light chain amyloidosis) - (<i>immunoglobulin light chain-λ or light chain-κ - Lλ or Lκ</i>) AH amyloidosis (Amyloid Heavy chain amyloidosis) - (<i>immunoglobulin heavy chain - IgG, IgA, IgM or IgD</i> classes) n of autopsy patients with Ala**: B-cell lymphoma n=1 ALκ, myeloma associated to RA n=1 ALκ (0.62 %) of 161, myeloma associated to SSc n=1 ALλ (8.33 %) of 12 [7]</p>
1.3	<p>Senile (derived from transthyretin of natural conditions) ATTRwt amyloidosis (Amyloid Transthyretin wild type amyloidosis [9]) - (<i>Transthyretin wild type - TTRwt</i>) n of autopsy patients with ATTRwta: n=2</p>
1.4	<p>Hemodialysis-associated Aβ2M amyloidosis (Amyloid β2M amyloidosis) - (<i>β2-microglobulin</i>) n* of surgical specimens (synovial membranes and femoral heads of both side in one patient) with Aβ2Ma: n=1</p>

1.5	<p>Hereditary forms of amyloidosis (genetically determined mutant variants - with or without in familial setting - pertain to flaws or accidental changes in gene sequence)</p> <p style="text-align: center;">n=0</p> <p style="text-align: center;">Familial Mediterranean fever - (FAM) (<i>serum amyloid A - SAA</i>)</p> <p>Familial amyloid polyneuropathy - (FAP) (determined by genomic point mutations leading to <i>Transthyretin mutant variants -TTRm</i> [9])</p> <p>AApoAI amyloidosis (Amyloid Apolipoprotein AI amyloidosis) - (<i>Apolipoprotein A I variants</i>)</p> <p>AApoAII amyloidosis (Amyloid Apolipoprotein AII amyloidosis) - (<i>Apolipoprotein A II variants</i>)</p> <p>AApoCII amyloidosis (Amyloid Apolipoprotein CII amyloidosis) - (<i>Apolipoprotein CII variants</i>)</p> <p>AApoCIV amyloidosis (Amyloid Apolipoprotein CIV amyloidosis) - (<i>Apolipoprotein CIV variants</i>)</p> <p>AGel amyloidosis (Amyloid Gelsolin amyloidosis) - (<i>Gelsolin variants</i>)</p> <p>ALys amyloidosis (Amyloid Lysozyme amyloidosis) - (<i>Lysozyme variants</i>)</p> <p>ACys amyloidosis (Amyloid Cystatin C amyloidosis) - (<i>Cystatin C variants</i>)</p> <p>AFib amyloidosis (Amyloid Fibrinogen amyloidosis) - (<i>Fibrinogen alfa variants</i>), etc.</p>
2.	<p style="text-align: center;">Organ (tissue)-limited (isolated, localized) amyloidosis</p>
	<p style="text-align: center;">Cerebral</p> <p>Aβ protein-related amyloidosis (Amyloid β-protein amyloidosis) - (<i>Aβ protein precursor, wild type or mutant variants</i>) in Alzheimer’s disease (AD)</p> <p style="text-align: center;"><i>Cerebral extravascular amyloidosis - “amyloid plaque”</i></p> <p style="text-align: center;"><i>Cerebral amyloid angiopathy (CAA)</i></p> <p style="text-align: center;">n of autopsy patients with AD: n=12</p> <p style="text-align: center;">n of autopsies with CAA (without clinical symptoms of AD in RA patients): n=2 (3.77 %) of 53</p> <p style="text-align: center;">Non-Aβ protein-related amyloid diseases n = 0</p> <p>APrP amyloidosis (Amyloid Prion Protein amyloidosis: kuru, fatal familial insomnia, Creutzfeldt-Jakob disease, Gerstmann-Sträussler-Scheinker disease, PrP cerebral angiopathy)</p> <p>APro amyloidosis (Amyloid Prolactin amyloidosis: pituitary prolactinomas, aging pituitary (<i>Prolactin - Pro</i>))</p>
2.1	<p style="text-align: center;">Dystrophic amyloidosis (Aging related)</p> <p style="text-align: center;">Localized to articular cartilage - Femoral head (<i>precursor?</i>)</p> <p style="text-align: center;">n of femoral heads with dystrophic amyloid deposits in RA autopsy patients: n=9 (16.98%) of 53</p> <p style="text-align: center;">Localized to articular cartilage - Acetabula (<i>precursor?</i>)</p> <p style="text-align: center;">n of acetabula with dystrophic amyloid deposits in RA autopsy patients: n=6 (14.63%) of 41</p> <p style="text-align: center;">Localized to articular capsule or ligaments (<i>Transthyretin wild type - ATTRwt</i> [1])</p> <p style="text-align: center;">Localized to temporal artery (internal elastic lamina) (<i>elastin?</i>)</p> <p style="text-align: center;">n* of surgical specimens with dystrophic amyloid deposits of temporal arteries in patients with polymyalgia rheumatica (PMR): n=55 (18.39%) of 299 [10]</p> <p style="text-align: center;">AMed amyloidosis localized to aorta (media) (? or <i>Lactadherin</i> [1])</p>

2.2	<p style="text-align: center;">Endocrine related</p> <p>AIAPP amyloidosis (Amyloid Islet Amyloid PolyPeptide amyloidosis) localized to islets of Langerhans (<i>Islet amyloid polypeptide</i> also called <i>amylin</i>) n of autopsy patients with AIAPPa in RA: n=16 (9.75 %) of 164</p> <p>AANF amyloidosis (Amyloid Atrial Natriuretic Factor amyloidosis) or Cardiac - atrial myocyte associated amyloidosis (<i>Atrial natriuretic factor</i>) n* of biopsies of the heart with AANFa: n=2 Localized to parathyroid gland (<i>parathormone prohormone?</i>)</p> <p>ACal amyloidosis (Amyloid Calcitonin amyloidosis) localized to medullary carcinoma of thyroid gland - C-cell thyroid tumors (<i>(Pro)Calcitonin</i>) n* of biopsies of thyroid gland with medullary carcinoma and ACala: n=2</p>
2.3	<p style="text-align: center;">Localized to epithelial tumors (<i>Kerato-epithelin</i>)</p> <p style="text-align: center;">n=0</p> <p style="text-align: center;">Basal cell carcinoma Calcifying epithelioma of Malherbe (<i>Pilomatrixoma</i>) Squamous cell carcinoma Calcifying epithelial odontogenic tumor of Pindborg</p>
2.4	<p style="text-align: center;">Localized amyloidosis caused by concentrated secretion, and/or of inflammatory origin</p> <p style="text-align: center;">Renal retention cysts (concentrated glomerular filtrate) - Aβ2M (<i>β2-microglobulin</i>) n of isolated Aβ2Ma: n=1 (0.63 %) of 159 autopsy patients with RA</p> <p style="text-align: center;">Pulmonary corpora amylacea - Aβ2M (<i>β2-microglobulin</i>) n of pulmonary corpora amylacea (Aβ2Ma): n=16 (4.73 %) of 338 lung specimens from 169 autopsy patients with RA</p> <p style="text-align: center;">Prostatic corpora amylacea - Aβ2M (<i>β2-microglobulin</i>) n of prostatic corpora amylacea (Aβ2Ma): n=6 (54.54 %) of 11 autopsy patients with RA</p> <p style="text-align: center;">Seminal vesicle (<i>concentrated secretion in seminal vesicles</i>) Colloid cyst of thyroid gland (concentrated glandular secretion)</p>
2.5	<p>Isolated AL λ-, or AL κ-chain amyloidosis (Isolated Amyloid Light λ-, or κ-chain amyloidosis) - (<i>immunoglobulin light chain-λ or light chain-κ - Lλ or Lκ</i>) caused by isolated (solitary) plasmocytoma or B-cell dyscrasia n* of biopsies (bucca, gingiva, vocal cord, trachea) with localized ALλ-chain amyloidosis: n=6 or ALκ-chain amyloidosis n=5</p>
2.6	<p style="text-align: center;">Alac amyloidosis (Amyloid Lactoferrin amyloidosis) (<i>Lactoferrin</i> [1]) localized to cornea</p>
2.7	<p style="text-align: center;">Alns amyloidosis (Amyloid Insulin amyloidosis) (<i>Insulin</i>) Iatrogenic, caused by local insulin injection n* of biopsy from abdominal wall with Alnsa: n=1</p>

Table 1: Main types of systemic and localized amyloidosis.

The number of autopsies (n) or biopsies (n*) with amyloid syndromes [6] discussed in this study is incorporated in each category, and their characteristics are summarized in sections "Results" or in "Discussion".

Remarks to table 1

Bold - abbreviations of amyloid fibril proteins with the name of amyloid syndromes are marked in bold

Italics - precursor proteins are indicated in parentheses

"Synonyms and terminology used in the past ...may be confusing" [1]; nonetheless in some cases the historical names of amyloid syndromes were mentioned in Table 1 (secondary, reactive, primary, senile, etc)

n - Number of autopsy patients (autopsies)

n* - Number of surgical specimens (biopsies)

** - The low prevalence of systemic primary ALa has been deceptive in our autopsy population. One recognized reason has been that patients with lymphoproliferative disorders were transferred to an institution specialized in hematology

All the bellow mentioned types of amyloid deposits are eosinophilic, congophilic and birefringent, showing a specific apple green color under polarized light. The “congophilia and birefringence is the gold standard” for diagnosis of amyloid deposits [1]. “There is no difference in the color of polarized light produced by biochemically heterogeneous amyloid deposits” [1], but according to our experience, the intensity of birefringence may be diverse in amyloid deposits side by side, even within the same slide.

The relations of systemic and isolated amyloid deposits to the blood vessels are demonstrate on figures 1.1 and 1.2.

