Archives of Gastroenterology and Hepatology

ISSN: 2639-1813

Volume 3, Issue 2, 2020, PP: 1-26

AA Amyloidosis of the Liver in Rheumatoid Arthritis – A Postmortem Clinicopathologic Study of 152 Autopsy Patients

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Abstract

*The **aim** of this study was to determine the prevalence and severity of systemic AA amyloidosis (**sAAa**) in rheumatoid arthritis (**RA**), to identify the prevalence and amount of amyloid A deposits in different tissue structures of the liver (**hAAa**), to outline the progression and spead of amyloid A deposition in different tissue structures of the liver, to asses the relationship between **sAAa** and **hAAa**, and to determine the role of **sAAa** and **hAAa** in mortality.*

***Patients and Methods:** One hundred fiftytwo (152) random autopsy patients with **RA** were studied. **RA** was confirmed clinically according to the criteria of the **ACR**.*

*The prevalence (existence) and severity (extent) of **sAAa** and **hAAa** was specified histologically. Amyloid A deposition was diagnosed histologically according to Romhányi by a modified (more sensitive) Congo red staining.*

The extent of amyloid A deposition was evaluated by semi-quantitative, visual estimation on a 0 to 3 plus scale.

*Demographics of different patient cohorts were compared with the Student t-probe. The possible relationship between **sAAa** and **hAAa** was analyzed with chi-squared ($\chi 2$) test.*

***Results:** **sAAa** complicated RA in **32** (21.05 %) of 152 patients. Branches of blood vessels of different calibers and various tissue structures of the liver were involved in **26** (81.25 % of 32, 17.11 % of 152) cases; **hAAa** was histologically excluded in **6** (18.75 % of 32) patients with **sAAa**.*

*There was a very strong positive relationship between **sAAa** (n=32) and **hAAa** (n=26) (c=1.0, $\chi 2$=111.9587, p <0.0000).*

*The **hAAa** does not appear to be a very serious, life-threatening complication of **RA**.*

***Conclusions:** **sAAa** and **hAAa** may develop in both sexes, and at any time in the course of **RA**.*

***sAAa** is related to the cardiovascular system, and **hAAa** is connected with it.*

*The amyloid A deposition in the liver starts after a latent stage, compared to **sAAa** of other organs, caused by not specified local protective mechanism.*

***sAAa** and **hAAa** is a progressive and cumulative process, involving in its early stage only a few structures, and increasingly more in later stages of the disease.*

*The chronology of amyloid A deposition allows an indirect assessment of the stage of **sAAa** or **hAAa**, which may have a prognostic value in biopsies.*

Amyloid A deposition on the liver cell plates may be an appropriate cleaning mechanism as well, but the role of the reticuloendothelial system (Kupffer cells, sinus endothelial, and perisinusoidal Ito cells) in blood clearance also cannot be ruled out.

Keywords: *Rheumatoid arthritis, systemic AA amyloidosis, AA amyloidosis of the liver*

Abbreviations

RA – Rheumatoid Arthritis

ACR = American College of Rheumatology

sAAa – **s**ystemic **AA a**myloidosis

hAAa – **h**epatic **AA a**myloidosis

rAAa – Uremia due to massive amyloid A deposition in the kidneys with renal insufficiency

cAAa – lethal outcome exclusively caused by cardiac amyloidosis or cardiac amyloidosis contributed to death

CoD – Cause of death

U – Uremia

Cl+ = clinically diagnosed

Cl- = clinically not diagnosed

SD – Standard **D**eviation

ND – No **D**ata

Introduction

Systemic AA amyloidosis (**sAAa**) is one of the main and most insidious complications of rheumatoid arthritis (**RA**) characterized by amyloid A deposition in various organs [1].

The **aim** of this study was to determine the prevalence and severity of systemic AA amyloidosis (**sAAa**) in rheumatoid arthritis (**RA**), to identify the prevalence and amount of amyloid A deposits in different tissue structures of the liver (**hAAa**), to outline the progression and spead of amyloid A deposition in different tissue structures of the liver, to asses the relationship between **sAAa** and **hAAa**, and to determine the role of **sAAa** and **hAAa** in mortality.

Patients and Methods

At the National Institute of Rheumatology **9475** patients died between 1969 and 1992; among them **161** with **RA** and all of them were autopsied [1]. **RA** was confirmed clinically according to the criteria of the American College of Rheumatology (**ACR**) [2].

Tissue samples of liver were available for histological evaluation in **152** patients.

The **sAAa** and **hAAa** was specified histologically, based on evaluation of five organs (kidneys, heart, pancreas, lungs, and liver).

Amyloid A deposition in different tissue structures of various organs was diagnosed histologically according to Romhányi [3] by a modified (more sensitive) Congo red staining [4]. Amyloid A deposits were identified in serial sections by immunohistochemical and histochemical methods [5-7)]. The prevalence (existence) and severity (extent) of amyloid A deposition were evaluated microscopically with an Olympus BX51 polarizing microscope.

The correlations were determined by Student (Welch) t-probe [8] comparing the age, sex, onset of **RA**, and duration of disease at the time of death with or without **sAAa** or **hAAa**, and with "**mild**" (**< 0.8** / patient) or "**severe**" amyloid A deposits (**0.8 ≤** / patient).

The relationship between **sAAa** and **hAAa**, and the role of **sAAa** and **hAAa** in mortality was analyzed with Pearson's chi-squared (χ^2) test [8].

Glossary of Definitions

"Prevalence of **sAAa**" concerns the proportion of amyloid A deposits in various organs of our autopsy population, and conveys information about the risk of complications. The **sAAa** was specified histologically based on the presence of amyloid A in blood vessels of different calibers or in different tissue structures of four organs (kidneys, heart, pancreas, and lungs) in each patient, and was compared with prevalence of **hAAa**.

"Prevalence of **hAAa**" concerns the dispersal of amyloid A deposits in blood vessels of different calibers or in different tissue structures of the liver.

Size of blood vessels [9] **in various organs**: ***arteriole*** (**a**) no internal or external elastic membrane, less than 500 micrometers in diameter, ***small artery*** (**A**) – internal elastic membrane present, but no external elastic membrane – 500-1000 micrometers in diameter, ***medium size artery*** (**AA**) – more than 1000 micrometers in diameter, internal and external elastic membrane present, ***venule*** (**v**) –, ***small vein*** (**V**) –, ***medium size vein*** (**VV**) – accompanying vessels of (a), (A) or (AA)

Interstitial collagen fiber (**I**) ***reticulin fiber*** *(collagen IV)* (**ret**) ***Basement membrane of biliary ducts*** – (**BM**) in the liver were not evaluated; the small biliary ducts and ductules of the portal triads were negative

except the larger ducts, which were only exceptionally present in tissue sections ***Nerves*** (**n**) in the liver "**Severity**" means different amounts of amyloid A 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 and 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

sAAa complicated **RA** in **32** (**21.05** %) of **152** patients. Branches of blood vessels of different calibers and various tissue structures of the liver were involved in **26** (**81.25** % of 32, 17.11 % of 152) cases; **hAAa** was histologically excluded in **6** (**18.75** % of 32) patients with **sAAa**.

There was a very strong positive relationship between **sAAa (n=32)** and **hAAa** (**n=26**) (c=1.0, χ^2=111.9587, p <0.0000).

In Table 1 are summarized the demographics, onset and duration of disease of the **total population** (n=**152**), **with** (n=**32**) and **without** (n=**120**) **sAAa**, **with** (n=**26**) and **without** (n=**6**) **hAAa**, furthermore **with mild** (n=**15**) and **severe** (n=**11**) amyloid A deposits of the **liver**.

Table1. *Sex, mean age with SD, range, onset and disease duration (in years) of 152 RA patients with sAAa and hAAa*

Sex	Number of autopsies	Mean age in years at death ± SD	Range (in years)	Mean age at onset of disease ± SD	Disease duration (in years) mean ± SD
RA patients (total)	**152**	**65.81±13.06**	**16 – 88**	**51.43±17.20**	**14.30±10.61**
Female	108	65.54±11.85	16 – 87	50.99±15.89	14.54±10.78
Male	44	66.45±15.62	19 – 88	52.56±20.15	13.69±10.12
with sAAa	**32 of 152**	**63.25±15.64**	**19 – 88**	**48.17±18.41**	**16.00±9.51**
Female	27	65.48±10.54	32 – 83	49.28±15.48	16.20±10.08
Male	5	51.20±28.18	19 – 88	41.25±30.07	14.75±4.44
without sAAa	**120 of 152**	**66.49±12.19**	**16 – 87**	**52.37±16.71**	**13.81±10.86**
Female	81	65.56±12.25	16 – 87	51.62±15.99	13.93±10.97
Male	39	68.41±11.83	20 – 87	53.97±18.05	13.56±10.61
with hAAa	**26 of 32**	**61.31±9.34**	**19 – 88**	**47.83±19.83**	**14.39±9.34**
Female	21	63.71±10.65	32 – 83	49.21±16.57	14.32±2.00
Male	5	51.20±28.18	19 – 88	41.25±30.07	14.75±4.44
without hAAa	**6 of 32**	**71.67±7.34**	**66 – 82**	**49.50±11.34**	**22.17±7.40**
Female	6	71.67±7.34	66 – 82	49.50±11.34	22.17±7.40
Male	0	–	–	–	–
with mild hAAa	**15 of 26**	**66.47±10.05**	**50 – 88**	**52.71±14.78**	**13.93±10.08**
Female	14	64.93±8.53	50 – 83	50.49±13.34	14.31±10.37
Male	1	88.00±0.0	88	79.00±0.0	9.0±0.0
with severe hAAa	**11 of 26**	**53.82±19.79**	**19 – 82**	**35.89±20.47**	**18.89±9.31**
Female	7	60.57±12.84	32 – 73	39.50±17.42	20.00±10.98
Male	4	42.00±25.86	19 – 82	28.67±23.92	16.67±3.40

Glossary to Table 1

RA – Rheumatoid **A**rthritis

sAAa – systemic **AA a**myloidosis

hAAa – hepatic **AA a**myloidosis (amyloid A deposits in the liver)

SD – Standard **D**eviation

Comparing **age, sex, onset of RA**, and **duration of disease** at the time of death there was **no significant difference in survival time, onset** or **duration of RA** between patient cohorts **with sAAa** (n=32) compared to the **total population** (n=152) (p <0.399, p< 0.397, p< 0.407), neither between **females** (p< 0.407, p< 0.981, p< 0.397) and **males** (p< 0.397, p< 0.482, p< 0.981), **with** (n=32) and **without sAAa** (n=120) (p< 0.290, p< 0.284, p< 0.303), neither between **females** (p< 0.976, p< 0.532, p< 0.359) and **males** (p< 0.291, p< 0.520, p< 0.721), **with sAAa** (n=32) and **with hAAa** (n=26) (p< 0.655, p< 0.950, p< 0.552), neither between **females** (p<0.578,p< 0.989, p< 0.552) and **males** (p< 1.00, p< 1.00, p< 1.00),

The average age (61.31 ys) of patients **with hAAa** (n=26) was significantly lower compared to the average age (71.67 ys) of the patients **without hAAa** (n=6) (p< **0.040**).

The onset of disease (p< 0.804), and disease duration (p< 0.075) between patient cohorts **with hAAa** (n=26) and **without hAAa** (n=6) was not significant, neither between **females** (p< 0.076, p< 0.965, p< 0.96); the **males** were not involved by **hAAa**, significance was not calculated.

The difference between patient cohorts **with mild** (n=15) and **severe hAAa** (n=11) was not significant (p< 0.085, p< 0.064, p< 0.266), neither between **females** (p< 0.469, p< 0.236, p< 0.0349); significance was not calculated between **males** because of zero divisor.

Amyloidosis developed in both sexes, and **at any time in the course of the disease** (Tables 1 and 2).

The relationship ("**p**"values of correlation) of demographics, onset and duration of disease between **RA patients with** and **without sAAa** or **hAAa** are summarized in Table 2.

Table2. *The statistical correlations ("p" values of significance) between female and male RA patients with and without sAAa or hAAa*

RA patients (Liver) n=152	Age	Onset of disease	Disease duration
RA n=152 versus **with sAAa n=32** of 152	**p <0.399**	**p <0.397**	**p <0.407**
Female n=**108** of 152 versus n=**27** of 32	p <0.407	p <0.981	p <0.397
Male n=**44** of 152 versus n=**5** of 32	p <0.397	p <0.482	p <0.981
with sAAa n=32 vs**. without sAAa n=120 of 152**	**p <0.290**	**p <0.284**	**p <0.303**
Female n=**27** of 32 versus n=**81** of 120	p <0.976	p <0.532	p <0.359
Male n=**5** of 32 versus n=**39** of 120	p <0.291	p <0.520	p <0.721
with sAAa n=32 vs**. with hAAa n=26** of 32	**p <0.655**	**p <0.950**	**p <0.552**
Female n=**27** of 32 versus n=**21** of 26	p <0.578	p <0.989	p <0.552
Male n=**5** of 32 versus n=**5** of 26	p <1.000	p <1.000	p <1.000
with hAAa n=26 vs**. without hAAa n=6 of 32**	**p <0.040**	**p <0.804**	**p <0.075**
Female n=**21** of 26 versus n=**6** of 6	p <0.076	p <0.965	p <0.081
Male n=**5** of 26 versus n=**0** of 6	-	-	-
with mild hAAa n=15 vs**. with severe hAAa n=11 of 26**	**p <0.085**	**p <0.064**	**p <0.266**
Female n=**14** of 15 versus n=**7** of 11	p <0.469	p <0.236	p <0.349
Male n=**1** of 15 versus n=**4** of 11	-	-	-

Glossary to Table 2

RA – Rheumatoid **A**rthritis

sAAa – systemic **AA a**myloidosis (amyloid A deposits in the kidneys, heart, pancreas, and lungs)

hAAa – hepatic **AA a**myloidosis (amyloid A deposits in the liver)

The deposition of amyloid A in different tissue structures of the liver is summarized in Table 3.

