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Research Article

Comparative Study of Gastric, Small and Large Intestinal AA Amyloidosis in Rheumatoid Arthritis - A Postmortem Clinicopathologic Study of 161 Autopsy Patients

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Abstract

Aim of the Study: The aim of this study was to determine the prevalence and severity of gastrointestinal AA amyloidosis (giAAa) in different sections (stomach, small and large intestine) of the GI tract, to assess the relationship between prevalence and severity of giAAa, to compare the progression (parallel development) of amyloid A deposition in the stomach, small and large intestine, and to clarify the value of gastrointestinal biopsy in these sections of the GI tract.

Patients: One hundred sixty-one (161) random autopsy patients with RA were studied. Our patients clinically diagnosed with RA fulfilled the criteria of the American College of Rheumatology (ACR).

Methods: The presence and amount of giAAa was specified histologically. AA deposition was diagnosed histologically according to Romhányi by a modified, more sensitive Congo red staining.

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

Demographics of different patient cohorts, furthermore prevalence and amount of amyloid A deposits in different section of GI tract were compared with the Student (Welch) T-test.

Results: Systemic AA amyloidosis (sAAa) complicated RA in 34 (23,13%) of 161 patients.

Tissue blocks of one or more segments of GI tract (stomach, small or large intestine) were available in 31 (91.18% of 34) patients.

Amyloid A deposition started earlier in the small intestine, where the amyloid A deposits were more frequent and massive, exceeding the prevalence and amount of amyloid A deposits in other sections of the GI tract.

Amyloid A deposition started on arterioles, small arteries and interstitial collagen (I) fibers at the earliest, and on the venules, myocytes and nerves at the latest.

Conclusion: giAAa developed in both sexes, at any time in the course of RA, and the amyloid A deposition involved stomach, small and large intestine, with different latency.

giAAa is a progressive and cumulative processes, involving in their early stage only a few structures, and increasingly more in later stages of the disease.

The chronology of AA deposition (histological description of development) on different tissue structures of all parts of the gastrointestinal tract allows an indirect assessment of the stage of the giAAa, which may have a prognostic value in biopsies.

giAAa does not appear to be a very serious, life-threatening complication of RA, rather it is an early complication of great clinical and pathological importance as an optimal biopsy site, especially the small intestine.

***Keywords**: Rheumatoid Arthritis; Gastrointestinal AA Amyloidosis; Stomach; Small Intestine; Large Intestine*

Abbreviations

RA: Rheumatoid Arthritis; ACR: American College of Rheumatology; GI Tract: Gastro-Intestinal Tract; AA: Amyloid A Protein; AAa: Amyloid A Protein Amyloidosis; sAAa: Systemic (generalized)AA Amyloidosis; giAAa: Gastrointestinal AA Amyloidosis; ALa: Amyloid Light Chain Amyloidosis; Senile or ATTRwta: Amyloid Transthyretin wild type amyloidosis; FMF: Familial Mediterranean Fever; Pr. n0/y: Protocol Number/Year; CoD: Cause of Death; U: Uremia; Cl+ = Clinically Diagnosed; Cl- = Clinically Not Diagnosed; SD: Standard Deviation; ND: No Data

Glossary of definitions

The "prevalence" of giAAa" concerns the presence of AA protein deposits in different sections of GI tract, and conveys information about the involvement of stomach, small and large intestine.

The "prevalence of giAAa" was specified histologically based on the presence of AA protein deposits in blood vessel walls of different calibers and on tissue structures of GI tract.

Size of blood vessels 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), reticular fiber (collagen III) (ret), Basement membrane of gastrointestinal glands - (BM), Nerves (n).

The "severity of giAAa" designates different amounts of AA deposition in blood vessel walls, and on different tissue structures of stomach, small and large intestine.

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) polarizing microscope.

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.

Introduction

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

To date 49 distinct precursor proteins have been identified in humans; 22 proteins appearing as systemic amyloidosis and 27 as localized forms [1].

Nomenclature of amyloidosis

The amyloidosis syndromes are named according to their fibrillar proteins [1]. For example, after AA protein (AA) deposits (precursor: serum AA - SAA, produced by hepatocytes) the disease is named AA amyloidosis (AA protein amyloidosis - AAa), after amyloid Light chain (AL) deposits (precursor: immunoglobulin light chain-λ or light chain-κ - Lλ or Lκ, 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 β2microglobulin (Aβ2M) deposits (precursor: β2-microglobulin, produced by lymphoid-cells) is assigned (Amyloid β2M amyloidosis - Aβ2Ma), etc.