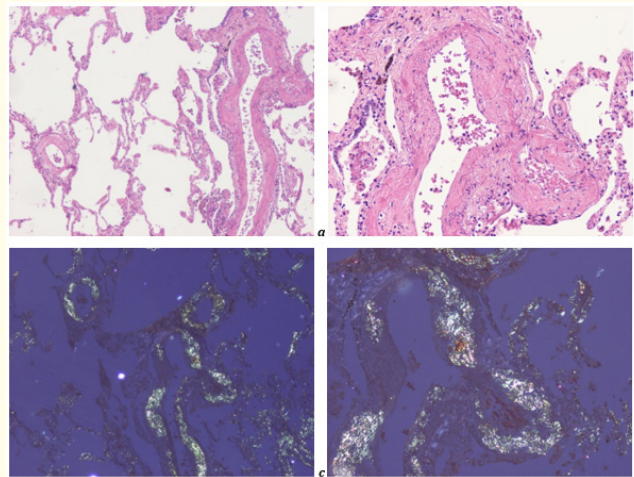


Figure 1.1a-d: Lung, upper lobe, systemic AL-κ amyloidosis; massive amyloid light chain κ deposits in the walls of arterioles and small artery and moderate amounts on interstitial collagen fibers.

All forms of systemic amyloidosis are connected to the circulation, and the vessel walls are always involved in any types of systemic amyloid deposition [2-4,6].

(a) HE, x40; (b) same as (a) HE, x100; (c) same as (a) Congo red staining, without alcoholic differentiation, covered with gum arabic, viewed under polarized light, x40; (d) same as (c) x100.

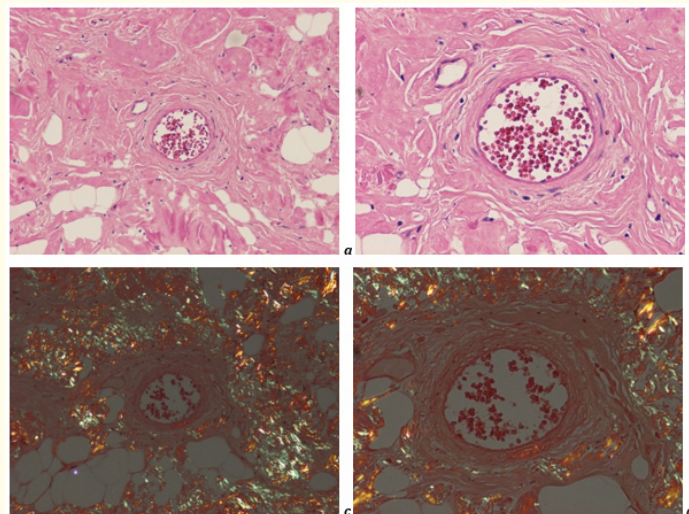


Figure 1.2a-d: Vocal cord, subepithelial localized primary amyloid AL-κ amyloid deposits; the vessel wall of a small artery is spared by AL-κ amyloid deposits within the confluent amyloid masses.

All forms of localized (isolated) amyloidosis are not connected to the circulation, and the vessel walls are always spared in any types of localized amyloid deposition [2- 4,6].

(a) HE, x100; (b) same as (a) x200; (c) same as (a) Congo red staining, without alcoholic differentiation, covered with gum arabic, viewed under polarized light, x100; (d) same as (c) x200.

Aim of the Study

The aim of this study was to summarize the pathogenesis and characteristics of sAA in RA based on previous studies [2,4,6,11-13]. Formal pathogenesis and the basic rules of sAA discussed in section "Results" were supported by selected examples of other types of systemic or localized amyloidosis to prove that these basic rules are of general validity.

Patients and Methods

Autopsy and biopsy material of patients with or without autoimmune disease was investigated for amyloidosis in this study. These patients died or were treated resp., at the National Institute of Rheumatology between 1963 and 1999.

The number of patients (autopsies and biopsies) studied in each category is incorporated in table 1.

Amyloidosis was diagnosed histologically according to Romhányi [14] by a modified (more sensitive) Congo red staining [15]. Amyloid deposits were identified in serial sections by immunohistochemical and histochemical methods [3,11,12].

The prevalence (existence) and severity (extent) of amyloid A deposits in various organs, and in blood vessels of different sizes and tissue structures were determined microscopically.

Selected cases were investigated with a JEM 100CX electron microscope.

RA [16], SLE [17], SSc [18] were confirmed clinically according to the criteria of the American College of Rheumatology (ACR), PsA according to Helliwell and Taylor [19] and PMR according to Bird., *et al* [20].

Glossary of definitions

"Prevalence" concerns the presence of amyloid deposits in blood vessels of different calibers or in different tissue structures of various organs

Size of blood vessels [21] in tissue samples

- Arteriole (a): No internal or external elastic membrane, < 500 micrometers in diameter.
- Small artery (A): Only internal elastic membrane present, vessels 500 - 1000 micrometers in diameter.
- Medium size artery (AA): Internal and external elastic membrane are present - vessel > 1000 micrometers in diameter.
- Venule (v), small vein (V), medium size vein (VV) -accompanying (a), (A) or (AA).
- Interstitial collagen fiber (I).
- Reticulin fiber (collagen IV) (ret).
- Basement membranes of ducts, etc (BM).
- Peripheral Nerves (n).

"Severity" indicates varying amounts of amyloid deposition in different tissue structures. Severity of amyloidosis was evaluated by semi-quantitative visual estimation on a 0 to 3 plus scale (based on the number of involved vessels or tissue structures/light microscopic field x40 lens of Olympus BX51).

Semi-objective score system of "severity":

- "0": No amyloid deposits.
- "1": Sporadic, minimal amyloid deposits in different tissue structures.
- "2": Less than five involved tissue structures.
- "3": Five or more involved tissue structures.

Remark: In case of AA or VV this corresponds to the absolute number of involved medium size vessels of a tissue sample, e.g. “0” none, “1” only one, “2” less than five, “3” 5 or more than five medium size vessels/tissue sample with a x20 objective lens.

Results

The golden rules of amyloid A deposition in RA

Rule 1

Amyloidosis is a progressive, cumulative process, involving in its early stage only a few structures in some organs, and increasingly more in the later stages of the disease [2,4,6,11-13].

This statement is based on quantitative differences of amyloid A deposits in various organs of 34 RA patients complicated by systemic AAa (sAAa) summarized in table 2 and figures 2.1-2.6.

	f/m	Pr n ^o /y	Kidney	Heart	Pancr	Liver	Lung	Avg	Total	Avg %	CoD	Cl+/-
1	f	155/87	0,000	0,000	0,000	0,000	0,083	0,017	0,083	0,553		
2	f	240/88	0,000	0,000	0,100	0,000	0,000	0,020	0,100	0,667		
3	f	76/79	0,250	0,000	*	0,000	0,000	0,063	0,250	2,083		
4	f	243/87	0,000	0,400	0,100	0,000	0,000	0,100	0,500	3,333	cAAa	
5	f	287/91	0,083	0,500	0,400	0,000	0,000	0,197	0,983	6,553	cAAa	
6	f	183/92	0,667	0,000	0,500	0,111	0,000	0,256	1,278	8,520		
7	f	266/78	0,333	0,500	*	0,111	0,167	0,278	1,111	9,258		
8	f	430/80	0,417	0,600	0,200	0,111	0,167	0,299	1,495	9,967	cAAa	
9	f	226/85	0,417	0,400	0,400	0,111	0,167	0,299	1,495	9,967		
10	f	395/76	0,250	1,200	0,000	0,111	0,167	0,346	1,728	11,520	cAAa	
11	f	162/78	0,083	1,100	0,550	0,111	0,000	0,369	1,844	12,293		
12	f	306/90	0,750	0,800	0,400	*	0,000	0,488	1,950	16,250	rAAa-U	Cl+
13	f	45/74	0,000	1,400	0,800	0,444	0,000	0,529	2,644	17,627	cAAa	
14	m	342/86	0,750	1,100	0,300	0,333	0,250	0,547	2,733	18,220	rAAa-U	
15	f	80/80	0,750	0,400	*	0,667	0,417	0,559	2,234	18,617	rAAa-U	Cl+
16	f	52/92	0,667	1,000	0,650	0,000	0,750	0,613	3,067	20,447		
17	f	322/81	0,833	0,700	1,000	0,556	0,167	0,651	3,256	21,707	cAAa	
18	f	203/88	0,667	1,000	0,700	0,167	0,750	0,657	3,284	21,893	rAAa-U	
19	f	39/76	1,500	0,400	1,200	0,667	0,333	0,820	4,100	27,333	rAAa-U	
20	f	90/85	0,917	1,400	0,100	1,778	0,000	0,839	4,195	27,967		
21	f	265/80	1,500	1,400	0,000	0,889	0,417	0,841	4,206	28,040	rAAa-U	Cl+
22	f	245/88	1,000	1,500	1,100	0,556	0,250	0,881	4,406	29,373	cAAa	
23	f	V/T	*	1,300	*	1,111	0,417	0,943	2,828	31,422	rAAa-U	
24	m	232/74	1,833	1,300	0,600	1,000	0,333	1,013	5,066	33,773	rAAa-U	Cl+
25	f	367/75	2,167	0,900	1,400	0,778	0,333	1,116	5,578	37,187	cAAa	
26	m	43/85	1,083	1,000	1,350	1,222	1,167	1,164	5,822	38,813	rAAa-U	
27	f	137/76	1,583	1,400	1,000	*	0,917	1,225	4,900	40,833	rAAa-U	Cl+
28	f	73/87	1,417	1,600	1,300	1,111	0,833	1,252	6,261	41,740	rAAa-U	Cl+