Table3. *Prevalence and extent of amyloid A deposits in tissue structures of 32 RA patients with and without hAAa arranged according to the increasing average amounts of amyloid A deposits /patient (horizontal lines), and decreasing values of amyloid A deposits/structures (vertical columns)*

	Pr n⁰/	Sex	a	A	VV	AA	V	ret	I	v	n	Avg	CoD	Cl-/Cl-	n=0	n+	Prev in %	Extent in %
1	76/79	**f**	0	0	0	0	0	0	0	0	0	**0,000**			**9**	**0**	**0**	**0**
2	155/87	**f**	0	0	0	0	0	0	0	0	0	**0,000**			**9**	**0**	**0**	**0**
3	243/87	**f**	0	0	0	0	0	0	0	0	0	**0,000**	**cAAa**		**9**	**0**	**0**	**0**
4	240/88	**f**	0	0	0	0	0	0	0	0	0	**0,000**			**9**	**0**	**0**	**0**
5	287/91	**f**	0	0	0	0	0	0	0	0	0	**0,000**	**cAAa**		**9**	**0**	**0**	**0**
6	52/92	**f**	0	0	0	0	0	0	0	0	1	**0,000**			**9**	**0**	**0**	**0**
7	395/76	**f**	1	0	0	0	0	0	0	0	0	**0,111**	**cAAa**		**8**	**1**	**11,11**	**3,704**
8	162/78	**f**	1	0	0	0	0	0	0	0	0	**0,111**			**8**	**1**	**11,11**	**3,704**
9	266/78	**f**	0	0	0	0	0	0	0	1	0	**0,111**			**8**	**1**	**11,11**	**3,704**
10	430/80	**f**	1	0	0	0	0	0	0	0	0	**0,111**	**cAAa**		**8**	**1**	**11,11**	**3,704**
11	226/85	**f**	1	0	0	0	0	0	0	0	0	**0,111**			**8**	**1**	**11,11**	**3,704**
12	183/92	**f**	1	0	0	0	0	0	0	0	0	**0,111**			**8**	**1**	**11,11**	**3,704**
13	203/88	**f**	1	0,5	0	0	0	0	0	0	0	**0,167**	**rAAa-U**		**7**	**2**	**22,22**	**5,556**
14	342/86	**m**	2	1	0	0	0	0	0	0	0	**0,333**	**rAAa-U**		**7**	**2**	**22,22**	**11,111**
15	45/74	**f**	2	2	0	0	0	0	0	0	0	**0,444**	**cAAa**	**ND**	**7**	**2**	**22,22**	**14,815**
16	322/81	**f**	2	2	0	0	0	0	1	0	0	**0,556**	**cAAa**		**6**	**3**	**33,33**	**18,519**
17	245/88	**f**	2	2	0	1	0	0	0	0	0	**0,556**	**cAAa**		**6**	**3**	**33,33**	**18,519**
18	39/76	**f**	3	2	0	1	0	0	0	0	0	**0,667**	**rAAa-U**		**6**	**3**	**33,33**	**22,222**
19	80/80	**f**	3	1	0	0	0	2	0	0	0	**0,667**	**rAAa-U**	**Cl+**	**6**	**3**	**33,33**	**22,222**
20	101/90	**f**	3	3	0	0	0	0	0	0	0	**0,667**	**rAAa-U**		**7**	**2**	**22,22**	**22,222**
21	367/75	**f**	3	3	0	1	0	0	0	0	0	**0,778**	**cAAa**		**6**	**3**	**33,33**	**25,926**
22	265/80	**f**	2	3	0	1	1	0	1	0	0	**0,889**	**rAAa-U**	**Cl+**	**4**	**5**	**55,56**	**29,63**
23	174/88	**f**	3	3	1	0	1	0	0	0	0	**0,889**	**rAAa-U**		**5**	**4**	**44,44**	**29,63**
24	232/74	**m**	3	3	0	0	0	3	0	0	0	**1,000**	**rAAa-U**	**Cl+**	**6**	**3**	**33,33**	**33,333**
25	53/87	**m**	3	2	2	1	1	0	0	0	0	**1,000**	**rAAa-U**	**Cl+**	**4**	**5**	**55,56**	**33,333**
26	V/T	**f**	3	2	3	0	2	0	0	0	0	**1,111**	**rAAa-U**		**5**	**4**	**44,44**	**37,037**
27	73/87	**f**	3	3	1	2	1	0	0	0	0	**1,111**	**rAAa-U**	**Cl+**	**4**	**5**	**55,56**	**37,037**
28	237/70	**f**	3	3	2	1	1	0	0	0,5	0	**1,167**	**rAAa-U**		**3**	**6**	**66,67**	**38,889**
29	43/85	**m**	3	3	0	2	0	3	0	0	0	**1,222**	**rAAa-U**		**5**	**4**	**44,44**	**40,741**
30	181/80	**m**	3	3	2	3	1	0	2	0	0	**1,556**	**rAAa-U**	**Cl+**	**3**	**6**	**66,67**	**51,852**
31	90/85	**f**	3	0	3	0	2	3	3	2	0	**1,778**			**3**	**6**	**66,67**	**59,259**
32	255/83	**f**	3	3	3	3	2	0	2	2	0	**2,000**	**rAAa-U**	**Cl+**	**2**	**7**	**77,78**	**66,667**
	Count		32	32	32	32	32	32	32	32	32	**32**	25	10	32	32	32	32
	Sum		58	44,5	17	16	12	11	9	5,5	0	**19,2**			204	84	933,33	640,74
	Avg		**1,81**	**1,39**	**0,53**	**0,5**	**0,38**	**0,34**	**0,28**	**0,17**	**0**	**0,601**					**29,167**	**20,023**
	SD		1,23	1,32	1,02	0,88	0,66	0,94	0,73	0,52	0,00	**0,57**						
	0 value		7	13	24	22	23	28	27	28	32	**6**			204		**6**	**6**
	+ value		25	19	8	10	9	4	5	4	0	**26**				84	**26**	**26**
	Prevalence %		**78,125**	**59,375**	**25,000**	**31,250**	**28,125**	**12,500**	**15,625**	**12,500**	**0,000**	**81,250**					**81,250**	
	Severity%		**60,417**	**46,354**	**17,708**	**16,667**	**12,500**	**11,458**	**9,375**	**5,729**	**0,000**	**20,023**						**20,02**
	Pr n⁰/	**Sex**	**a**	**A**	**VV**	**AA**	**V**	**ret**	**I**	**v**	**n**	**Avg**	**CoD**	**Cl-/Cl-**	**n=0**	**n+**	**Prev in %**	**Sev in %**

Remarks to Table 3

Pr n^0/y – Protocol number / year

Prevalence (Prev) in %: positive ("+") cases in % of "count" (for example: 26 "+" in % of 32=81.250%

Severity (Sev) in %: "Avg" in % of maximum "3" value of severity (for example: Avg=0.601 in % of "3"=20.023%)

CoD: Cause of **D**eath: **rAAa** – Uremia due to massive amyloid A deposition in the kidneys with renal insufficiency (n=**17**), **cAAa** – lethal outcome exclusively caused by cardiac amyloidosis (n=**3**) (430/80, 322/81, 45/74); **cAAa** – cardiac amyloidosis only contributed to the death (n=**5**) (243/87, 287/91, 367/75, 395/76, 245/88); further CoD were: autoimmune vasculitis, septic infection, peritonitis, bronchopneumonia, pulmonary embolism

Cl+: Clinically recognized (assigned) – **Cl-**: Clinically not recognized (not assigned)

f: female, **m**: male

ND – no data (the tissues samples of the liver were not available in two patients)

SD – Standard **D**eviation

Abbreviations

arteriole (**a**), small artery (**A**),medium size artery (**AA**), venule (**v**), small vein (**V**), medium size vein (**VV**), interstitial collagen fiber (**I**), reticulin fiber (collagen IV) (**ret**), nerve (**n**)

Special structures such as basement laminas of portal bile ducts and ductules were negative for amyloid A deposition, and are not considered in Table 3

Amyloid A deposition was exceptionally found on the basal lamina of common hepatic ducts.

The ***prevalence and amount of amyloid A deposits /patients*** with **hAAa** run parallel to each other (see trend lines of increment in Figure 3.1), except in the terminal stage of the disease, in which the increment was exponential. In some patients there was a considerable difference between prevalence and severity of **hAAa** (Figure 3.1).

Figure 3.1 demonstrates the prevalence and amount of **hAAa** in 32 **RA** patients.

Figure3.1 **Prevalence and extent of amyloid A deposits of the liver in 32 patients with hAAa** arranged according to the increasing amount of amyloid **A deposits /patient**

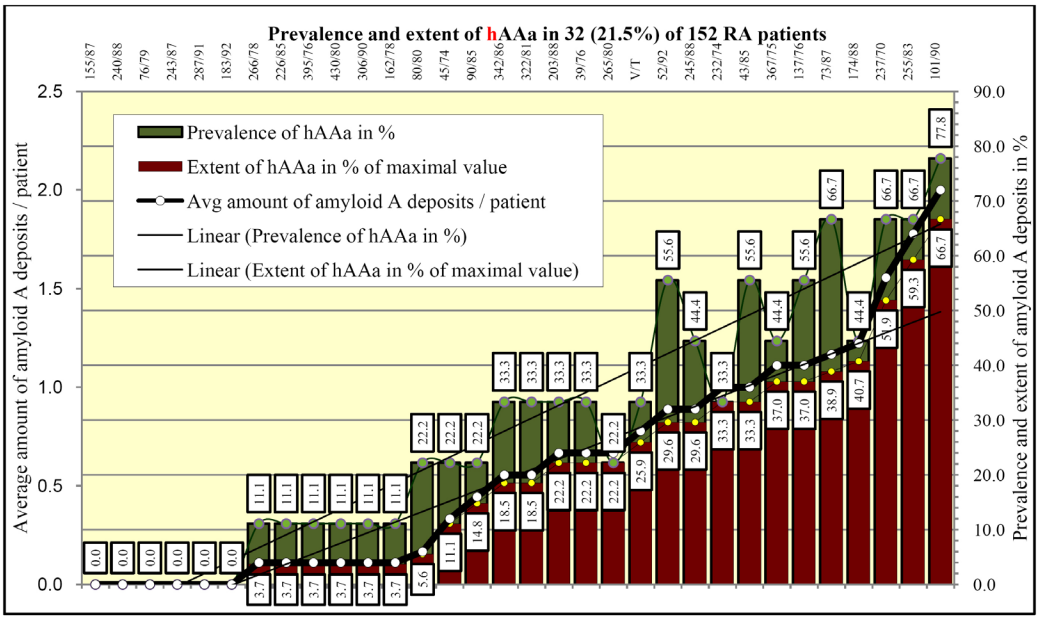


Figure3.1. *The prevalence and amount of amyloid A deposits in the liver (hAAa) changed basically parallel, but with considerable differences in some patients (Table 3).*

The ***prevalence and amount of amyloid A deposits in different tissue structures*** of the liver run parallel to each other with a few exceptions. The frequently involved tissue structures showed marked deposits of

amyloid. Deposits were less striking in less frequently involved tissue structures.

Figure 3.2 demonstrates the prevalence and amount of amyloid A deposits in different tissue structures of the liver.

Figure 3.2 **Prevalence and extent of amyloid A deposits in different tissue structures of the liver** arranged according to the the decreasing values of amount of amyloid **A deposits/structures**

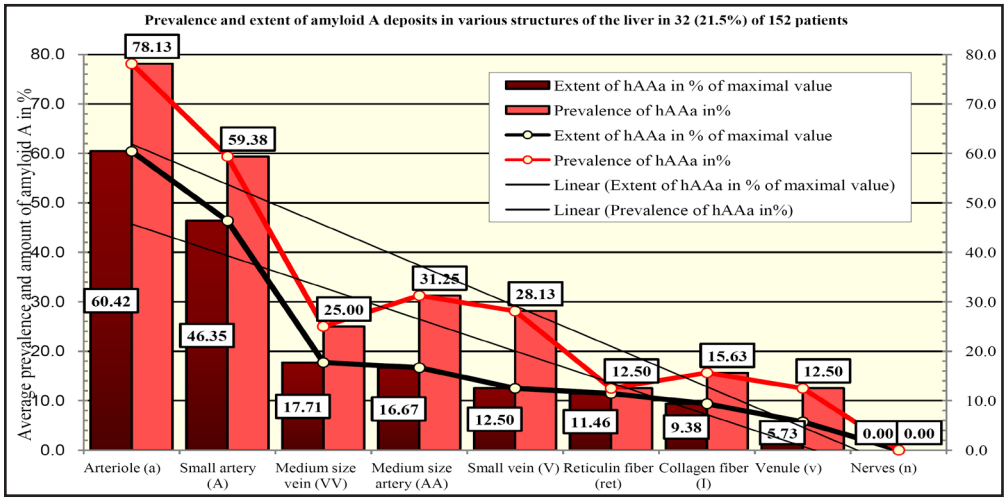


Figure3.2. *Prevalence and amount of amyloid A deposits of the liver (hAAa) run basically parallel in different tissue structures, except the medium size (VV), and medium size arteries (AA), furthermore reticulin (ret) and collagen (I) fibers, in which the sequence of amyloid deposition was inverse (Table 3).*

Detectable amounts of amyloid A deposits in different tissue structures of the liver did not appear simultaneously. In the early stage of systemic amyloidosis there were histologically detectable amyloid deposits only in a few structures (arterioles, small arteries, medium size arteries and veins). On other structures of the liver amyloid deposits were seen only in advanced or late stages of amyloidosis with pronounced involvement of the former. In our patient population the nerves of the liver were not involved (Table 3).