Histological characteristics of systemic and localized amyloidosis

Systemic amyloidosis is related to the cardiovascular system and its precursors become generalized via the bloodstream (the blood vessels are always involved), 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 (the blood vessels are not involved) [2-4].

All forms of amyloidosis connected to the circulation are systemic, with constant involvement of the blood vessels, and all forms of amyloidosis not connected to the circulation are isolated (localized)*; without involvement of the blood vessels [2-4].

Histologically all 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 the microscopic diagnosis of amyloid deposits [6].

Prevalence, clinical signs and symptoms of AAa

Forty-five percent (45%) of generalized (systemic) types of amyloidosis (sAAa) are secondary or reactive AAa [7].

In the United States and the Western world, ALa is the most prevalent type of systemic amyloidosis, but hereditary types of amyloidosis are diagnosed with increasing frequency [8].

Clinical signs and symptoms of amyloidosis vary, depending on the affected organ.

AA deposits affect most commonly the gastrointestinal tract (GI tract), pancreas, kidneys, heart [5].

Based on the high occurrence of gastrointestinal amyloidosis, we - like others -prefer a gastrointestinal (buccal or rectal) biopsy for correct diagnosis [5, 9] using an „appropriate staining procedure” [10].

[1]Senile amyloid is an altered derivate of wild type Transthyretin (TTRwt) present in natural conditions, and senile amyloidosis is a synonym of ATTRwt amyloidosis.

Systemic and localized forms of amyloidosis are progressive cumulative processes and have distinct dynamics.

All systemic amyloid depositions start somewhere and sometime, and in the early stages of the disease they are limited to a few structures in some organs only, in fact (literally) they are “localized”, and are only potentially systemic.

The notion of localized amyloid deposition may also be relative. Isolated B-cell dyscrasia causes localized AL amyloid deposition (without involvement of the vessels). In some cases, because of progression of the underlying diseases, the originally localized AL depositions affect the lymphatic and/or blood vessels and the process becomes "regional" or "widespread", i.e. more or less "systemic" [5]. This is indirect evidence that a localized proliferative process has acquired the features of malignancy (turned into a malignancy).

Data of the literature

The number of articles on amyloidosis is very high (exceeding three thousand).

Several authors discuss the role of chronic microbial infections, chronic reactive inflammatory diseases, autoimmune (inflammatory) diseases, chronic cachectic diseases or malignancies in AAa, others talk about the role of serum AA (SAA) or discuss the role of elevated plasma level of serum amyloid P (SAP) in amyloid A protein formation, etc.

However, to the best of our knowledge, there is no study dealing with the formal pathogenesis of amyloid deposition, progression (development) of amyloidosis or the rate of amyloid A deposition from a histopathological point of view, apart from our previous publications [11-13].

Objective of the Study

The aim of this study was to determine the prevalence and severity of gastrointestinal AA amyloidosis (giAAa) in different segments (stomach, small and large intestine) of the GI tract, to assess the relationship between prevalence and severity of giAAa, to compare the progression (parallel development) of amyloid A deposition in the stomach, small and large intestine, and to clarify the value of gastrointestinal biopsy in these sections of GI tract.

Methodology

Patients (Autopsy population)

At the National Institute of Rheumatology 9475 patients died between 1969 and 1992; among them 161 (1.699%) with RA, who were autopsied [4,5].

Our patients who were clinically diagnosed with RA fulfilled the criteria of the American College of Rheumatology (ACR) [14].

The patient's history and protocols were clinically controlled by the co-author Ágnes Apáthy rheumatologist, neurologist, the autopsies and histopathologic reports were by Miklós Bély.

Methods

The definition "GI tract" refers only to the stomach, small and large intestine; other sections, e.g. oral cavity, pharynx, esophagus were not included.

Histology, immunohistology, and histochemistry

The presence and amount of giAAa in stomach, small and large intestine was specified histologically according to Romhányi [15] by a modified, more sensitive Congo red staining [16]. AA deposits were identified in serial sections by immunohistochemical [17], and histochemical methods [18-20].

Statistics

Demographics of different patient cohorts were evaluated with the Student (Welch) T-test comparing the age, sex, onset, and duration of RA at the time of death with or without sAAa, and with giAAa, including gastric, small and large intestine AAa [21].

The "p" cutoff for significance was < 0.05.

Comparing two cohorts of data, the most severe criteria of excel calculator were used; two-tailed distribution ("2"), and non-equal variation ("not homoscedastic -3").

The mean age of the patients was variable (between minimal and maximal value of age), and the mean (average) amount of the AA deposits showed a "normal distribution"; typical bell-shaped curve of Gauss.