29	f	174/88	1,917	1,000	1,800	0,889	0,917	1,305	6,523	43,487	rAAa-U	
30	f	237/70	2,250	*	*	1,222	0,917	1,463	4,389	48,767	rAAa-U	
31	f	101/90	1,917	2,400	2,000	0,667	0,833	1,563	7,817	52,113	rAAa-U	
32	f	255/83	2,083	1,600	1,650	2,000	0,917	1,650	8,250	55,000	rAAa-U	Cl+
33	m	53/87	2,667	2,300	1,900	1,000	1,167	1,807	9,034	60,227	rAAa-U	Cl+
34	m	181/80	1,917	1,500	2,900	1,556	1,750	1,925	9,623	64,153	rAAa-U	Cl+
Count			33	33	29	32	34	34	34	34	25	9
Sum			32,67	32,10	24,40	19,28	14,59	26,09				
Average			0,990	0,973	0,841	0,602	0,429	0,767				
SD			0,783	0,611	0,724	0,567	0,445	0,533				
0 values n			4	4	3	6	9	0				
+ values n			29	29	26	26	25	34				
Prevalence %			87,88	87,88	89,66	81,25	73,53	100,00				
Severity %			33,00	32,42	28,05	20,08	14,30	25,58				
	Sex	Pr n ⁰ /y	Kidney	Heart	Pancr	Liver	Lung	Avg	Total	Avg %	CoD	Cl+/-

Table 2: Prevalence and extent of amyloid A deposits in 5 organs of 34 RA patients with sAAa arranged according to the increasing values of average amounts of amyloid A deposits/patient (horizontal lines) and amyloid A deposits/organ (vertical columns).

Remarks to table 2

Pr n⁰/y - Protocol number/year

CoD: Cause of death: rAAa-U - Uremia due to massive amyloid A deposition in the kidneys with consecutive renal insufficiency (n=17), cAAa - lethal outcome exclusively caused by cardiac amyloidosis (n=3);
cAAa - cardiac amyloidosis contributed to the death in further 5 Cases

Cl+: Clinically recognized; Cl-: Clinically not recognized; f: female, m: male; Avg – Average; SD - Standard Deviation;
* - tissue blocks were not available

Legend to table 2

In 5 (14.71 %) of 34 RA patients with sAAa the amyloid A deposits were minimal or moderate (less than 0.2), representing an early stage of systemic amyloidosis.

The progress of the disease was characterized in 12 (35.29 %) patients with amyloid A deposits between 0.256 - 0.651, and in 12 (35.29 %) patients between 0.657 - 1.305; these represent advanced and late stages of sAAa (70.58 %).

The terminal (end) stage amyloid A deposition progressed rapidly and the amount of amyloid A deposits in 5 (14.71 %) patients was between 1.463 - 1.925.

The increment in early, advanced and late stages sAAa showed basically linear growth, representing nearly the same rate and equal speed of amyloid A deposition in various organs. In the terminal end stage amyloid A deposition progressed rapidly and the growth curve showed an exponential increment (Table 2 and Figures 2.1 - 2.3).

The distribution of RA patients with sAAa arranged according to mean "severity" ("average amounts" of amyloid A deposits/patient") in early, advanced, late and terminal stages showed the curve of Gauss (Figure 2.1).

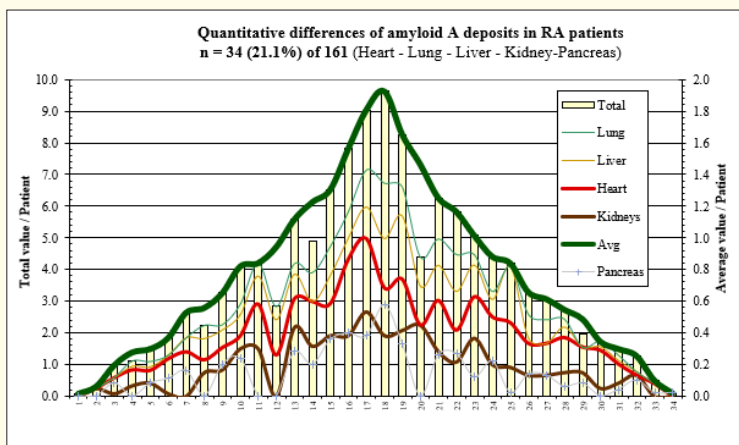


Figure 2.1: Distribution of RA patients in early, advanced, late and terminal stages of sAA at death.

Remarks to figure 2.1

In 5 patients the “total value” of deposited amyloid A is lower compared to the “average value”, because 1 tissue sample of kidney (VT/85), 1 of heart (237/70), 5 of pancreas (76/79, 266/78, 80/80, V/T and 237/70), and 2 of liver (137/76 and 306/90) of 34 RA patients with sAA were not available for evaluation (consequently the “total value” of severity in these patients is lower).

Distribution of lethal cases of 34 RA patients with sAA (Table 2), according to increasing values of amyloid A deposits (“average amount of amyloid A deposits/patient”) is demonstrated in figures 2.2 and 2.3.

The relationship of severity, mortality, and clinical recognition of amyloidosis in 34 RA patients with sAA is demonstrated in figure 2.3.

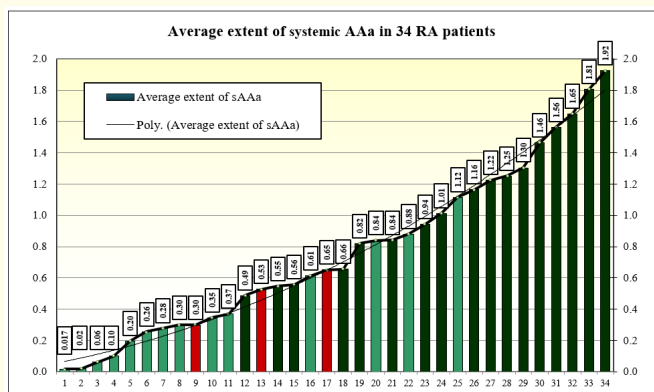


Figure 2.2: Distribution of lethal cases of 34 RA patients with AAa according to increasing “severity” (“average amount of amyloid A deposits/patient”).

Legend to figure 2.2

IRA patients with sAAa at death - according to increasing “severity” (“average amount of amyloid A deposits/patient”) - shows basically a linear growth curve with an exponential increment in the end stage.

Seventeen (50.0%) of 34 patients with AAa - (‘dark green’) - died of renal insufficiency and uremia caused by massive amyloid A deposition of the kidney, mostly in late and terminal stages of AAa, 3 (8.82 %) patients - (‘dark red’) - died of circulatory failure caused by AAa, and 14 (41.18 %) of 34 patients with AAa died of other causes such as peritonitis, septic infection, etc (Table 2).

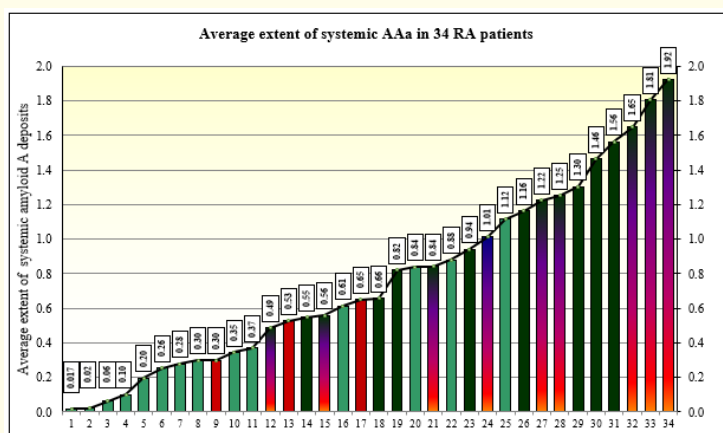


Figure 2.3: Relationship between severity, mortality and clinical recognition of sAAa according to increasing “severity” (“average amount of amyloid A deposits/patient”).

Legend to figure 2.3

sAAa was clinically diagnosed in 9 (26.47%) - (‘red-blue’) - of 17 patients mostly in late and end stages of amyloidosis with massive renal amyloid A deposits and lethal outcome- (‘dark green’) - and was missed in 25 (75.53%) of 34 patients.

cAAa - (‘dark red’) - or its pathogenic role in mortality was not recognized clinically.

The amount of amyloid A deposits in various organs increased gradually and showed basically a linear growth curve in early, advanced and late stages of amyloidosis. At the end stage of sAAa the mean amount of amyloid A deposits showed an increased accumulation; amyloid A deposition progressed rapidly and the growth curve showed an exponential increment (Table 2 and Figure 2.4).

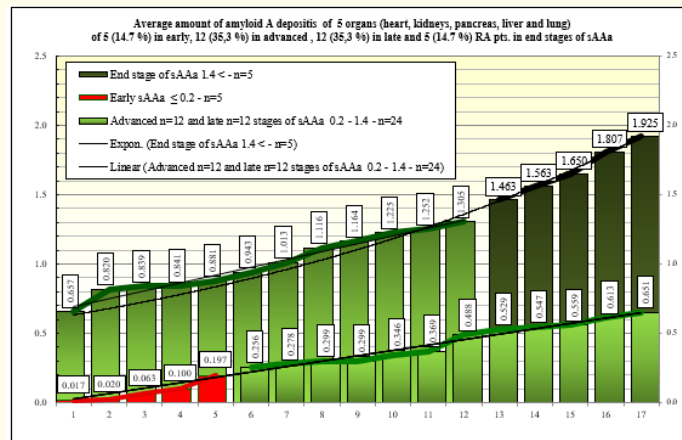


Figure 2.4: Mean amount of amyloid A of 5 organs in early, advanced, late and end stages of sAAa according to increasing "severity" ("average amount of amyloid A deposits/patient").

Legend to figure 2.4

Distribution of sAAa in 34 RA patients with early, advanced, late and end stages.