The amount of deposited amyloid A in various tissue structures of the liver was different and increased simultaneously. The proportion of deposited amyloid A was constant and independent of the stage of amyloidosis. The tendency of increment (probability of increasing amounts of amyloid A deposits) in various structures of the liver are demonstrated in Figure 3.3.

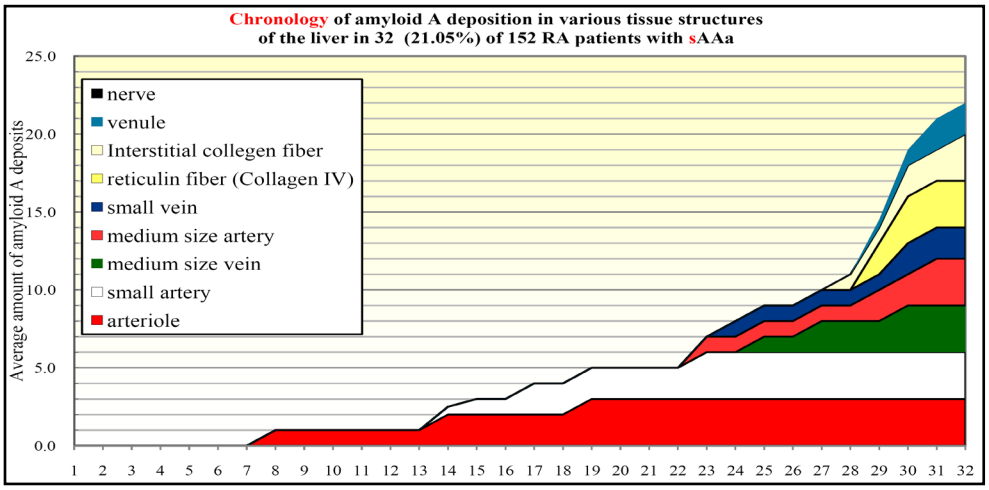


Figure3.3. *Tendency of amyloid A deposition on different tissue structures of the liver arranged according to their increasing severity and onset. Amyloid A deposition did not start at the same time on different tissue structures of the liver. The amount of amyloid A deposits on different tissues increased simultaneously, the rate of tendency (probability of deposition) was constent and independent of the stage of amyloidosis.*

In **6** (18.75 %) of 32 **RA** patients with systemic AA amyloidosis (**sAAa**) there was no amyloid A deposition in the liver, these represent a latent stage of hepatic amyloidosis (the amount of amyloid A deposits was: **0.00**).

In **15** (46.875 %) of 32 **RA** patients with **hAAa** the average amount of amyloid A deposits of the liver was less than **<0.8**, and was regarded "**mild**"; in **11** (34.375 %) of 32 patients it was more than **0.8 ≤**, and was considered "**severe**" (Table 3).

In patients with **mild** and **severe hAAa** the amyloid A deposition in the liver after an early latent stage ran basically parallel, and showed a linear growth curve; development of **hAAa** was continuously steady, except for the end stage of the disease with extreme severe increment of amyloid A deposits (Table 3 and Figure 3.4).

Figure 3.4 **Mean amount of amyloid A of the liver in early, advanced, late and end stages of hAAa** according to increasing "severity" ("average amount of amyloid A deposits/patient")

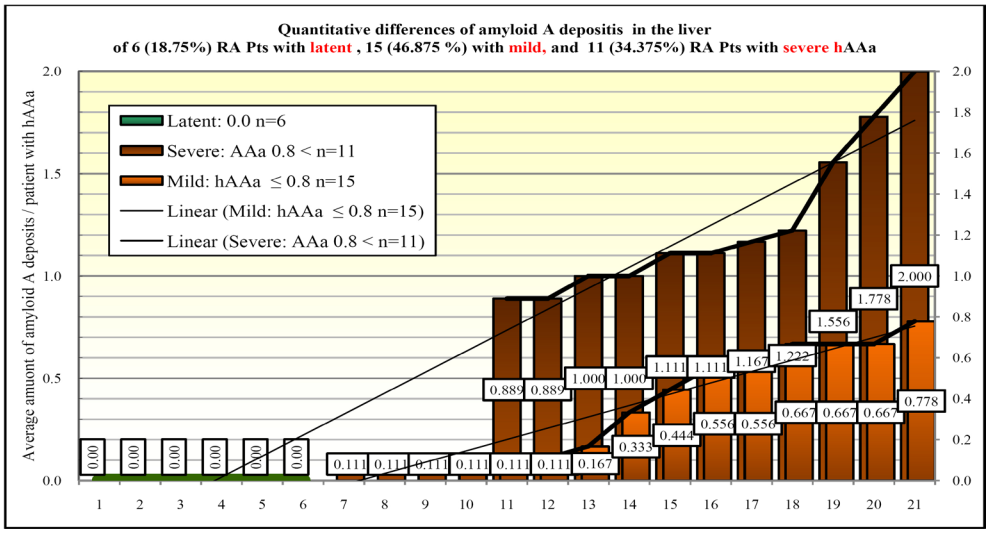


Figure3.4. *Cohort of 32 RA patients with hAAa. In 6 (18.75 %) of 32 RA patients complicated with sAAa there was no amyloid A deposition in the liver, these represent a latent stage of hepatic amyloidosis (the amount of amyloid A deposits was: 0.00); in 15 (46.875 %) patients with mild amyloidosis the amount of amyloid A deposits was: 0.111 – 0.778, and in 11 (34.375 %) patients with severe hepatic amyloid A deposition the amount of amyloid A deposits was: 0.898 – 2.00 (Table 3).*

In the last 3 of these 11 patients with severe hAAa the amyloid A deposition was more severe and exceeded the average amount of 1.5, these represent the terminal (end) stage of hepatic amyloidosis.

The increment with mild and severe hAAa showed a basically linear growth curve, representing the same rate of amyloid A deposition, except in 3 patients with extreme severe hAAa; in these the increment was exponential.

Table 4 and Figures 4.1-4.4 summarize the **quantitative differences** of **amyloid A deposits** in different tissue structures of the kidneys, heart, pancreas, and lungs in **32 RA** patients with **sAAa**.

Table4. *Prevalence and extent of amyloid A deposits in tissue structures of 32 RA patients with sAAa arranged according to the increasing average amounts of amyloid A deposits/patient with sAAa (horizontal lines), and decreasing values of amyloid A deposits/organs (vertical columnes) (Only comparable structures were considered; glomeruli, myocardiocytes, basement membranes were not)*

	Sex	Pr n⁰/y	Kidneys	Heart	Pancreas	Lungs	sAAa	0/Pts	+/Pts	Prevalence	Severity	CoD	Cl+/-
#			Avg	Avg	Avg	Avg	Avg	sAAa	sAAa	/Pts in%	/Pts in%		
1	**f**	155/87	0	0	0	0,111	**0,028**	35	1	**2,78**	**0,93**		
2	**f**	240/88	0	0	0,111	0	**0,028**	35	1	**2,78**	**0,93**		
3	**f**	76/79	0,333	0	ND	0	**0,111**	24	3	**11,11**	**3,70**		

4	**f**	243/87	0	0,333	0,111	0	**0,111**	32	4	**11,11**	**3,70**	**cAAa**	
5	**f**	287/91	0,111	0,556	0,444	0	**0,278**	29	7	**19,44**	**9,26**	**cAAa**	
6	**f**	183/92	0,889	0	0,556	0	**0,361**	28	8	**22,22**	**12,04**		
7	**f**	266/78	0,444	0,556	ND	0,111	**0,37**	18	9	**33,33**	**12,34**		
8	**f**	226/85	0,556	0,667	0,222	0,111	**0,389**	24	12	**33,33**	**12,97**		
9	**f**	395/76	0,222	1,222	0	0,222	**0,417**	26	10	**27,78**	**13,88**	**cAAa**	
10	**f**	430/80	0,556	0,444	0,444	0,222	**0,417**	23	13	**36,11**	**13,88**	**cAAa**	
11	**f**	162/78	0,111	1,111	0,611	0	**0,458**	26	10	**27,78**	**15,28**		
12	**f**	80/80	0,667	0,444	ND	0,333	**0,481**	18	9	**33,33**	**16,04**	**rAAa-U**	**Cl+**
13	**f**	45/74	0	1,444	0,889	0	**0,583**	25	11	**30,56**	**19,44**	**cAAa**	ND
14	**f**	90/85	0,889	1,444	0,111	0	**0,611**	23	13	**36,11**	**20,37**		
15	**m**	342/86	0,667	1,111	0,333	0,333	**0,611**	21	15	**41,67**	**20,37**	**rAAa-U**	
16	**f**	322/81	1	0,778	1,111	0,222	**0,778**	19	17	**47,22**	**25,93**	**cAAa**	
17	**f**	203/88	0,667	1,111	0,778	0,556	**0,778**	15	21	**58,33**	**25,93**	**rAAa-U**	
18	**f**	39/76	1,333	0,444	1,333	0,222	**0,833**	16	20	**55,56**	**27,77**	**rAAa-U**	
19	**f**	265/80	1,556	1,444	0	0,333	**0,833**	20	16	**44,44**	**27,78**	**rAAa-U**	**Cl+**
20	**f**	V/T	ND	1,444	ND	0,333	**0,889**	9	9	**50,00**	**29,62**	**rAAa-U**	
21	**f**	52/92	0,889	1,111	0,722	0,889	**0,903**	17	19	**52,78**	**30,09**		
22	**f**	245/88	1	1,444	1,222	0,333	**1**	14	22	**61,11**	**33,33**	**cAAa**	
23	**m**	232/74	1,778	1,444	0,667	0,333	**1,056**	14	22	**61,11**	**35,18**	**rAAa-U**	**Cl+**
24	**m**	43/85	1,111	1,111	1,389	1	**1,153**	9	27	**75,00**	**38,43**	**rAAa-U**	
25	**f**	367/75	2,222	0,889	1,556	0,333	**1,25**	11	25	**69,44**	**41,67**	**cAAa**	
26	**f**	73/87	1,444	1,778	1,444	0,778	**1,361**	12	24	**66,67**	**45,37**	**rAAa-U**	**Cl+**
27	**f**	174/88	1,889	1	1,889	0,778	**1,389**	10	26	**72,22**	**46,30**	**rAAa-U**	
28	**f**	237/70	2,333	ND	ND	0,778	**1,556**	6	12	**66,67**	**51,85**	**rAAa-U**	
29	**f**	255/83	1,889	1,778	1,833	0,778	**1,57**	8	28	**77,78**	**52,32**	**rAAa-U**	**Cl+**
30	**f**	101/90	2,222	2,333	2,222	0,667	**1,861**	4	32	**88,89**	**62,03**	**rAAa-U**	
31	**m**	181/80	2,222	1,667	2,889	1,333	**2,028**	5	31	**86,11**	**67,59**	**rAAa-U**	**Cl+**
32	**m**	53/87	2,667	2,333	2,111	1	**2,028**	4	32	**88,89**	**67,59**	**rAAa-U**	**Cl+**
33		**Count**	31	31	27	32	32	32	32	32	32		
		Sum	31,667	31,441	24,998	12,109	26,520	580	509	1491,660	883,910		
		Avg	**1,0215**	**1,0142**	**0,9259**	**0,3784**	**0,8288**			**46,614**	**27,622**		
		SD	0,81	0,64	0,79	0,37	0,57						
		0 value	4	4	3	8	0	580					
		+ value	**27**	**27**	**24**	**24**	**32**		509				
		Prev %	**87,097**	**87,097**	**88,889**	**75,000**	**100,000**			**46,740**			
		Sev %	**34,051**	**33,808**	**30,862**	**12,614**	**27,625**				**27,625**		
	Sex	**Pr n^0/y**	**Kidneys**	**Heart**	**Pancreas**	**Lungs**	**sAAa**	**0/Pts**	**+/Pts**	**Prevalence**	**Severity**	**CoD**	**Cl+/-**
#			**Avg**	**Avg**	**Avg**	**Avg**	**Avg**	**sAAa**	**sAAa**	**/Pts in%**	**/Pts in%**		

Remarks to Table 4

Pr n⁰/y – Protocol number / year

Prevalence (Prev) / patient in %: positive ("+") cases in % of "count" (for example in case of 155/87: One "+" in % of 36 =2.78 %)

Severity (Sev) in %: "Avg" in % of maximum "3" value of severity (for example in case of 155/87: Avg=0.028 in % of "3"=0.93%)

CoD: Cause of **D**eath: **rAAa** – Uremia due to massive amyloid A deposition in the kidneys with renal insufficiency (n=**17**), **cAAa** – lethal outcome exclusively caused by cardiac amyloidosis (n=**3**) (430/80, 322/81, 45/74); **cAAa** – cardiac amyloidosis only contributed to the death (n=**5**) (243/87, 287/91, 367/75, 395/76, 245/88); further patients died of autoimmune vasculitis, septic infection, peritonitis, bronchopneumonia, pulmonary embolism

Cl+: Clinically recognized (assigned) – **Cl-**: Clinically not recognized (not assigned)

f: female, **m**: male

ND – no data

SD – **S**tandard **D**eviation

The ***prevalence and amount of amyloid A deposits / patients*** with **sAAa** run basically parallel to each other (see trend lines of increment in Figure 4.1). In some patients there was a moderate difference between prevalence and severity of **sAAa** (Figure 4.1).

Figure 4.1 demonstrates the prevalence and amount of amyloid A deposits in **32 RA** patients with **sAAa**.

Figure 4.1 **Prevalence and extent of amyloid A deposits in 32 (21.05 %) of 152 patients with sAAa** arranged according to the increasing amount of amyloid **A deposits /patient**

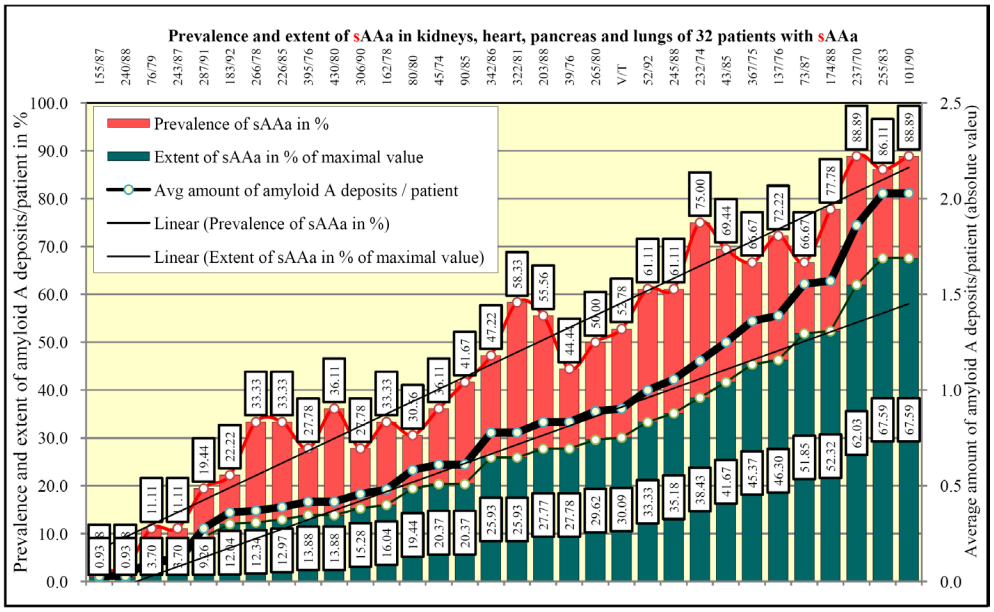


Figure4.1. *The prevalence and extent of amyloid A deposits in the kideneys, heart, pancreas and lungs (sAAa) changed basically parallel with moderate differences in some patients (Table 4).*

The amyloid A deposition in the kidneys, heart, pancreas, and lungs changed parallel with average severity of **sAAa** (see trend lines of increment).

Figure 4.2 demonstrates the tendency of amyloid A deposition in various organs.

Figure 4.2 **Amyloid A deposition in the kidneys, heart, pancreas, and lung compared to the average value of sAAa**

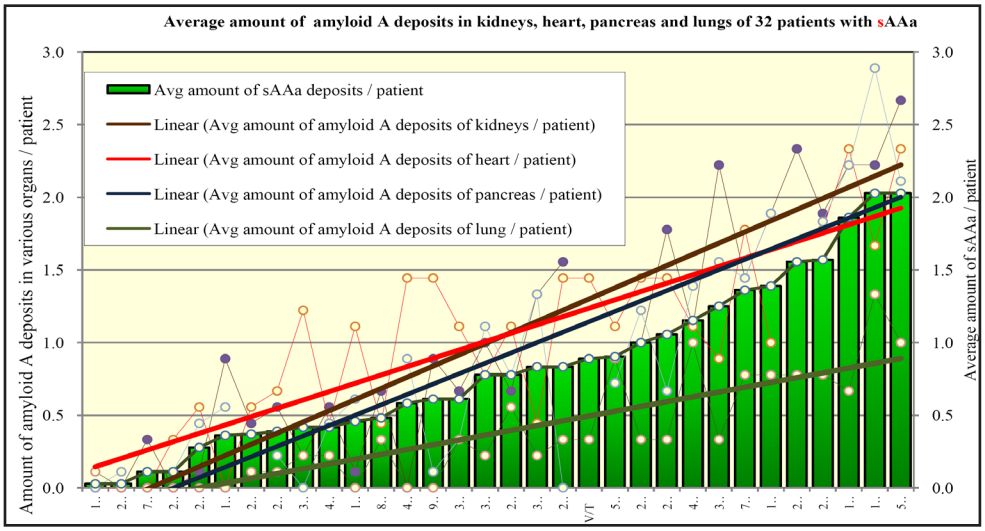


Figure4.2. *The amount of amyloid A deposits in the kidneys, heart, pancreas, and lungs changed basically parallel with the average value of sAAa (see trend lines) with considerable individual differences (Table 4). (This was mainly due to the contingency of sampling and the limitations of the semi-objective evaluation method)*

Comparing **sAAa** to **hAAa**, the amyloid A deposition in the liver started later than systemic amyloid A deposition in other organs of **RA** patients. Development of **sAAa** and **hAAa** was steady and showed consistently a linear growth curve. In the terminal stage of the disease the increment in various organs (**sAAa**) and in the liver (**hAAa**) was exponential (Tables 3 and 4).

Amyloid A deposition in the liver (**hAAa**) compared to other organs of **RA** patients (**sAAa**) is demonstrated on Figure 4.3

Figure 4.3 **Cohort of 32 RA patients with sAAa and hAAa** according to increasing "severity" ("average amount of amyloid A deposits/patient")

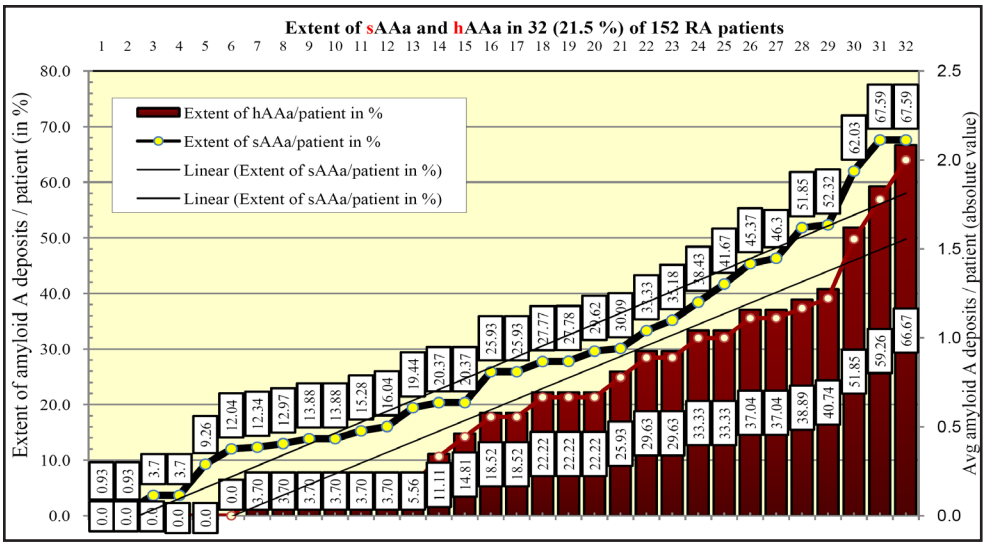


Figure4.3. *"Average amount of amyloid A deposits/patient" with sAAa of four organs and hAAa at death according to increasing values of amyloid A deposits.*
sAAa showed basically a linear growth curve, except in the terminal stage of the disease, in which the increment was exponential.
The amount of amyloid A deposits in the liver (hAAa) increased gradually after a latent stage, and showed basically a lineal growth curve like that of systemic amyloid A deposition. The advanced stage of hAAa was characterized by an intensive amyloid A deposition, and in the late (terminal) stage by an abrupt exponential increment of amyloid A.

In **17** (53.125 %) of 32 **RA** patients with **sAAa**, the average amount of amyloid A deposits/patients was less than **<0.8**, and was regarded "**mild**"; in **15** (46.875 %) of 32 patients was more than **0.8 ≤**, and was considered "**severe**" (Table 4).

In patients with **mild** and **severe sAAa**, the amyloid A deposition in various organs of 32 **RA** patients ran basically parallel, and showed a linear growth curve; development of **sAAa** was continuously steady, except for the end stage of the disease with extremely severe amyloid A deposits (Table 4 and Figure 4.4).

Figure 4.4 **Mean amount of amyloid A in early, advanced, late and end stages of sAAa in 32 RA patients** according to increasing "severity" ("average amount of amyloid A deposits/patient")

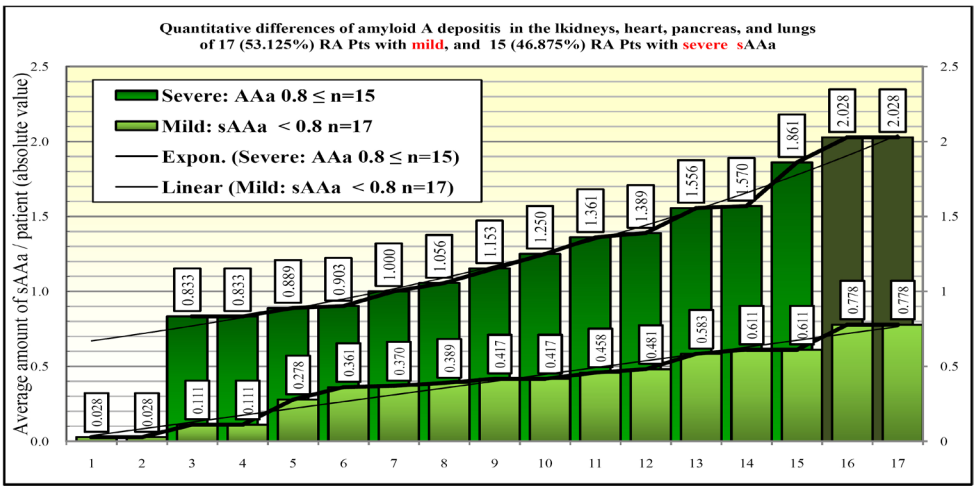


Figure4.4. *Cohort of 32 RA patients with sAAa. In 17 (53.125 %) patients with mild amyloidosis the amount of amyloid A deposits was: 0.028 – 0.778, and in 15 (46.876 %) patients with severe systemic amyloid A deposition the amount of amyloid A deposits was: 0.833 – 2.028 (Table 4).*

In the last 2 of these 15 patients with severe sAAa the amyloid A deposition was extremely severe and exceeded the average amount of 2.0, these represent the terminal (end) stage of systemic amyloidosis.

The increment of mild and severe sAAa showed a basically linear growth curve, representing the same rate of amyloid A deposition, except in 2 patients with extremely severe sAAa; in these the increment was exponential.

Table 5 and Figures 5.1-5.2 summarize the **quantitative differences** of **amyloid A deposits** on different tissue structures of the kidneys, heart, pancreas, and lungs in **32 RA** patients with **sAAa**, compared to the amyloid A deposits in the liver in **32 RA** patients with **hAAa**.