The increment in early, advanced and late stages of sAAa showed a basically linear growth curve, representing nearly the same rate of amyloid A deposition.

In the terminal end stage amyloid A deposition progressed rapidly and the growth curve showed an exponential increment.

The speed of amyloid deposition in early, advanced and late stage of amyloidosis (with minimal, moderate or severe amyloid deposits) was the same; amyloid deposition progressed rapidly only in terminal (end) stage of the disease.

The prevalence and mean amount of amyloid A deposits run basically parallel in various organs of RA patients complicated by sAAa. The frequently involved organs showed marked deposits of amyloid A. Deposits were less striking in less frequently involved organs (Table 2 and Figure 2.5).

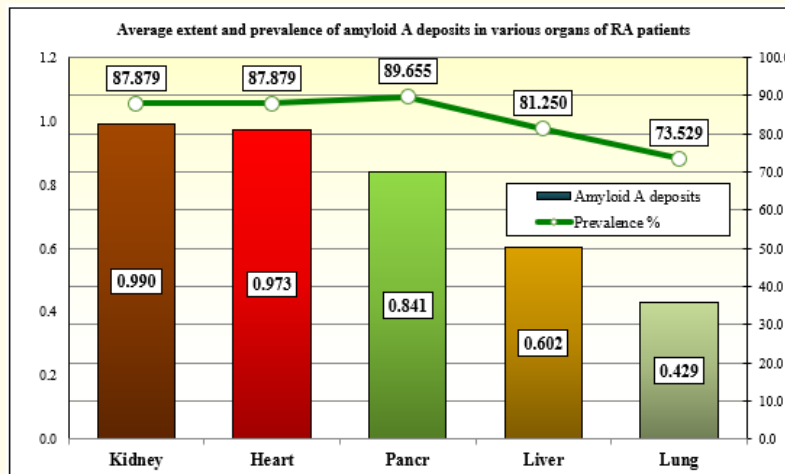


Figure 2.5: Prevalence and extent of amyloid A deposits in various organs (kidney, heart, pancreas, liver and lung) with sAAa.

Legend to figure 2.5

The prevalence and amount of amyloid A deposits run basically parallel in various organs of RA patients with sAAa.

The amount of amyloid A deposits was different in various organs and increased simultaneously, but the proportion of deposited amyloid A was nearly constant and independent of the stage of amyloidosis.

The quantitative differences of amyloid A deposits in various organs (kidneys, heart, pancreas, liver and lungs) of 34 RA patients with sAAa are demonstrated in figure 2.6.

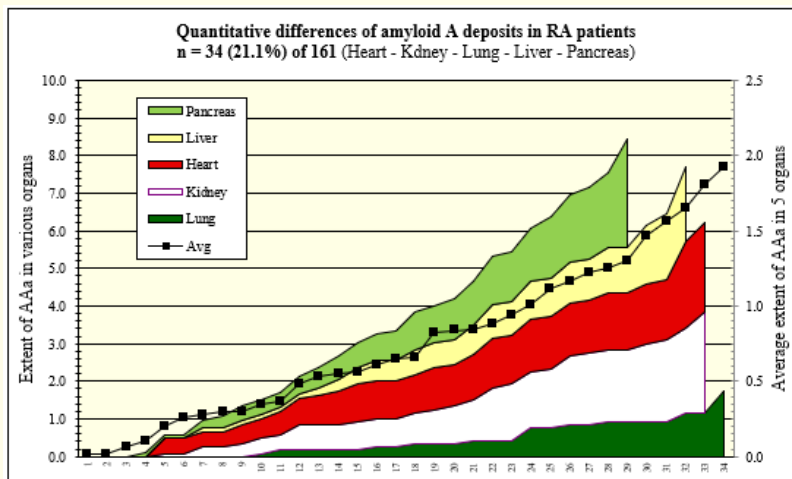


Figure 2.6: Severity and chronology of amyloid A deposition in various organs of 34 RA patients with sAA arranged according to increasing “severity”/organ.

Legend to figure 2.6

The linear, progressive and cumulative character of amyloid A deposition is demonstrated in the kidneys, heart, liver, pancreas and lungs in 34 RA patients with sAA. The amount of deposited amyloid A protein occurred basically parallel in each organ (except the end stage of amyloidosis), but amyloid A deposition did not start at the same time. The proportion of deposited amyloid A was essentially constant in all organs and independent of the stage of amyloidosis.

Figures 2.7 and 2.8 demonstrate early and advanced stages of cAAa, figures 2.9 and 2.10 early and advanced stages of rAAa. Original magnifications correspond to the 24 x 36 mm transparency slide - the correct height: width ratio is 2:3.

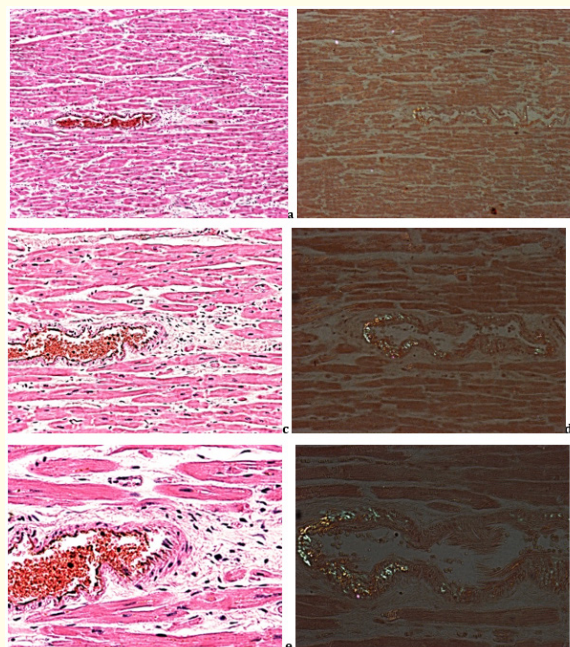


Figure 2.7a-f: Heart, intramural region of myocardium, early stage of cAAa.

Detectable deposits of Amyloid A are present only in the wall of arterioles.

- (a) HE, x40; (b) same field as (a) stained with Congo red according to Romhányi without alcoholic differentiation, covered with gum Arabic, and viewed under polarized light, x40; (c) Same field as (a) HE, x100
- (d) Same field as (b), x100; (e) Same field as (a) HE, x200; (f) Same field as (b), x200.

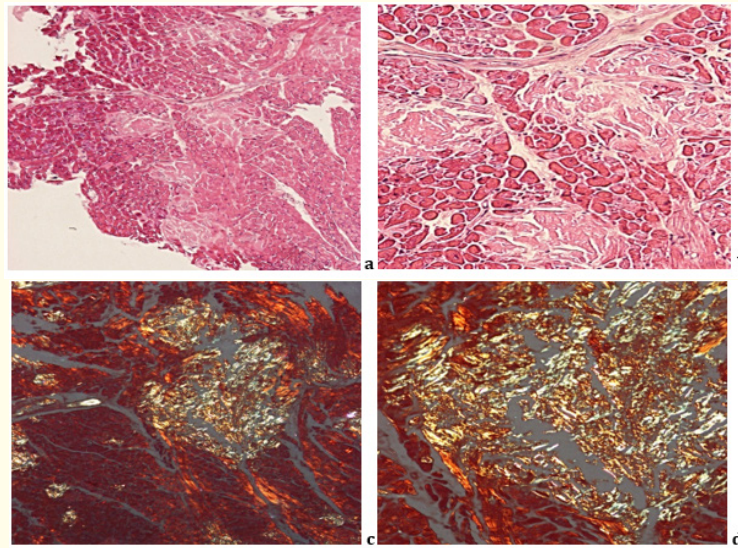


Figure 2.8a-d: RA, heart, intramural region of myocardium, advanced stage of cAAa.

Amyloid A deposits are within the wall of arterioles, intramural interstitial collagen fibers and myocardiocytes.

(a) HE, x40; (b) Same field as (a) HE, x100; (c) Same field as (a) stained with Congo red according to Romhányi without alcoholic differentiation, covered with gum Arabic, and viewed under polarized light, x40; (d) Same field as (c) x100.

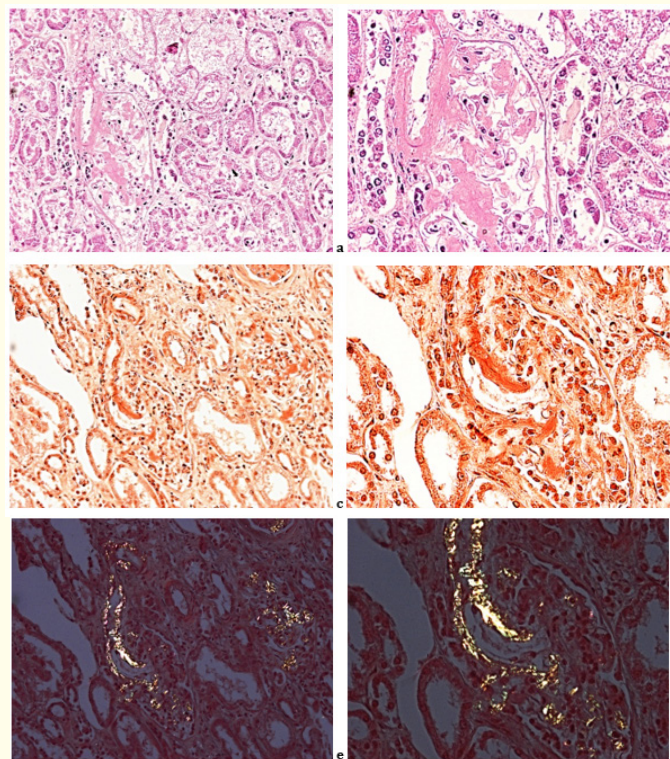


Figure 2.9a-f: RA, Kidney, early stage of rAAa.

Pronounced amyloid A deposits are present in the wall of preglomerular arterioles, with minimal involvement of glomeruli.

(a) HE, x100; (b) Same field as (a) HE, x200; (c) Same field as (a) stained with Congo red according to Romhányi, x100; (d) Same field as (c) x200; (e) Same field as (a) stained with Congo red, without alcoholic differentiation, covered with gum arabic, and viewed under polarized light, x100; (f) Same field as (e) x200.

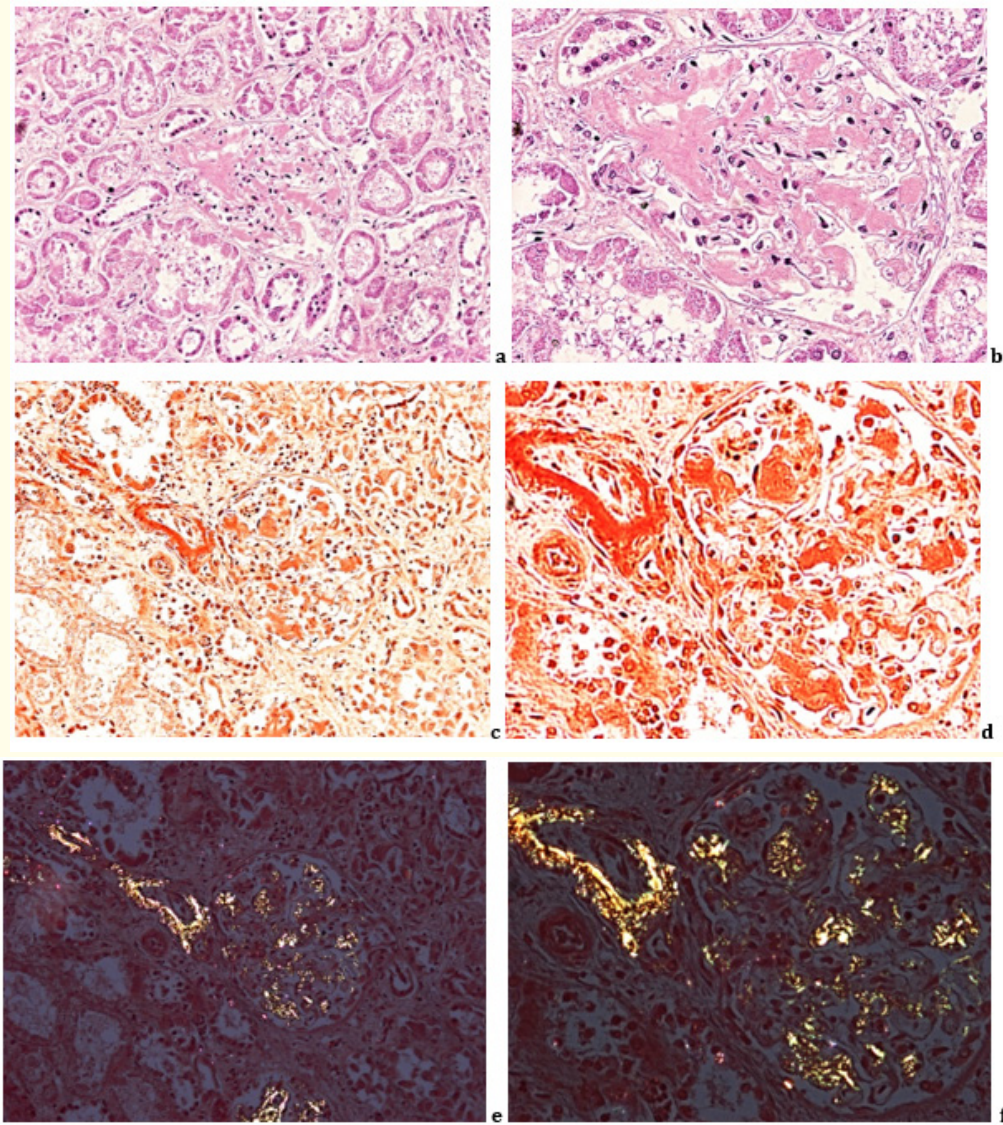


Figure 10a-f: RA, Kidney, a more advanced stage of rAAa in comparison with figures 2.9a-f.

Massive Amyloid A deposits are present in the wall of preglomerular arterioles, with more pronounced involvement of glomeruli in comparison with figures 2.9a-f.

(a) HE, x100; (b) Same field as (a) HE, x200; (c) Same field as (a) stained with Congo red, according to Romhányi, x100; (d) Same field as (c), x200; (e) Same field as (a) stained with Congo red, according to Romhányi, without alcoholic differentiation, covered with gum arabic, and viewed under polarized light, x100; (f) Same field as (e), x200.

The printed size may be different, therefore it is necessary to indicate the original magnifications corresponding to a fixed size (in case of transparency slides this is the 24 x 36 mm analogue positive).

Rule 2

During the progression of AAA, amyloid A protein deposition begins in the organs and tissue structures that are frequently involved and later show marked deposition of amyloid. Where deposits are infrequent or less marked, deposition starts later [4,6,13].

Amyloid A deposition starts at the most common sites (the most frequently involved tissue structures) in the most frequently involved organs [4,6,13]. Amyloid A deposition starts in the wall of blood vessels of the gastrointestinal tract [4,6,13].

2a

The most common sites of amyloid A deposition were the wall of blood vessels (capillaries, arterioles, small arteries), collagen fibers, glomeruli and myocardiocytes followed by the basement lamina, and reticulin fibers (collagen IV in fatty tissue). Deposition of amyloid in the wall of medium-sized blood vessels began later. The involvement of venules, small and medium size veins overlapped but appeared to be increased in the presence of stasis. Deposition of amyloid in peripheral nerves was a late phenomenon (Table 3 and figure 3.1) tract [4,6,13].

Quantitative differences (prevalence and extent) of amyloid A deposits in various tissue structures of 6 organs (kidney, heart, pancreas, liver, lungs and brain) with sAA are summarized in table 3 and figure 3.1.

Tissue structures n = 187*	Prevalence in%	(+) cases of 187	Extent of AA in%	
Arteriole	68,984	128 of 187	1,548	51,60
Small artery	57,219	107 of 187	1,134	37,80
Collagen fiber	49,733	93 of 187	0,840	28,00
Glomerulus	48,485	16 of 33	1,212	40,40
Myocardiocytes	39,394	13 of 33	0,515	17,17
Reticulin fiber	35,829	67 of 187	0,647	21,57
Basal lamina**	34,884	45 of 129	0,612	20,40
Small vein	32,086	60 of 187	0,487	16,23
Medium size artery	30,481	53 of 187	0,457	15,23
Medium size vein	28,342	57 of 187	0,489	16,30
Venule	25,134	47 of 187	0,369	12,30
Nerves	5,882	11 of 187	0,102	3,40

Table 3: Quantitative differences (prevalence and extent) of amyloid A deposits in various tissue structures of 6 organs (kidney, heart, pancreas, liver lungs, and brain) with sAA according to decreasing “prevalence”/structure.

Remarks to table 3

*- Some tissue samples [one of kidney, one of heart, five of pancreas, two of liver and eight of brain] of 34 RA patients with sAA amyloidosis were not available for evaluation (n: 6 x 34 = 204 - 17 = 187).

“Prevalence in %” means the positive cases of 187 examined samples

**-. The basal lamina was evaluated in the kidneys (basal lamina of collecting ducts and basal lamina of cortical convoluted tubules, in the lungs (peribronchial and peribronchiolar basal lamina) and in the pancreas (periductal basal lamina).

“Extent in %” means the average amount of deposited amyloid A protein in percents of maximum 3 value.

The extent of deposited amyloid was evaluated on a 0 - 3 scale.

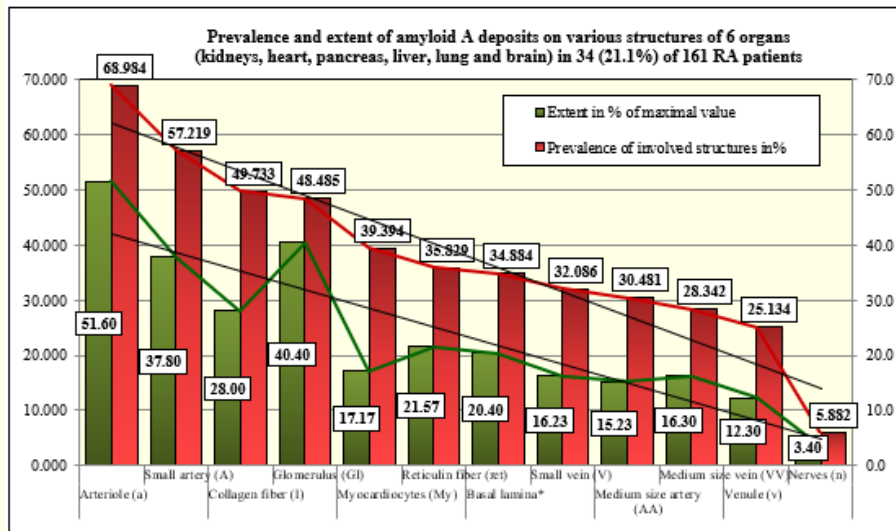


Figure 3.1: The prevalence and amount of amyloid A deposits run basically parallel in blood vessels and in different tissue structures of various organs with sAA. Frequently involved tissue structures showed marked deposits of amyloid. Deposits were less marked in less frequently involved tissue structures at death.