Table5. *Prevalence and extent of amyloid A deposits in tissue structures of the kidneys, heart, pancreas, lungs, and amyloid A deposits of the liver arranged according to the decreasing prevalence of amyloid A deposits/organs (horizontal sections) and decreasing amount of amyloid A deposits/structures (based on the section with sAAa)*

Kidney	a	A	I	ret	V	VV	AA	v	n	Avg
Count	31	31	31	31	31	31	31	31	31	**31**
Sum	66	51	43	33	22	26	21	16	7	**32,78**
Avg	**2,129**	**1,645**	**1,387**	**1,065**	**0,71**	**0,839**	**0,677**	**0,516**	**0,226**	**1,057**
SD	1,18	1,20	1,02	1,26	0,90	1,00	1,01	0,85	0,62	**0,89**
0 value	4	7	7	16	17	15	18	20	26	**4**
+ value	**27**	**24**	**24**	**15**	**14**	**16**	**13**	**11**	**5**	**27**
Prevalence %	**87,097**	**77,419**	**77,419**	**48,387**	**45,161**	**51,613**	**41,935**	**35,484**	**16,129**	**87,097**
Severity %	**70,968**	**54,839**	**46,237**	**35,484**	**23,656**	**27,957**	**22,581**	**17,204**	**7,527**	**35,245**
Heart	**a**	**A**	**I**	**ret**	**V**	**VV**	**AA**	**v**	**n**	**Avg**
Count	31	31	31	31	31	31	31	31	31	**31**

Sum	61	39	50	39	27	19	10	31	7	**33,667**
Avg	**1,968**	**1,258**	**1,613**	**1,258**	**0,871**	**0,613**	**0,323**	**1,000**	**0,226**	**1,086**
SD	0,98	0,86	1,02	0,89	0,85	0,88	0,54	0,86	0,72	**0,80**
0 value	4	7	4	7	13	19	22	10	28	**4**
+ value	**27**	**24**	**27**	**24**	**18**	**12**	**9**	**21**	**3**	**27**
Prevalence %	**87,097**	**77,419**	**87,097**	**77,419**	**58,065**	**38,710**	**29,032**	**67,742**	**9,677**	**87,097**
Severity %	**65,591**	**41,935**	**53,763**	**41,935**	**29,032**	**20,430**	**10,753**	**33,333**	**7,527**	**36,201**
Pancreas	**a**	**A**	**I**	**ret**	**V**	**VV**	**AA**	**v**	**n**	**Avg**
Count	27	27	27	27	27	27	27	27	27	**27**
Sum	52,5	41,5	18	26	22	25,5	24,5	11	4	**25**
Avg	**1,944**	**1,537**	**0,667**	**0,963**	**0,815**	**0,944**	**0,907**	**0,407**	**0,148**	**0,926**
SD	1,21	1,30	0,88	0,98	0,99	1,06	1,14	0,75	0,60	**0,79**
0 value	6	9	15	11	14	13	15	20	25	**3**
+ value	**21**	**18**	**12**	**16**	**13**	**14**	**12**	**7**	**2**	**24**
Prevalence %	**77,8**	**66,7**	**44,4**	**59,3**	**48,1**	**51,9**	**44,4**	**25,9**	**7,4**	**88,9**
Severity %	**64,8**	**51,2**	**22,2**	**32,1**	**27,2**	**31,5**	**30,2**	**13,6**	**4,9**	**30,9**
Lung	**a**	**A**	**I**	**ret**	**V**	**VV**	**AA**	**v**	**n**	**Avg**
Count	32	32	32	32	32	32	32	32	32	**32**
Sum	34	24	27	3	5	1	12	2	1	**12,111**
Avg	**1,063**	**0,75**	**0,844**	**0,094**	**0,156**	**0,031**	**0,375**	**0,063**	**0,031**	**0,378**
SD	0,88	0,84	0,81	0,30	0,45	0,18	0,55	0,25	0,18	**0,37**
0 value	10	16	12	29	28	31	21	30	31	**8**
+ value	**22**	**16**	**20**	**3**	**4**	**1**	**11**	**2**	**1**	**24**
Prevalence %	**68,75**	**50**	**62,5**	**9,375**	**12,5**	**3,125**	**34,38**	**6,25**	**3,125**	**75**
Severity %	**35,42**	**25**	**28,13**	**3,125**	**5,208**	**1,042**	**12,5**	**2,083**	**1,042**	**12,62**
sAAa	**a**	**A**	**I**	**ret**	**V**	**VV**	**AA**	**v**	**n**	**Avg**
Count	121	121	121	121	121	121	121	121	121	**121**
Sum	213,5	155,5	138	101	76	71,5	67,5	60	19	**100,22**
Avg	**1,764**	**1,285**	**1,140**	**0,835**	**0,628**	**0,591**	**0,558**	**0,496**	**0,157**	**0,828**
SD	1,13	1,10	1,00	1,02	0,86	0,91	0,86	0,79	0,56	**0,71**
0 value	24	39	38	63	72	78	76	80	110	**19**
+ value	97	82	83	58	49	43	45	41	11	**102**
Prevalence %	**80,17**	**67,77**	**68,6**	**47,93**	**40,5**	**35,54**	**37,19**	**33,88**	**9,091**	**84,3**
Severity %	**58,82**	**42,84**	**38,02**	**27,82**	**20,94**	**19,7**	**18,6**	**16,53**	**5,234**	**27,61**
sAAa	**a**	**A**	**I**	**ret**	**V**	**VV**	**AA**	**v**	**n**	**Avg**
hAAa	**a**	**A**	**I**	**ret**	**V**	**VV**	**AA**	**v**	**n**	**Avg**
Count	32	32	32	32	32	32	32	32	32	**32**
Sum	58	44,5	9	11	12	17	16	5,5	0	**19,22**
Avg	**1,813**	**1,391**	**0,281**	**0,344**	**0,375**	**0,531**	**0,5**	**0,172**	**0**	**0,601**
SD	1,23	1,32	0,73	0,94	0,66	1,02	0,88	0,52	0	**0,57**
0 value	7	13	27	28	23	24	22	28	32	**6**
+ value	25	19	5	4	9	8	10	4	0	**26**
Prevalence %	**78,13**	**59,38**	**15,63**	**12,5**	**28,13**	**25**	**31,25**	**12,5**	**0**	**81,25**
Severity %	**60,42**	**46,35**	**9,38**	**11,46**	**12,5**	**17,71**	**16,67**	**5,73**	**0**	**20,02**
sAAa	**a**	**A**	**I**	**ret**	**V**	**VV**	**AA**	**v**	**n**	**Avg**

Remarks to Table 5

sAAa – systemic **AA a**myloidosis

hAAa – hepatic **AA a**myloidosis (amyloid A deposits in the liver)

SD – Standard **D**eviationa

Abbreviations

arteriole (**a**), small artery (**A**),medium size artery (**AA**), venule (**v**), small vein (**V**), medium size vein (**VV**), interstitial collagen fiber (**I**), reticulin fiber (collagen IV) (**ret**), nerve (**n**) – special structures such as glomeruli, myocardiocytes, basement laminas were not considered

The ***prevalence and amount of amyloid A deposits in different tissue structures*** in the kidneys, heart, pancreas, and lungs run parallel to each other. The frequently involved tissue structures showed marked deposits of amyloid. Deposits were less striking in less frequently involved tissue structures. Differences were only found for medium size veins (**VV**) and arteries (**AA**), in which the sequence was inverse (Tables 4 and 5; Figure 5.1).

Figure 5.1 demonstrates the prevalence and amount of amyloid A deposits in different tissue structures of the kidneys, heart, pancreas, and lungs.

Figure 5.1 **Prevalence and extent of amyloid A deposits in different tissue structures of the kidneys, heart, pancreas, and lungs** arranged according to the the decreasing amyloid **A deposits/ structures**

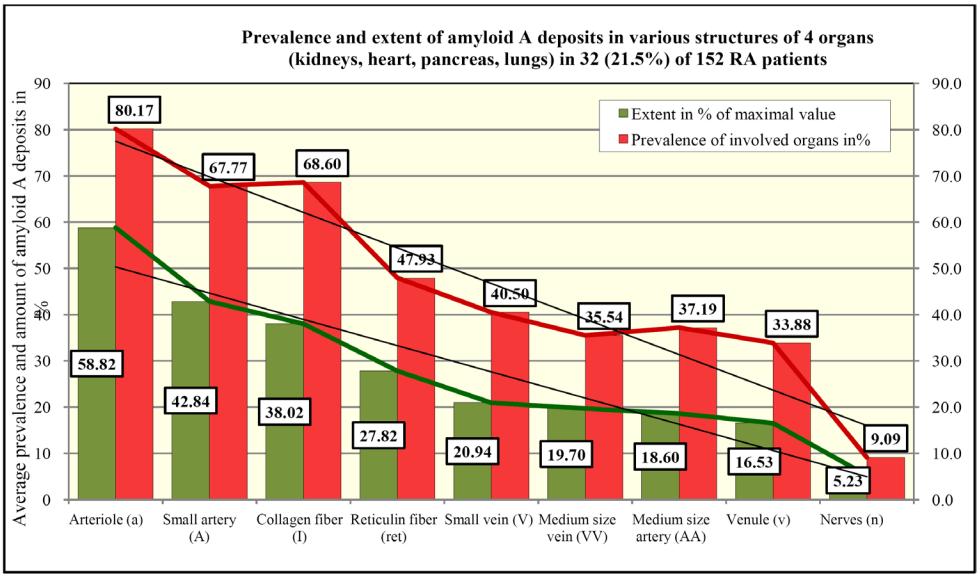


Figure5.1. *The prevalence and amount of amyloid A deposits in different tissue structures in the kidneys, heart, pancreas and lungs (sAAa) run basically parallel except the medium size veins (VV) and medium size arteries (AA), in which the sequence was inverse (Table 4 and 5).*

The ***prevalence and amount of amyloid A deposits in the kidneys, heart, pancreas, and lungs*** run more or less parallel to each other. The frequently involved organs showed marked deposits of amyloid. Deposits were less striking in less frequently involved organs. Deposition of amyloid A on arterioles and small arteries was an early phenomenon of **sAAa**, while the involvement of venules and nerves was characteristic for the end stage of amyloidosis (Table 5).

Quantitative differences of amyloid A deposits on various tissue structures in the kidneys, heart, pancreas and lung are demonstrated in Figure 5.2.

Figure 5.2 **Average amount of amyloid A deposits in the kidneys, heart, pancreas and lungs in 32 RA patients with sAAa** arranged according to the decreasing values of **sAAa/organs** (from bottom to top) and **sAAa/structures** (from left to right)

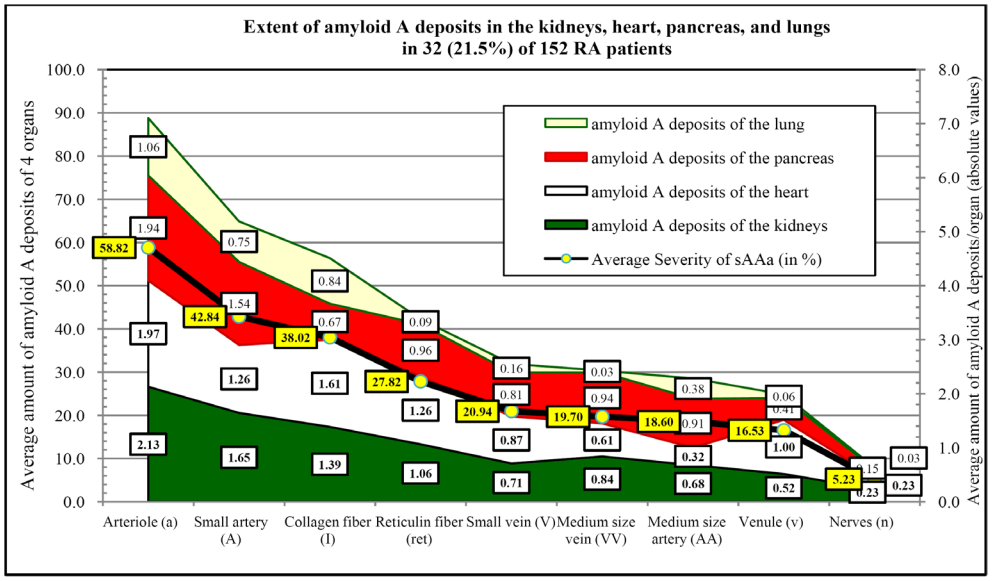


Figure 5.2. The linear progressive accumulation of amyloid A deposition is demonstrated in blood vessels of different sizes or on collagen and reticulin fibers in kidneys, heart, pamcreas, and lungs of 32 RA patients with sAAa. Amyloid A deposition in the kidneys, heart, pancreas and lungs changed basically parallel to each other. The trend of amyloid A deposition was constant and independent of the stage of amyloidosis, and demonstrated the progressive cumulative character of amyloid A deposition. The proportion of deposited amyloid A was more or less constant (Table 5).

Comparing the extent of **sAAa** and **hAAa**, there was a great difference in amount A deposits on interstitial collagen (**I**), reticulin fibers (**ret**), or in small veins (**V**); in these structures the prevalence and extent of amyloid A deposis were inverse (Table 5)

Figure 5.3 demonstrates the quantitative differences of **sAAa** and **hAAa** in different tissue structures.

Figure 5.3 **Average amount of sAAa and hAAa on different tissue structures** arranged according to the decreasing values of **sAAa/structures**

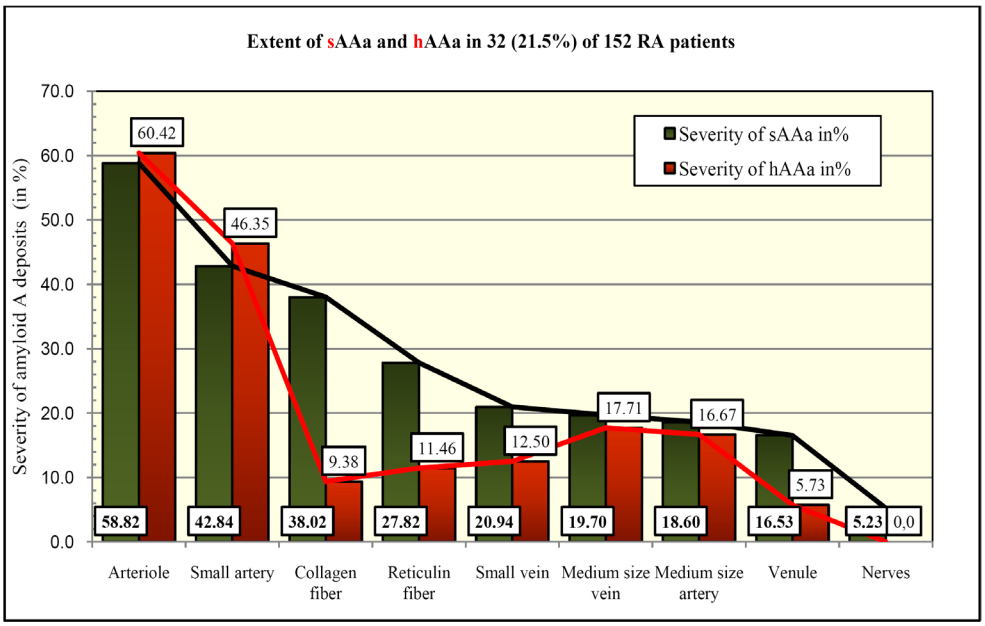


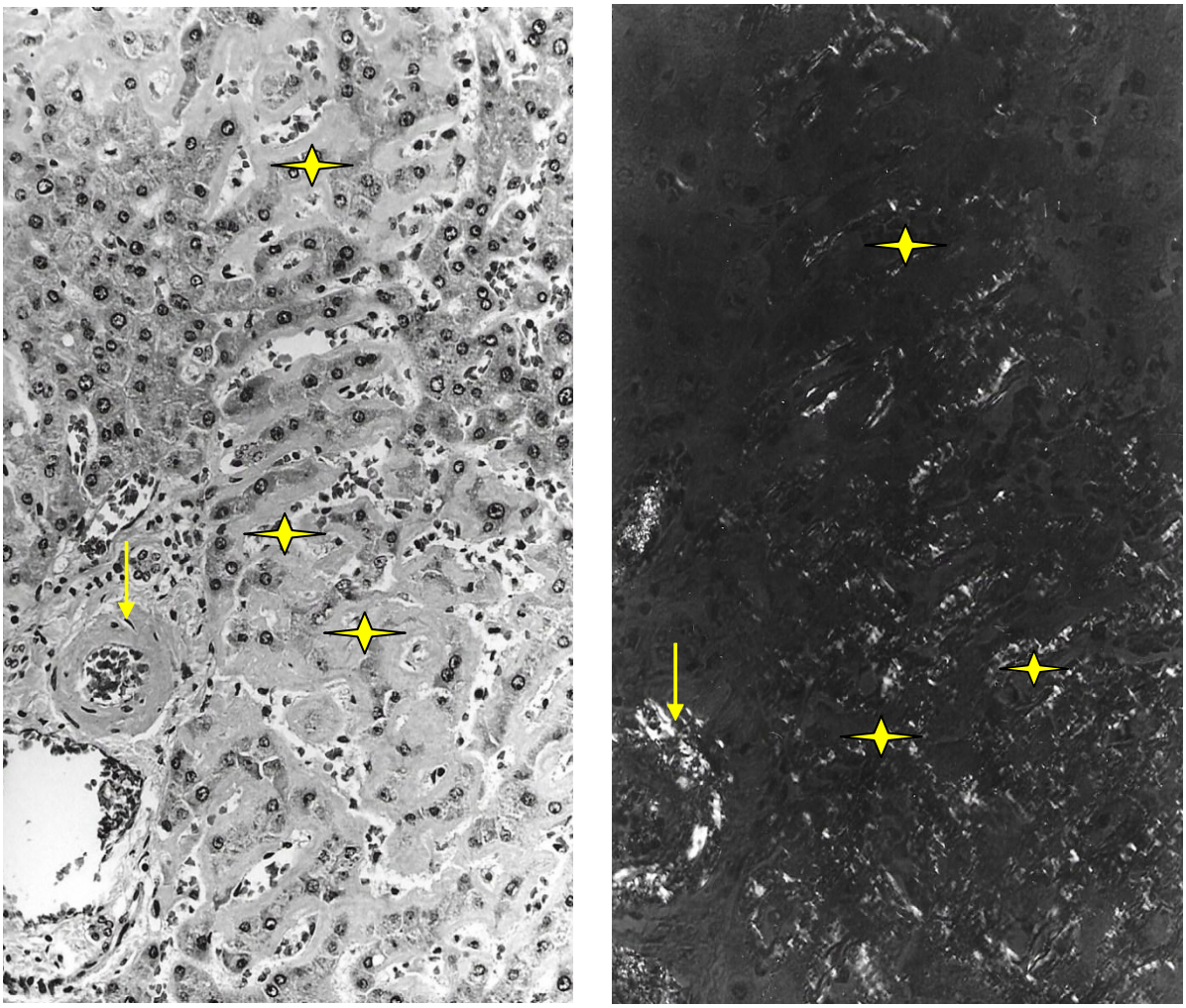
Figure5.3. *The prevalence and amount of amyloid A deposits in different tissue structures of the kidneys, heart, pancreas and lungs compared to the liver showed inverse sequence on interstitial collagen (I), reticulin fibers (ret) (Table 5).*

There was a definite discrepancy between the amounts of sinusoidal amyloid A deposition in tissue samples stained by hematoxylin-eosin and observed by light microscopy versus Congo red staining viewed under polarized light. The intensity of birefringence of amyloid deposits stained by Congo red was relatively moderate under polarized light compared to the massive sinusoidal amyloid deposition observed by traditional staining and light microscopy (Figures 6-7).

Figures 6 and 7 demonstrate the extent of amyloid A deposits in the liver by traditional HE and Congo red staining, viewed by light microscopy and under polarized light respectively.

Original magnifications correspond to the 24x36 mm transparency slide; the correct height: width ratio is 3:2. The printed size may be different; therefore it is necessary to indicate the original magnifications.

RA-sAAa 80-80 x50



a **b**

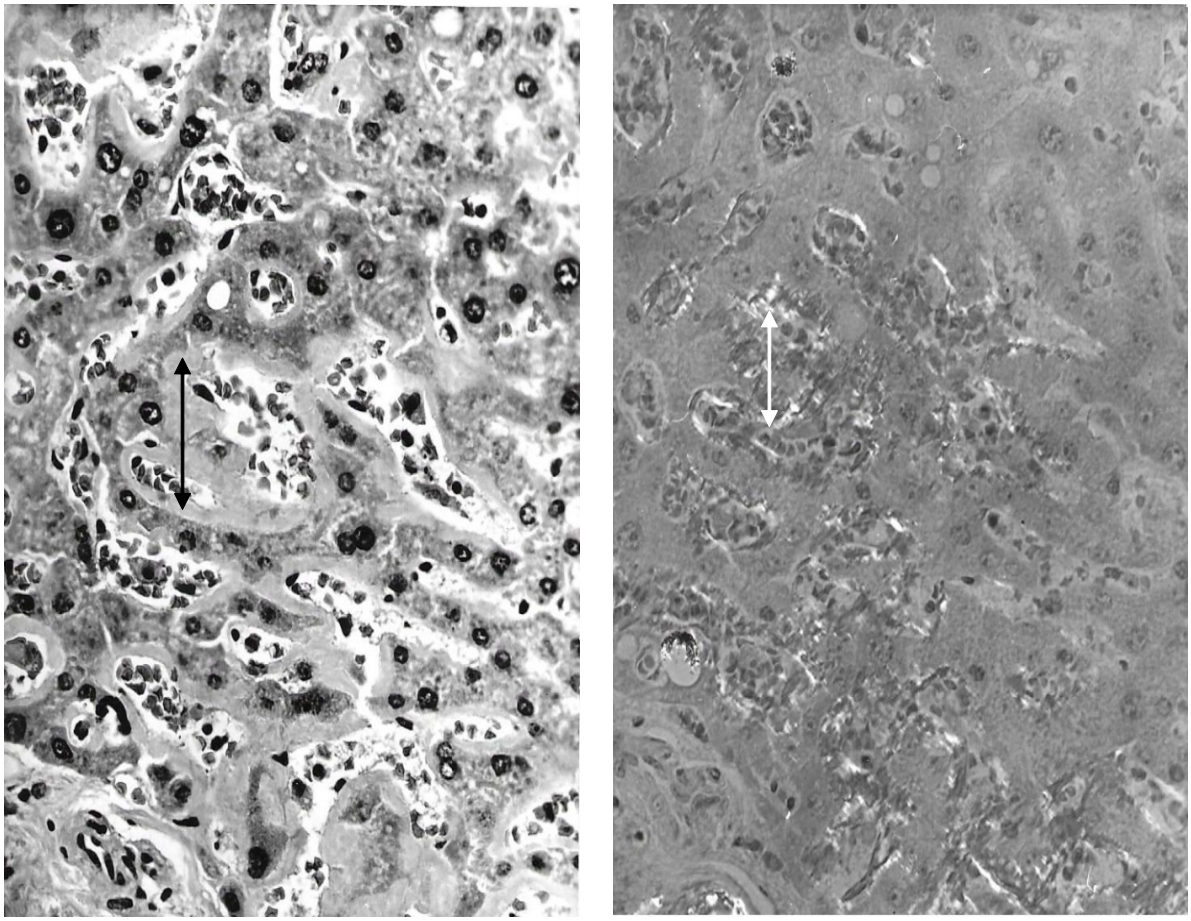
Figure 6a-b. Rheumatoid arthritis, liver with portal triad and perilobular amyloid deposits within spaces of Disse, advanced stage of hAAa

(a) Massive amyloid A deposits within the perisinusoidal areas of Disse along the reticulin fibers (stars) and in the wall of a blood vessels (arrowhead indicate an arteriole of a portal triad), liver cell plates are atrophic, HE, x125

(b) same as (a) Congo red staining, without alcoholic differentiation, covered with gum Arabic, viewed under polarized light, x125

The intensity of birefringence is relatively moderate compared to the massive deposition of amyloid A by traditional staining and ligh microscopy

RA-sAAa 80-80 x200



a b

Figure 7a-b. *Rheumatoid arthritis, liver with panlobular amyloid deposits within spaces of Disse, late stage of hAAa*
(a) The atrophic liver cell plates are covered by massive amyloid A deposits within the perisinusoidal spaces of Disse, sinusoidal endothelium is demaged, HE, x50
(b) same as (a) Congo red staining, without alcoholic differentiation, covered with gum Arabic, viewed under polarized light, x50
The intensity of birefringence is relatively moderate compared to the massive amyloid A deposits demonstrated by traditional staining and ligh microscopy

Mortality of systemic AA amyloidosis (sAAa)

sAAa was lethal in **15** (46.875 % of 32) patients due to massive amyloid A deposition in the kidneys, leading to renal insufficiency and uremia. Cardiac amyloid A deposition led to death in **3** (9.375 % of 32) patients, in further **5** (15.625 % of 32) cases **cAAa** was associated with systemic vasculitis of autoimmune origin (**AV**), atherosclerosis (**Ath**) or occlusive bronchiolitis and played an additive role, contributing to the lethal outcome. **Fourteen** (43.75 % of 32) patients with **sAAa** died of other causes such as autoimmune vasculitis, peritonitis, septic infection, etc.

sAAa was clinically diagnosed, exclusively the cases with massive renal amyloid A deposits, in **7** (21.875 %), and missed in **25** (78.125 %) of 32 patients.

cAAa or its pathogenic role in mortality was not recognized (Table 6).

The basic disease, complication(s) and associated diseases of **32 RA** patients with **sAAa** and the mortality of **rAAa** and **cAAa** are summarized in Table 6.

Amyloid A deposits in the liver did not play a direct role in lethal outcome in our **RA** patient's population with **sAAa**.

Table6. *Mortality of renal (n=15) and cardiac amyloidosis (n=3) in 32 RA patients with sAAa arranged according to the increasing values of average amounts of systemic amyloid A deposits/patient (horizontal lines)*