2b

The deposition of amyloid A began in the gastrointestinal tract followed by that in the kidneys, heart, spleen, adrenal glands and liver. The pancreas, lungs, thyroid, aorta, muscle, synovial membranes, lymph nodes, peripheral nerves, bones and skin were less frequently involved. Amyloid A deposits were not found in the brain (other intracranial tissues like the pituitary gland and meninges may be involved) (Table 4 and figure 4.1) [4,6].

The quantitative differences of amyloid A deposits in 17 organs of 34 RA patients complicated by sAA are summarized in table 4 and figure 4.1.

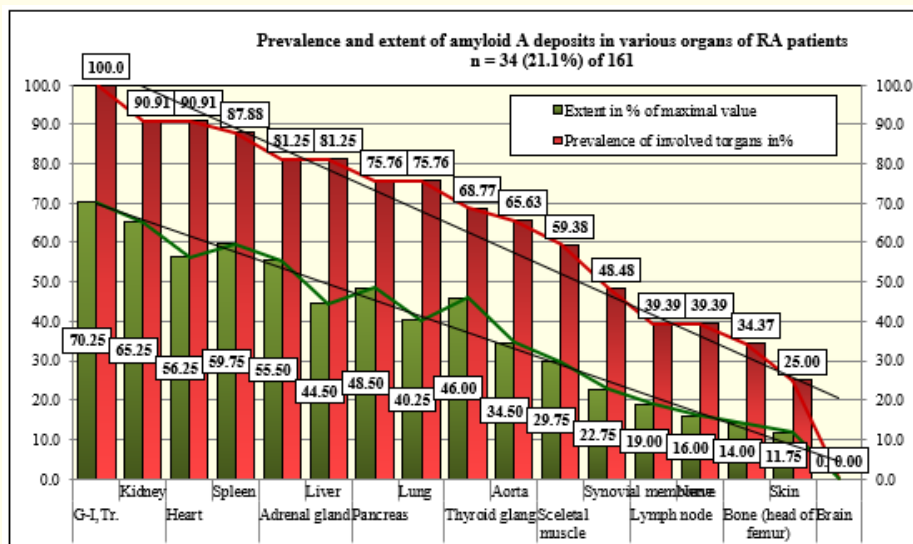


Figure 4.1: The prevalence and amount of amyloid A deposits run basically parallel in various organs of RA patients with sAA. Frequently involved organs show marked deposits of amyloid. Deposits were less marked in less frequently involved tissue structures and organs at death.

Organ	(+ cases N of samples*	Prevalence %	Extent of AAa	
			Average**	%
Gastrointestinal tract	32 of 32	100%	2.81	70.3
Kidney	30 of 33	90.91%	2.61	65.3
Heart	30 of 33	90.91%	2.25	56.3
Spleen	29 of 33	87.88%	2.39	59.8
Adrenal gland	26 of 32	81.25%	2.22	55.5
Liver	26 of 32	81.25%	1.78	44.5
Pancreas	25 of 33	75.75%	1.94	48.5
Lungs	25 of 33	75.75%	1.61	40.3
Thyroid gland	22 of 32	68.77%	1.84	46.0
Aorta	21 of 32	65.63%	1.38	34.5
Skeletal muscle	19 of 32	59.38%	1.19	29.8
Synovial membrane	16 of 33	48.48%	0.91	22.8
Lymph node	13 of 33	39.39%	0.76	19.0
Nerve	13 of 33	39.39%	0.64	16.0
Bone (head of femur)	11 of 32	34.37%	0.56	14.0
Skin	8 of 32	25.00%	0.47	11.8
Brain	0 of 33	0.0%	0.00	0.0

Table 4: Quantitative differences (prevalence and extent) of amyloid A deposits in different organs [5,6].

Remarks to table 4

*: Some tissue blocks were not available for evaluation.

** : In some of earlier studies the extent of amyloid A deposits was evaluated on a 0 to 4 pluss scale; “severity: 1-3” was defined as described in “glossary” (se above), and “4” indicated a subjective impression in case of diffuse, massive, general involvement of the organs [2,13].

Rule 3

Prevalence and severity of amyloid A deposition are different aspects of the same phenomenon usually running parallel to each other in different organs, in different blood vessels and in various tissue structures (Tables 2-4 and figures 2.5, 3.1 and 4.1) [4,6,13].

Rule 4

The rate (prevalence) and amount (quantity) of amyloid A deposition are characterized (accompanied) by qualitative differences of deposited amyloid [4,6,11,12].

Rule 5

The early stage of amyloid deposition is characterized by minimal, loose, more “soluble” amyloid deposits while the later stages are characterized by massive, dense, less “soluble” amyloid deposits.

The histochemical differences between recent and older deposits point to the age-dependent quality of deposited amyloid (Figures 5.1 and 5.2).

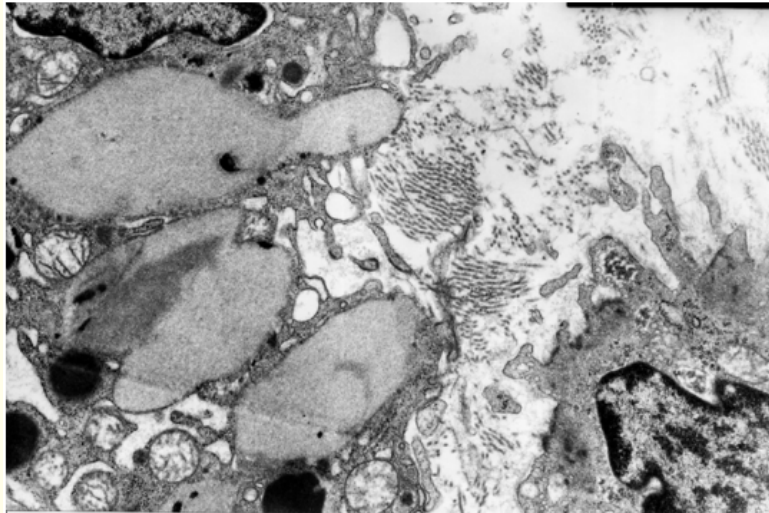


Figure 5.1: Rheumatoid arthritis, colon, submucosa.

Amyloid A lakes are surrounded by or incorporated into macrophages.

Fine filamentous structure of "fresh, loose" amyloid deposits alternating with condensed, tortuously winding "old, dense" fragmented ones.

Electron micrograph, original (O) magnification corresponds to the 60x90mm negative: x6600.

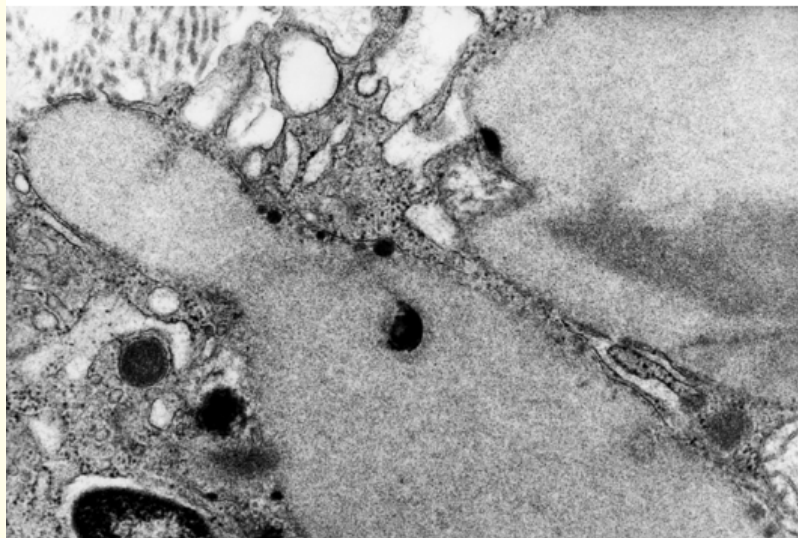


Figure 5.2: Rheumatoid arthritis, colon, submucosa.

Electron micrograph, same area as figure 2, O: x16000.

Resolution of recent, loose amyloid deposits was more likely during the early stages of deposition (Figures 5.3 and 5.4) [4,6,11,12].

The ultrastructural characteristics of “fresh, loose” and of “old, dense” amyloid A protein deposits are demonstrated in figures 5.1 and 5.2. These observations confirm the histochemical differences between recent and older deposits (Figure 5.3).

Amyloidosis is a stage-dependent phenomenon. Amyloid A deposition starts earlier in the wall of arterioles (amyloid is older, more dense and resistant), and begins later in the wall of small arteries, etc. (amyloid deposits are relatively younger, loose and sensitive). The fresh amyloid A deposits in the wall of small arteries are looser and more soluble (sensitive) than the old ones in arterioles. The histochemical characteristics of “fresh, loose” and of “old, dense” amyloid A protein deposits are demonstrated in figure 5.3.

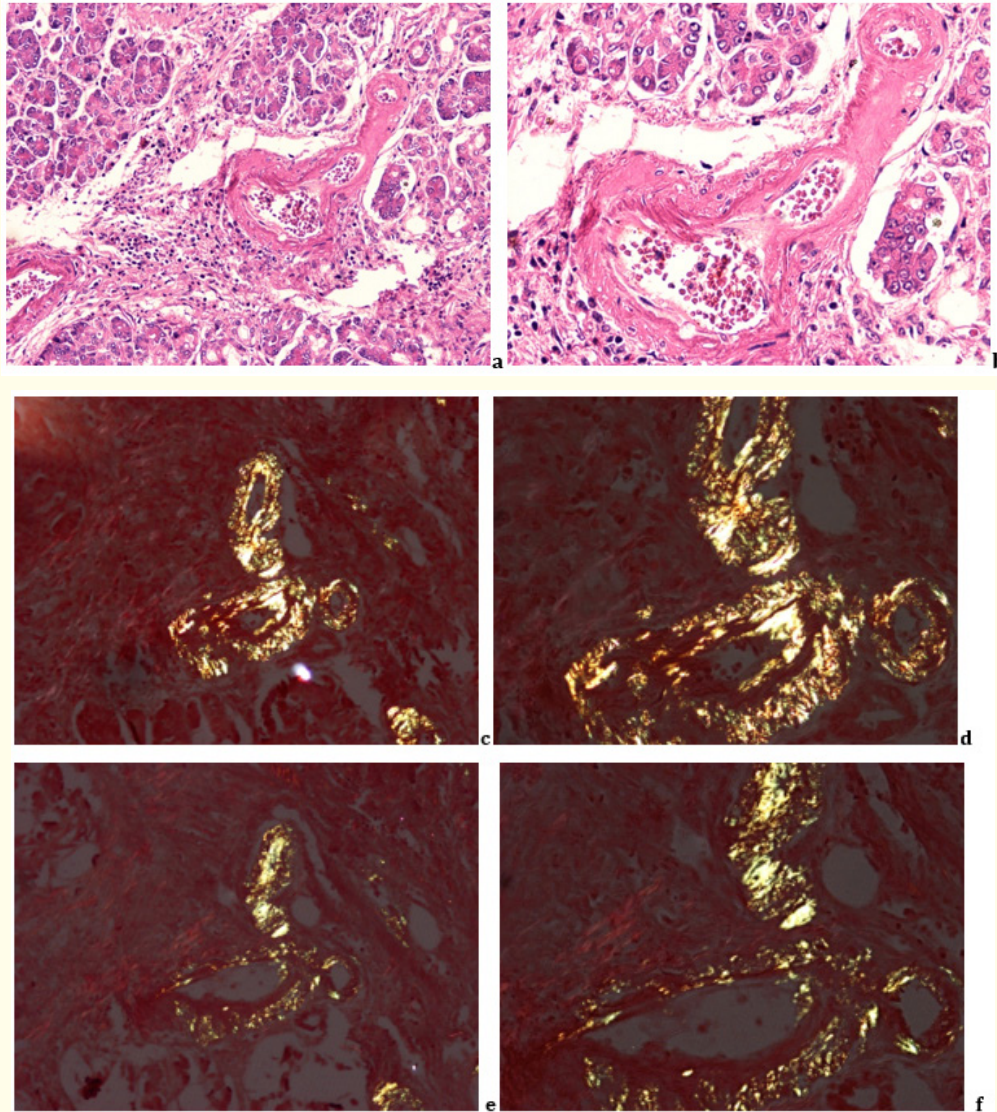


Figure 5.3a-f: Rheumatoid arthritis, pancreas, amyloid A deposits in the wall of arterioles and a small artery.

Amyloid A deposition starts earlier in the wall of arterioles and the small arteries become involved later. The old amyloid A deposits in the wall of arteriole are denser (resistant), and the fresh amyloid A deposits in the wall of small artery are looser (sensitive), and more soluble.

(a) HE, x 100; (b) Same field as (a) x200; (c) Same field as (a), stained with Congo red, according to Romhányi after KMnO_4 oxidation for 30 sec, x100; (d) Same field as (c) x200; (e) Same field as (a), stained with Congo red, according to Romhányi after KMnO_4 oxidation for 3 min, x100; (f) Same field as (e) x200.

Discussion

In the early 1940's amyloid was regarded to be a homogeneous substance, based on its light microscopic appearance in haematoxylin-eosin stained sections. Amyloid stained with Congo red exhibited strong birefringence of apple green color viewed under polarized light. From this observation Romhányi (1949) concluded that amyloid should have a specially oriented ultrastructure in which the amyloid subunits are oriented along their long axis [22]. This hypothesis was later confirmed by Alan S Cohen (1959) who introduced electron microscopy into the research of amyloidosis and demonstrated the fibrillar ultrastructure of amyloid [23]. The own birefringence of oriented amyloid fibrils is weak and not detectable. The bipolar Congo red molecules are oriented parallel to the surface of the amyloid filaments or fibrils, resulting an additive and linear (axis parallel) positive birefringence viewed under polarized light. The intensive additive linear positive birefringence of apple green color is specific for amyloid filaments [15,24].

In agreement with Cohen (1968) who declared that "there are no laboratory abnormalities specific to or unique for amyloid" furthermore, "there is no one finding in the blood, urine, electrocardiogram or x-ray that is specific for amyloidosis", "the diagnosis should be based upon a biopsy using an „appropriate staining procedure" [25] and so "congophilia and birefringence remain the gold standard" for the diagnosis of amyloid deposits [1]. Using a less sensitive staining method some positive cases with minimal deposits remain undetected. A more specific method potentially detects more cases and reveals earlier stages. Amyloidosis in most studies was diagnosed with different methods (Toluidine blue, Crystal violet, Syrius red, Congo red staining according to Romhányi, Bennhold's, Puchtler's, Bély's Congo red method, etc.) of diverse specificities and sensitivities, therefore it is difficult to estimate the real prevalence of amyloidosis syndromes. Congo red staining according to Bennhold [26] or Puchtler is widely used [27]. Congo red staining according to Romhányi is less well known and less commonly used in the English language literature [14,28], but in our experience, with a small modification this is the best one [15].

All forms of systemic amyloidosis are related to the cardiovascular system and become generalized via the bloodstream, while organ- or tissue-limited isolated amyloidosis are not directly related to the systemic circulation and remain a localized process [2-4,6].

"Systemic" and "localized" notions should be interpreted in their dynamics; all systemic amyloid deposition starts somewhere and sometime, and in the early stages of disease it is limited to a few structure in some organs only, in fact (literally) "localized". The precise (exact, accurate) interpretation of the term "systemic" is that the amyloid deposition is only „potentially systemic".

The notion of localized amyloid deposition may also be relative. Isolated B-cell dyscrasia causes localized amyloid deposition. In some cases, because of progression of the disease, the originally localized AL depositions affect the blood vessels and the process becomes "regional" or "widespread", e.g. more or less "systemic" [6]. This is indirect evidence that a proliferative process has acquired features of a malignancy.

The precursor polypeptides (SAA, L or L κ , TTRwt, TTRm, β 2M) of systemic amyloidosis spread via the bloodstream and are deposited throughout the body. The level of precursors in the blood depends on the production and/or elimination or, more succinctly, on the dynamics of these two processes (production-filtration-reabsorption and catabolism of precursor polypeptides may be different).

The rate and amount of systemic amyloid deposits existing in blood vessels and different tissue structures of various organs may be linked to the differences in blood supply per unit volume. Many factors may modify the rate and amount of amyloid deposition [2,13].

Organs directly supplied by precursors show more pronounced amyloid deposition than organs, which receive them indirectly - like lymph nodes or liver, filtered by gastrointestinal tract.

Amyloid deposition in the lungs is also influenced by motion of the organs: amyloid deposition starts in the less mobile peripheral and peribronchiolar areas of the lobules - like sludge in standing water, and the more mobile centrilobular areas are involved later.

The more massive deposition in the basal lamina of collecting ducts in the papillary (less oxygenated) region in contrast to the periglomerular cortical areas of the kidneys suggest the possible role of hypoxemia.

In the heart the role of relative hypoxemia is supported by the fact that amyloid A deposits are present earlier and are more prominent in the relatively less oxygenated subepicardial regions of the myocardium in comparison with its deeper, more supplied central portions.

Renewable structures, like germinal centers of lymph follicles or zona glomerulosa of the adrenal gland or newly formed tissue structures (like periurethral glands of hyperplastic prostate) are showing lesser amounts of amyloid deposition in comparison with the mantle zone of lymph follicles and deeper cortical zones of the adrenal gland, or normal (preexisting) prostatic tissue.

Barriers may block amyloid A deposition, for example in the brain (but not in the pituitary and meninges); this is characteristic for all types of systemic amyloidosis.

Incidental elimination of deposited amyloid (AA, AL λ , AL κ or A β 2M) by macrophages seems possible [6].

By electron microscope the macrophages and giant cells (histiocytes and multinucleated histiocytes) showed different phagocytic activity indicated by incorporated amyloid masses bordered by membranes. Cellular reaction around the amyloid A (AA) deposits is usually absent or minimal in comparison to AL deposition. AL λ deposits are accompanied by more prominent cellular reaction of macrophages, giant cells and fibroblasts than the AL κ deposits which are surrounded by moderate cellular reaction only. A β 2M deposits looks like the most degradable amyloid deposits accompanied with extreme intensity of cellular reaction [6].

Amyloid deposition in the wall of arteries (arterioles, small and medium size arteries) correlated consequently with interstitial deposition of amyloid A on collagen fibres ($c=0.2113 \chi^2=81.8970$)¹ and with follicular type of amyloidosis of the spleen ($c=0.1793 \chi^2=3.1975$)* in different cohorts of RA patients [2,13]. Amyloid deposition in the wall of veins (medium size, small veins and venules) correlated with deposition of amyloid A on reticulin fibers (Collagen IV) of fat tissue ($c=0.4001 \chi^2=166.1351$)*, and with diffuse type of amyloidosis of the spleen ($c=0.70586 \chi^2=17.0563$)*, which underline the role of venous stasis in development of amyloidosis [2,13].

The “progressive cumulative tendency” of amyloid deposition (Rule 1) is a characteristic of all forms of systemic amyloidosis; in the early stage of amyloidosis syndromes only a few structures are involved in some organs, and later on increasingly more structures are involved in most organs.

Deposition of precursors starts “at the most common sites” “in the most frequently involved organ” (Rule 2) is also a true basic principle, but regarding the starting point of amyloid deposition there are considerable differences between distinct entities of systemic amyloidosis.