	f/m	Pr n⁰ / year	Basic disease	Complication 1	Complication 2	Cause of death	CoD	Cl+/Cl-	Associated disease(s)	sAAa	hAAa
1	**f**	155/87	**bTu**	Ependymoma, **sAAa**	Vertebral fracture	Pulmonary embolism		**Cl-**	RA-Ath-HT	**0,028**	**0,000**
2	**f**	240/88	**RA**	**sAAa, AV**	Coronary arteriolitis	Myocardiocytolysis		**Cl-**	Tb-F-mTb	**0,028**	**0,000**
3	**f**	76/79	**RA**	**sAAa**, Duodenal ulcer	Perforation	Peritonitis		**Cl-**		**0,111**	**0,000**
4	**f**	243/87	**RA**	**sAAa, AV,** Coronary arteritis	Coronary arteriolitis	Circulatory failure, **cAAa**	**cAAa**	**Cl-**		**0,111**	**0,000**
5	**f**	287/91	**RA**	**sAAa**, Nodular epicarditis		Circulatory failure, **cAAa**	**cAAa**	**Cl-**	Operated breast Ca	**0,278**	**0,000**
6	**f**	183/92	**RA**	**sAAa**, Colitis		Peritonitis, Sepsis		**Cl-**		**0,361**	**0,111**
7	**f**	266/78	**RA**	**sAAa**		Lethal SI		**Cl-**	Ath	**0,370**	**0,111**
8	**f**	226/85	**RA**	**sAAa**	mTu-*sporadic. vasculitis	Parafocal pneumonia		**Cl-**	Ca Bralveolare-Ath	**0,389**	**0,111**
9	**f**	395/76	**RA**	**sAAa, AV,** Coronary arteritis	Coronary arteriolitis	Myocardiocytolysis-**cAAa**	**cAAa**	**Cl-**	TbFc-mTb	**0,417**	**0,111**
10	**f**	430/80	**RA**	**sAAa**		**cAAa**	**cAAa**	**Cl-**	Ca of gallbladder	**0,417**	**0,111**
11	**f**	162/78	**RA**	**sAAa,** Gastric ulcer-Bleeding	Perforation-Peritonitis	Lethal SI		**Cl-**		**0,458**	**0,111**
12	**f**	80/80	**RA**	**sAAa**		**rAAa-U**	**rAAa-U**	**Cl+**	Ath	**0,481**	**0,667**
13	**f**	45/74	**RA**	**sAAa**		Circulatory failure	**cAAa**	**ND**		**0,583**	**0,444**
14	**f**	90/85	**RA**	**sAAa, AV**		Myocardial necrosis		**Cl-**	Ath-DM	**0,611**	**1,778**
15	**m**	342/86	**RA**	**sAAa**		**rAAa-U**	**rAAa-U**	**Cl-**	Ac Neurinom-Ath	**0,611**	**0,333**
16	**f**	322/81	**RA**	**sAAa**		**cAAa**	**cAAa**	**Cl-**		**0,778**	**0,556**
17	**f**	203/88	**RA**	**sAAa**, Femoral vein thrombosis	Femoral artery thrombosis	**rAAa-U**	**rAAa-U**	**Cl-**		**0,778**	**0,167**
18	**f**	39/76	**RA**	**sAAa**		**rAAa-U**	**rAAa-U**	**Cl-**	Ath	**0,833**	**0,667**
19	**f**	265/80	**RA**	**sAAa**		**rAAa-U**	**rAAa-U**	**Cl+**	Ca of pancreas	**0,833**	**0,889**
20	**f**	V/T	**RA**	**sAAa**		**rAAa-U**	**rAAa-U**	**Cl-**		**0,889**	**1,111**
21	**f**	52/92	**Ath-Hy**	**sAAa**	Myocardial fibrosis	Bronchopneumonia		**Cl-**	RA	**0,903**	**0,000**
22	**f**	245/88	**RA**	**sAAa**, Bronchiolitis obliterans	Multifocal pneumonia	Multifocal pneumonia, **cAAa**	**cAAa**	**Cl-**	DM	**1,000**	**0,556**
23	**m**	232/74	**RA**	**sAAa**		**rAAa-U**	**rAAa-U**	**Cl+**		**1,056**	**1,000**
24	**m**	43/85	**RA**	**sAAa, AV**		**rAAa-U**	**rAAa-U**	**Cl-**	DM- HT	**1,153**	**1,222**
25	**f**	367/75	**RA**	**sAAa**		Myocardial necrosis-**cAAa**	**cAAa**	**Cl-**	Ath-DM	**1,250**	**0,778**
26	**f**	73/87	**RA**	**sAAa**		**rAAa-U**	**rAAa-U**	**Cl+**		**1,361**	**1,111**
27	**f**	174/88	**RA**	**sAAa**		**rAAa-U**	**rAAa-U**	**Cl-**		**1,389**	**0,889**
28	**f**	237/70	**RA**	**sAAa**		**rAAa-U**	**rAAa-U**	**Cl-**	Ath	**1,556**	**1,167**
29	**f**	255/83	**RA**	**sAAa**		**rAAa-U**	**rAAa-U**	**Cl+**	DM	**1,570**	**2,000**
30	**f**	101/90	**RA**	**sAAa**		**rAAa-U**	**rAAa-U**	**Cl-**	HT	**1,861**	**0,667**
31	**m**	181/80	**RA**	**sAAa**		**rAAa-U**	**rAAa-U**	**Cl+**	Neurinom	**2,028**	**1,556**
32	**m**	53/87	**RA**	**sAAa**		**rAAa-U**	**rAAa-U**	**Cl+**		**2,028**	**1,000**

Remarks to Table 5

Basic disease: underlying disease related to death

Complication: consequence of basic disease leading directly to death (**sAAa**, etc)

Associated (Accompanying) disease: important disorder without direct causal role in death

Pr n⁰/y – Protocol number / year

CoD: Cause of **D**eath: **rAAa** – Uremia due to massive amyloid A deposition in the kidneys with renal insufficiency (n=**17**), **cAAa** – lethal outcome exclusively caused by cardiac amyloidosis (n=**3**) (430/80, 322/81, 45/74); **cAAa** – cardiac amyloidosis only contributed to the death (n=**5**) (243/87, 287/91, 367/75, 395/76, 245/88); further patients died of consequence of autoimmune vasculitis, septic infection, peritonitis, bronchopneumonia, pulmonary embolism

hAAa – had no direct role in lethal outcome of RA patients with **sAAa**

f: female, **m**: male

Cl+: – Clinically recognized **sAAa** in **7** (21.875 %) of 32 patients

Cl-: – Clinically not recognized **25** (78.125 %) of 32 patients

*sporadic not specific vasculitis associated to Ca

bTu: – benign tumor (Ependymoma)

AV: – systemic vasculitis of autoimmune origin

SI: – lethal septic infection

Ath: – Atherosclerosis

HT: – Hypertension

Tb – Post-primary (**Fc** – fibrocaseous) tuberculosis

mTb – active miliary dissemination of **Tb**

DM – adult type II diabetes mellitus

Myocardiocytolysis – Multifocal microinfarction of myocardium

Discussion

Numerous early autopsy studies discuss the prevalence of **sAAa** in **RA,** its role in mortality, but only a few of these mention involvement of the liver (Table 7) [11 – 35].

Table7. *Prevalence and mortality of sAAa in RA autopsy patients – the role of rAAa, cAAa, and hAAa in lethal outcome (Extended data published by Miklós Bély and Ágnes Apáthy in: Journal of Clinical Trials in Cardiology, 2019; 6(1): 1-20. Doi: 10.15226/2374-6882/6/1/, in press [10])*

References	Year of Publication	Autopsy n=	Prevalence of sAAa n – %	Mortality of sAAa n – %	Prevalence of rAAa n – %	Prevalence of cAAa n – %	Prevalence of hAAa n – %
Bayles	1943 [11]	23	ND	3 of 23 – 13.0%	Nm	Nm	Nm
Baggenstoss and Rosenberg	1943 [12]	30	2 – 6.6%	1 of 30 – 3.3%	Nm	Nm	1 of 30 – 3.3%
Rosenberg and Baggenstoss	1943 [13]	30	2 – 6.6%	1 of 30 – 3.3%	1/2 – 50.0%	Nm	Nm
Young and Schwedel	1944 [14]	33	5 – 15.2%	0 of 33 – 0%	2/5 – 40.0%	Nm	2/5 – 100.0%
Unger et al.	1948 [15]	58	4 – 6.9%	ND	4/4 – 100.0%	1/4 – 25.0%	4/4 – 100.0%
Teilum and Lindahl	1954 [16]	28	17 – 60.7%	7 of 28 – 25.0%	14/17 –82.35%	1/17 –5.88%	6/17 –35.29%
Gedda	1955 [17]	45	11 – 24.4%	9 of 45 – 20.0%	9/11 – 81.81%	Nm	1/11 – 9.09%
Sinclair and Cruickshank	1956 [18]	16	4 – 25.0%	0 of 16 – 0%	3/4 –75.0%	1/4 –25.0%	2/4 –50.0%
Missen and Tailor	1956 [19]	47	8 – 17.0%	4 of 47 – 8.5%	4/8 –50.0%	2/8 –20.0%	8/8 –100.0%
Lebowitz	1963 [20]	62	6 – 10.0%	ND	Nm	Nm	Nm
Sokoloff	1964 [21]	19	0 – 0%	0 of 19 – 0%	ND	ND	ND

Cohen	1968 [22]	42	11 – 26%	ND	"most common" /**19**	"may be present" /**19**	ND
Karten	1969 [23]	95	1 – 1.05%	ND	ND	ND	ND
Gritsman	1969 [24]	15	6 – 40.0%	ND	Nm	Nm	Nm
Ozdemir et al.	1971 [25]	47	1 – 2.1%	ND	1/47 –2.13%	1/46 –2.17%	1/47 –2.13%
Gardner	1972 [26]	142	17 – 11.97%	ND	ND	"may be present"	"may be present"
Püschel	1973 [27]	143	15 – 10.5%	ND	Nm	Nm	Nm
Vroninks et al.	1973 [28]	62	3 – 4.84%	0 of 62– 0%	Nm	Nm	Nm
Hajzok et al.	1976 [29]	16	7 – 43.7%	ND	Nm	Nm	Nm
Eulderink	1976 [30]	111	ND	6 of 111– 5.4%	Nm	Nm	Nm
Rainer et al.	1978 [31]	79	ND	4 of 79– 5.0%	Nm	Nm	Nm
Boers et al. *	1987 [32]	132	14 – 10.6%	ND	*14/132 – 10.6%	ND	ND
Bély	1993 [33]	161	34 – 21.1%	17 of 161– 11%	29/**33** – 87.87%	29/**33** – 87.87%	26/**32** – 81.25%
Suzuki et al.	1994 [34]	81	17 – 21.0%	6 of 81– 7.4%	Nm	Nm	Nm
Bély and Apáthy**	2006 [35]	234	48 – 20.5%	20/234 – 8.5%%	41/46 – 89.13%	ND	ND

Remarks to Table 7

Amyloid deposits were identified with different staining methods methods of diverse specificities and sensitivities: Toluidine blue, Crystal violet, Syrius red, Congo red staining according to Romhányi, Bennhold's, Puchtler's, Bély's Congo red method [1]

ND – No **D**ata

Nm – Not **m**entioned

*** –** 132 patients with **RA –** only kidneys were discussed (Boers et al) [32]

Red – different cohorts or number of autopsied patients published by authors in referenced study

**** –** Prevalence of renal, cardiac or hepatic involvement by AA amyloidosis was not mentioned in the original publication and had been determined retrospectively

It is difficult to estimate the true prevalence of **sAAa** in **RA** since it depends on the specificity and sensitivity of the demonstration technique. Using a less sensitive staining method some positive cases remain undetected. A more specific method potentially detects more cases, and reveals earlier stages. Unspecific staining methods may be misleading about the prevalence and mortality of **sAAa**.

Amyloidosis was diagnosed with different methods of diverse specificities and sensitivities in most early publications. In most of these early studies the prevalence or mortality of **sAAa** seems to be over or underestimated compared to our results, presumably due to the limited microscopic examination of various organs, or unspecific staining methods (Table 7) [11-35]. Unfortunately only a few studies specify the relationship between **sAAa** and amyloid A deposition in various organs, including kidneys, heart or liver, etc. According to our best knowledge a detailed analysis regarding the rate of amyloid A deposition in the liver, and its relationship to **sAAa** and mortality has not been available in the literature. Systemic amyloidosis is associated with the cardiovascular system, and amyloid A deposition in the liver is connected with it, as evidenced by the close association between them. ($c=1.0$, $\chi^2=111.9587$, $p<0.0000$).

The precursor of amyloid A protein fibrils is the serum amyloid A (**SAA**) polypeptide [36, 37]. **SAA** is produced by the liver, spread via the bloodstream, and is deposited in target organs throughout the body. The level of precursors in the blood depends on the production and/or elimination of amyloid proteins or, more succinctly, on the dynamics of these two processes. Excretion of circulating **SAA** is plausible by the kidneys; **SAA** level is elevated in chronic renal failure [38, 39], and resolution or endogenous degradation of deposited amyloid A is also possible [40]. Less understood is the clearance, and catabolism of circulating **SAA** by the liver itself. Amyloid A deposition on the liver cell plates may be an appropriate cleaning mechanism as well. Periferolobular (zone 1) deposition may result from

the blood inflow from the branches of portal veins and arteries, while the centrilobular (zone 3) deposition may be due to retrograde flow in cardiac insufficiency and stasis.

There was an inverse relationship between prevalence and extent of amyloid A deposits on collagen and reticulin fibers of liver indicated by the intensity of birefringence in tissue sections stained with Congo red and viewed under polarized light (Figure 3.2). Oriented deposition of amyloid molecules on collagen fibers is more pronounced than the irregular deposition on the surface of liver cell plates. The Congo red molecules are oriented parallel to the surface of the amyloid filaments and – viewed under polarized light – produce an intensive, additive, linear (parellel axis) positive birefringence. This intensive additive linear positive birefringence is specific for amyloid filaments, and in a dark microscopic field even small amounts of amyloid deposits may give a detectable birefringence, in contrast to the less intensive irrregular deposition on the surface of liver cell plates.

The average amount of amyloid A deposits is consequently inverse on collagen and reticulin fibers of liver compared to the systemic amyloid A deposition of other organs, except the pancreas; in the pancreas the amyloid A deposition on reticulin fibers of peripancreatic fat tissue was more pronounced, than on interstitial collagen fibers (Table 5 and Figure 5.3). The enhanced amyloid A deposition on reticulin fibers may be related to the sinusoidal clearing effect of the liver. The role of the reticuloendothelial system (Kupffer cells, sinus endothelial, and perisinusoidal Ito cells) in blood clearance also cannot be ruled out (Figures 8-10).

Ito cells store vitamin A in lipid droplets and are the main fibrogenic cell type of the liver [41]. Sigmund and co workers concluded (2016) that "**SAA** may modulate fibrogenic responses in the liver in a positive and negative fashion by inducing inflammation, proliferation and cell death of Ito cells" [42].

Electron microscopic characteristics of perisinusoidal Ito cells are demonstrated in Figures 8-10.