The starting points of amyloid deposition may be influenced by qualitative differences of precursors, and by the special affinities of precursors to different tissue structures [7,29]. In our previous study the skin of SSc patients was involved more frequently by AL λ deposition, than by AA deposition; the differences between prevalence of AL λ and AA deposition ($p < 0.0001$), furthermore between the amounts of deposited AL λ and AA fibril proteins ($p < 0.0002$) were significant [7]. In SSc patients the high prevalence and extent of AL λ deposits on reticulin (collagen IV) fibers in contrast to AA deposition is explained by the qualitative differences of immunoglobulin L λ and SAA precursors as well [29].

Qualitative changes of collagen fibers may also have a role. Qualitative changes of collagen fibers have been proven in the skin, endocardium, fascia and bladder of SSc patients in comparison to the normal population; the concentrations of collagen cross-link pyridinoline have been elevated in all cases, especially in the skin [30,31].

¹The data correspond to 37 of 215 RA patients complicated by AAa [13]

The amyloid deposition of the same type starts on different tissue structures of different organs in distinct basic diseases [8,32].

According to our data the skin of PsA patients was involved more frequently by AA deposition (50.0%), than the skin of RA patients (10.83%); the prevalence ($p < 0.0000026$), and amount of amyloid A deposits ($p < 0.00027$) was significantly higher in the skin of PsA, than in the skin of RA patients [8]. The average prevalence and the total amounts of amyloid A deposits on reticulin (collagen IV) fibers in the same organs (kidneys, heart, liver, lung, and skin) of PsA and RA patients showed a similar tendency, but these differences were not significant (average prevalence of 5 organs: 41.67% versus 30.39%; $p < 0,365$ - NS and average amount of 5 organs: 0.83 versus 0.56; $p < 0,214$ - NS) [32]. Possible qualitative changes of reticulin fibers cannot be ruled out in PsA, like in SSc [30,31].

The rate and amount of amyloid deposition are different aspects of the same phenomenon usually running parallel to each other in blood vessels and in different tissue structures of various organs (Rule 3), which is a feature of all forms of systemic amyloidosis.

The qualitative differences of fresh (recent) and old amyloid deposits (Rule 4) is a rule of formal pathogenesis of systemic amyloidosis; the amyloidosis syndromes are characterized by minimal, loose, more "soluble" amyloid deposits in early stage of amyloid deposition while massive, dense, less "soluble" amyloid deposits are seen in advanced, late and terminal stages of amyloid deposition (Rule 5). The qualitative differences of fresh (recent) and old amyloid deposits have been proved by long series of electron microscopic and histochemical studies [4,6,11,12].

The precursor polypeptide (serum amyloid A - SAA) of amyloid A (AA) protein fibrils are regarded now as one of the most sensitive indicators for assessing inflammatory activity [33]. SAA proteins are produced predominantly by the liver [34]. SAA is involved in several chronic inflammatory diseases, such as RA, AAa, atherosclerosis, etc. [35]. Prolonged elevation of SAA is not specific for AAa, and does not necessarily indicate tissue deposition of amyloid A [36].

Systemic (secondary, reactive) amyloid A deposition (AA amyloidosis - AAa) occurs in a wide spectrum of chronic inflammatory diseases [37], such as chronic microbial infections: tuberculosis [38], leprosy [39], fibrocystic lung diseases, bronchiectasis, lung abscess [40, 41], chronic osteomyelitis [41], chronic xanthogranulomatous pyelonephritis [42], chronic mesenteric lymphadenitis, decubitus, etc. chronic reactive inflammatory diseases: ankylosing spondylitis [41], psoriatic arthropathies [8,41], Reiter's syndrome, etc. autoimmune (inflammatory) diseases: rheumatoid arthritis [6, 41], juvenile chronic arthritis [41], adult Still's disease, systemic lupus erythematoses [41], progressive systemic sclerosis [7], Crohn's disease [43], ulcerative colitis [44], polymyositis[41], polymyalgia rheumatica or giant-cell arteritis (GCA) [45], etc., and in association with chronic cachectic diseases or malignancies: renal cell carcinoma, ovarian carcinoma, hepatocellular adenoma, bronchial carcinoma, Hodgkin's disease, cardiac (atrial) myxoma, etc.

The precursor polypeptides (immunoglobulin light chain- λ or light chain- κ - L λ or L κ) and (immunoglobulin heavy chain of IgG, IgA, IgM or IgD classes) are produced by the B-cells of bone marrow. In multiple myeloma or B-cell dyscrasia the B-cells produce abnormal antibodies that can't be broken down leading to light or heavy chain amyloidosis (AL or AH amyloidosis). AL or AH amyloidosis can affect primarily the heart (dyspnea, orthopnea, fatigue, leg edema, syncope), kidneys (albuminuria, leg edema), skin (thickening, easy bruising, periorbital ecchymosis) nerves (paresthesias), liver (hepatomegaly, elevated alkaline phosphatase level) and gastrointestinal track (dysphagia, loss of appetite) accompanied with weight loss and fatigue [46]. Characteristic hallmarks are macroglossia and periorbital ecchymosis [46].

Senile systemic amyloidosis or according to its current name: ATTRwt amyloidosis (Amyloid Transthyretin wild type amyloidosis) is the result of abnormal transthyretin precursor (Transthyretin wild type - TTRwt) deposition, or precisely defined, ATTRwt (amyloid Transthyretin wild type) protein fibril deposition. Transthyretin is a physiological transport protein in the serum and cerebrospinal fluid that carries the thyroid hormone thyroxine (T4) and retinol-binding protein bound to retinol (transports thyroxine and retinol).

Senile systemic amyloidosis may be used only for normal-sequence ATTRwta occurring in advanced age [47].

ATTRwta is a common condition, affecting to some degree 25% of the population older than 80 years [48]. According to Barreton, *et al.* (1999) senile amyloidosis does not occur under 65 years [49].

In agreement with Sekijima Y (2011) the senile, non-hereditary systemic amyloidosis is usually associated with cardiac disease, but TTR deposition is not limited to the heart, and is also found in other organs [50].

The hereditary transthyretin amyloidosis (ATTRma) or familial amyloid polyneuropathy (FAP) is determined by genomic point mutations resulting transthyretin mutant variants (TTRm), and ATTRm (amyloid Transthyretin mutant) protein fibril deposition. It is "insufficient to identify a mutation in the gene of a candidate amyloid protein without confirming the variant changes in the amyloid fibril protein" [1]. With other words, to detect mutations in the gene of transthyretin protein does not necessarily mean that the actual amyloid deposits are transthyretin mutant variants (TTRm) and are amyloid Transthyretin mutant protein fibrils (ATTRm). Exact identification of amyloid deposits is required by histochemical methods or by determination of amino acid sequence.

According to the Amyloidosis 2016 Nomenclature Guidelines, "when genomic DNA is sequenced, a genomic DNA sequence is the preferred reference and when a protein sequence is reported, an amino acid sequence is the preferred reference" [1]. Identification of amino acid sequence is not always available, so from pathological point of view immunohistochemical or histochemical identification may also be helpful.

The main form of hereditary amyloidosis syndromes is the familial Mediterranean fever (FMF). It is an autosomal recessive disorder. All ethnic groups are responsive to FMF, but first of all people of Mediterranean origin including Sephardic Jews, Mizrahi Jews, Armenians, Azerbaijanis, Arabs, Kurds, Greeks, Turks, and Italians. The function of polymorphonuclear leucocytes is damaged, and the disease is characterized by repeated inflammation: fever, abdominal pain, chest pain, achy swollen joints, red rash especially below the knees, muscle aches and swollen, tender scrotum. The precursor polypeptide is the serum amyloid A (SAA), and the predilection of amyloid A (AA) protein fibrils deposition are the same organs like in AAa: gastrointestinal tract, kidneys, heart, spleen, thyroid gland, etc.

Long term hemodialysis may be associated with A β 2M amyloidosis (Amyloid β 2M amyloidosis). The precursors of amyloid β 2M (A β 2M) protein fibrils are the β 2M (β 2-microglobulin) polypeptides. The β 2M polypeptides are found on the cell membrane of all nucleated mammalian cells (excluding red blood cells). β 2M polypeptides are synthesized by lymphoid cells (first of all by T-cells, but in smaller amount by B-cells also) [51-53]. Under normal circumstances the β 2M is excreted by the kidneys. In chronic renal failure the clearing (filtration - reabsorption - tubular catabolism) of β 2-microglobulin is reduced [54]. In long-term hemodialysed patients with chronic renal failure the elevated β 2M serum level results in A β 2M protein fibrils deposition with systemic A β 2Ma (amyloid β 2Microglobulin amyloidosis). Osteo-articular structures, cervical intervertebral discs, the large bones close to the joint spaces, synovial membranes, carpal tunnel tissue are preferred sites [55,56], but other organs and viscera may be involved as well [57-59]. The risk of cardiovascular involvement is increased in A β 2Ma [60].

The presented golden rules are true for all forms of localized amyloidosis as well, except for the location of isolated amyloidosis. Detailed discussion of localized amyloidosis syndromes goes beyond the scope of this study.

Conclusions

All forms of amyloidosis connected to the circulation are systemic, and all forms of amyloidosis not connected to the circulation are isolated (localized).

The golden rules of formal pathogenesis of systemic and localized amyloidosis syndromes are:

- Amyloidosis is a progressive, cumulative process, involving in its early stage only a few structures in some organs, and increasingly more in the later stages of the disease

- The rate and amount of systemic amyloid deposition may be linked to the differences in blood supply per unit volume
- Amyloid protein deposition begins in the organs and tissue structures that are frequently involved and later show marked deposition of amyloid, but where deposits are infrequent or less marked, deposition starts later
- Prevalence and severity of amyloid deposition are different aspects of the same phenomenon usually running parallel to each other, and
- The quantitative differences of amyloid deposits are accompanied by qualitative differences as well.

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Volume 6 Issue 5 May 2019

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