The original magnification (O) of electron microphotographs correspond to the 60x90 mm negatives

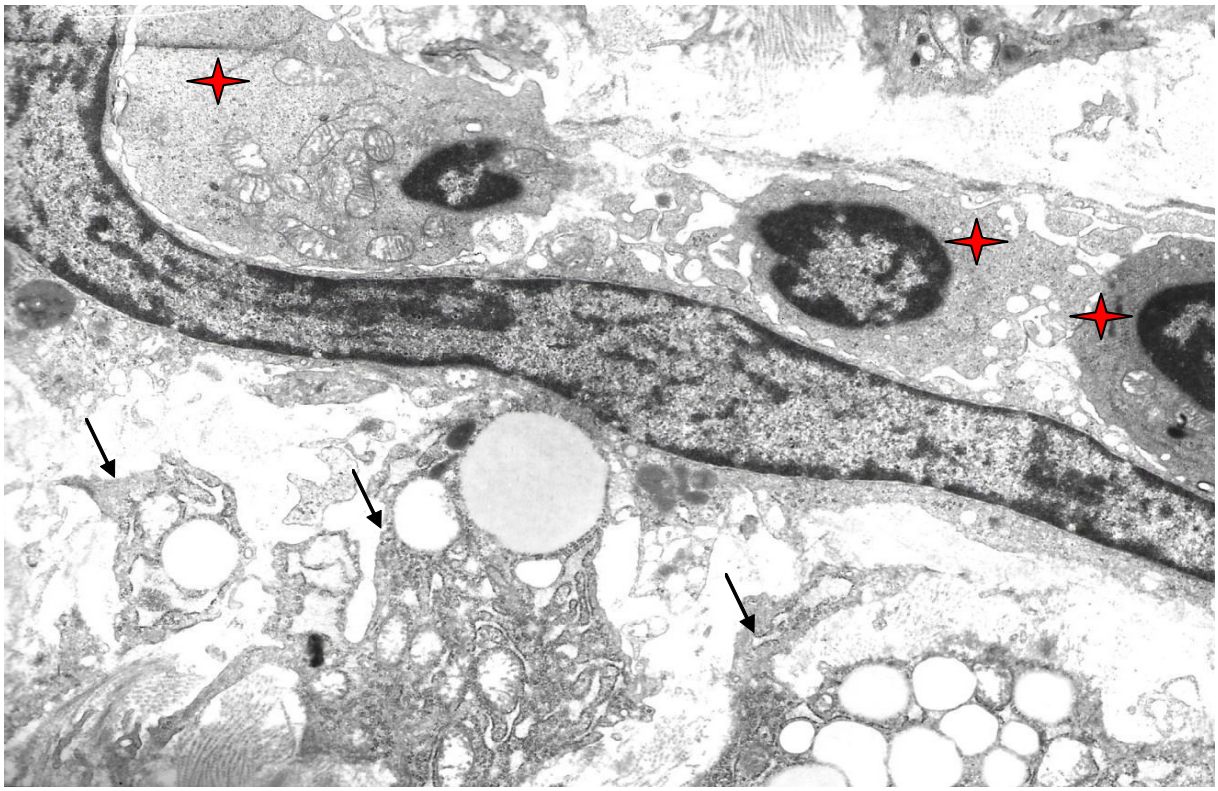


Figure8. *Liver, atrophic liver cell between lymphoid cells (stars), and lipid droplet containing perisinusoidal Ito cells (arrow heads), O: x3300*

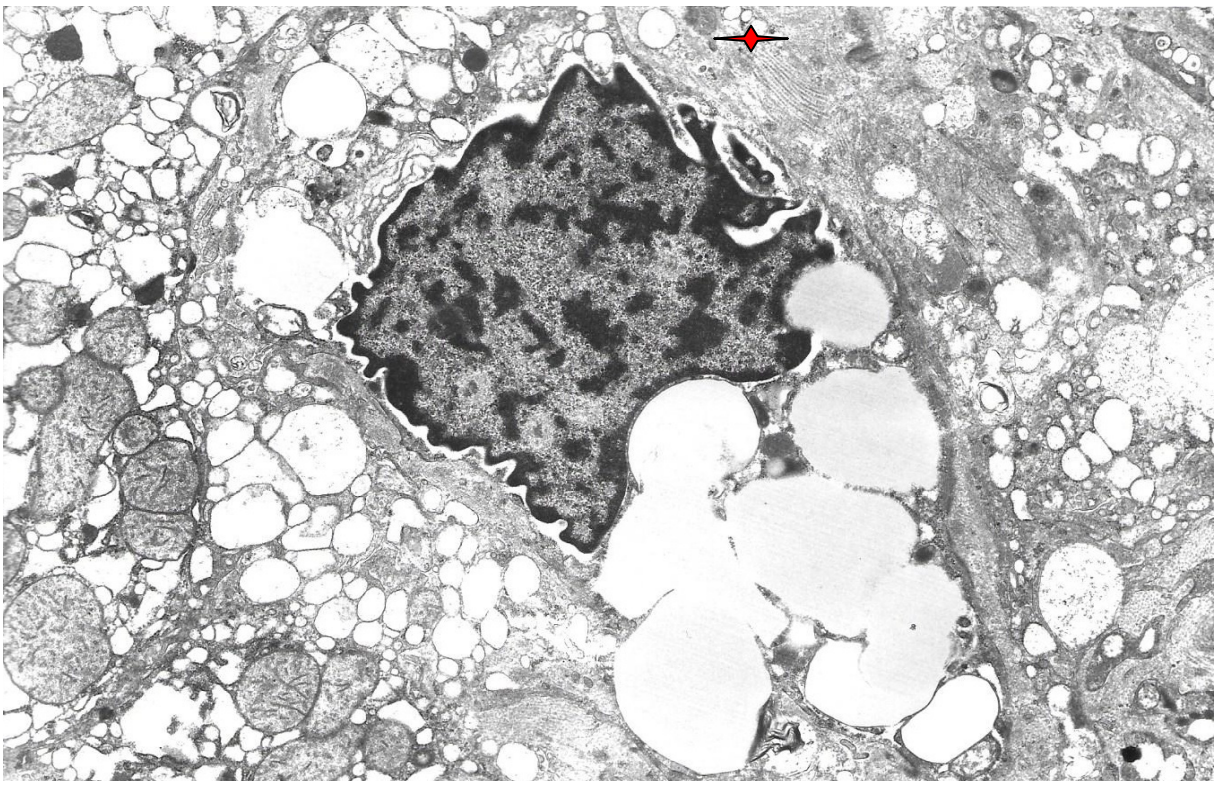


Figure9. *Liver, extended Disse space, transformation of lipid containing Ito cells into myofibroblast, with collagen fibers (stars) in starting perisinusoidal fibrosis, O: x3300*

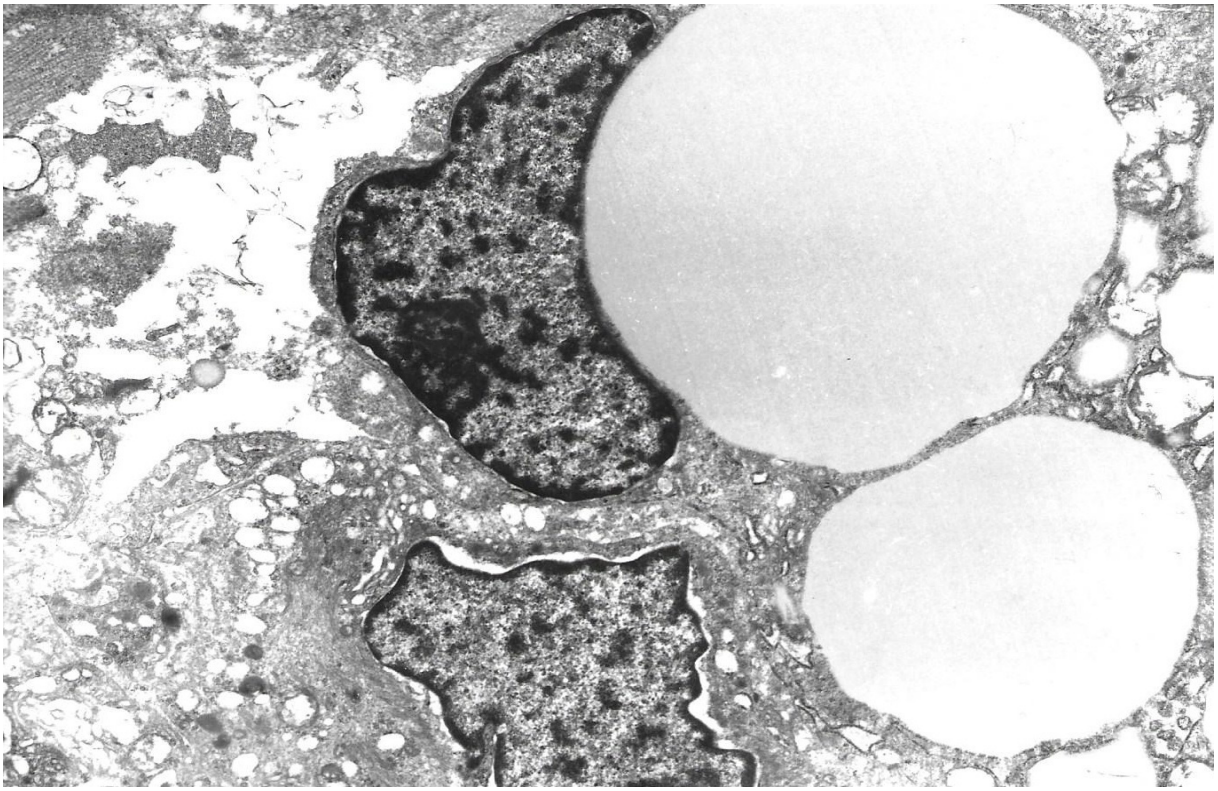


Figure10. *Liver, Ito cells with large lipid droppets, and deformed nucleus, O: x3300*

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 [1, 33, 43-44] (Figures 3 and 4).

The degree of amyloid A deposition in the liver also demonstrates this progressive cumulative process. Amyloid A deposition in the liver is delayed compared to the systemic manifestation of amyloidosis in other organs (Figure 4.3). This latency may be caused by an unspecified local protective mechanism, e.g. deposits on liver cell plates, and/or active phagocytic activity of Kupffer cells, and sinusoidal endothelial cells, etc.

Prevalence and ***severity*** of amyloid A deposits on different tissue structures of the liver or of various organs signify different aspects of the same pathological process which usually run parallel to each other (Figures 3.1 and 4.1).

Development of ***mild*** and ***severe*** amyloidosis represents the same process, based on the linear growth course of amyloid A deposition (Figures 3.4 and 4.4).

The ***increment*** of amyloid A deposits ***in early, advanced*** and ***late stages of*** **hAAa** and **sAAa** show basically linear growth, ***representing nearly the same rate*** and ***equal speed of amyloid A deposition*** in various organs. In the ***end stage*** amyloid A deposition progresses rapidly and the growth curve shows an exponential increment (Figures 3.4 and 4.4). The rapid increment in the end stage may be caused by the massive renal amyloidosis and the exhausted excretion capacity of the kidneys.

Mild and severe deposition of amyloid A is determined basically by the production of precursors. Quantitative differences in production of serum amyloid A may be related to a "benign" or "aggressive" clinical course of RA, which may be due to genetic and/or other factors.

Amyloid A deposition starts in the most frequently involved structures of the most frequently involved organs (Figures 5.1-5.3) [1, 33, 43-44]. The relatively constant rate of amyloid A deposition in different structures of various organs, e.g. the chronology (sequence) of amyloid A deposition allows histologically an indirect assessment of the stage of **sAAa**.

The **sAAa** can be asymptomatic or may present nonspecific symptoms. Clinical suspicion of **AA** amyloidosis is raised in case of unexplained weight loss, fatigue, anemia, impaired renal function, restrictive cardiomyopathy, hepatomegaly, or gastrointestinal complaints (malabsorption, malnutrition, diarrhea, constipation disturbed motility, bleeding) or reduced respiratory capacity; a biopsy is needed for confirmation [45].

From a prognostic point of view, amyloid A deposition in the liver does not appear to be a very serious, life-threatening complication of **RA**.

Conclusion

sAAa and **hAAa** may develop in both sexes, and at any time in the course of **RA**.

sAAa is related to the cardiovascular system, and **hAAa** is connected with it.

The amyloid A deposition in the liver starts after a latent stage, compared to **sAAa** of other organs, caused by not specified local protective mechanism.

sAAa and **hAAa** is a progressive and cumulative process, involving in its early stage only a few structures, and increasingly more in later stages of the disease. Amyloid A deposition starts in the most frequently involved structures of the most frequently involved organs.

The chronology of amyloid A deposition allows an indirect assessment of the stage of **sAAa** or **hAAa**, which may have a prognostic value in biopsies.

Amyloid A deposition on the liver cell plates may be an appropriate cleaning mechanism as well, but the role of the reticuloendothelial system (Kupffer cells, sinus endothelial, and perisinusoidal Ito cells) in blood clearance also cannot be ruled out.

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Citation: **Miklós Bély, Ágnes Apáthy.** *AA Amyloidosis of the Liver in Rheumatoid Arthritis – A Postmortem Clinicopathologic Study of 152 Autopsy Patients. Archives of Gastroenterology and Hepatology. 2020; 3(2): 1-26.*