The correlation existing between the sAAa and giAAa was calculated with Pearson's chi-squared (χ^2) test, including gastric, small and large intestine AAa [21].

Results

Demographics of patients with systemic and/or gastrointestinal amyloidosis

sAAa complicated RA in 34 (23.13%) of 161 patients.

Tissue blocks of one or more segments of GI tract (stomach, small or large intestine) were available in 31 (91.18% of 34) patients; stomach in 28, small intestine in 23, and large intestine in 24 of 31 patients (the tissue blocks of GI tract were not available in 3 of 34 patients with sAAa).

There was a very strong positive relationship between sAAa (n = 34) and giAAa (n = 31) (c = 1.0, $\chi^2 = 137.60$, $p < 0.000$), like between giAAa (n = 31) and gastric AAa (n = 28) (c = 1.0, $\chi^{2\,=\,135.92,\ p\,<\,0.000)}$, giAAa (n = 31) and small intestine AAa (n = 23) (c = 1.0, $\chi^2 = 106.54$, $p < 0.000$), furthermore between giAAa (n = 31) and large intestine AAa (n = 24) (c = 1.0, $\chi^{2\,=\,112.25,\ p\,<\,0.000)}$.

Table 1 summarizes the demographics, onset and duration of disease of the total population (n = 161), with (n = 34) and without (n = 127) sAAa, furthermore with giAAa (n = 31), including stomach (n = 24), small intestine (n = 23), and large intestine (n = 24) AAa.

Clinical and histological diagnosis	Number of autopsies	Mean age in years at death ± SD	Mean age at onset of RA ± SD	Mean duration of RA (in years) ± SD
RA patients (total)	**161**	**65,32 ± 12,99**	**50,83 ± 17,02**	**14,43 ± 10,55**
Female	116	64,95 ± 11,84	50,19 ± 15,78	14,79 ± 10,70
Male	45	66,27 ± 15,67	52,57 ± 20,15	13,46 ± 10,22
With sAAa	**34 of 161**	**62,41 ± 15,82**	**47,61 ± 18,33**	**15,58 ± 9,51**
Female	29	64,34 ± 11,27	48,56 ± 15,54	15,70 ± 10,06
Male	5	51,20 ± 31,51	41,25 ± 34,72	14,75 ± 5,12
Without sAAa	**127 of 161**	**66,10 ± 12,07**	**51,77 ± 16,59**	**14,09 ± 10,86**
Female	87	65,15 ± 12,08	50,79 ± 15,92	14,49 ± 10,97
Male	40	68,15 ± 11,94	53,94 ± 18,05	13,30 ± 10,71
With giAAa*	**31 of 34**	**63,32 ± 14,77**	**48,29 ± 16,68**	**16,00 ± 9,66**
Female	26	65,65 ± 8,25	49,46 ± 12,62	16,21 ± 10,29
Male	5	51,20 ± 31,51	41,25 ± 34,72	14,75 ± 5,12
With gastric[2]* AAa	**28 of 31**	**64,25 ± 14,31**	**49,15 ± 17,01**	**15,00 ± 9,86**
Female	24	65,63 ± 8,60	50,59 ± 12,58	15,05 ± 9,94
Male	4	56,00 ± 34,21	41,25 ± 34,72	14,75 ± 5,12
With small intestinal AAa[3]*	**23 of 31**	**63,13 ± 15,09**	**455,68 ± 17,72**	**17,27 ± 10,28**
Female	19	64,63 ± 8,45	46,67 ± 13,01	17,83 ± 11,14
Male	4	56,00 ± 34,21	41,25 ± 34,72	14,75 ± 5,12
With large intestinal AAa[4]*	**24 of 31**	**62,50 ± 16,09**	**46,71 ± 18,36**	**16,95 ± 10,09**
Female	20	66,30 ± 8,71	48,78 ± 13,64	17,61 ± 10,68
Male	4	43,50 ± 30,47	34,33 ± 39,00	13,00 ± 4,58

Table 1: *Sex, mean age with SD, range, onset and disease duration (in years) of 161 RA patients with sAAa and giAAa.*

*Glossary to table 1: RA: Rheumatoid Arthritis; sAAa: Systemic AA amyloidosis; giAAa: Gastrointestinal AA amyloidosis; SD: Standard Deviation; *: The tissue blocks of GI tract were not available in 3 of 34 patients with sAAa; 2*: Tissue blocks of stomach were available only in 28 of 31 patients with giAAa; 3*: Tissue blocks of small intestine were available only in 23 of 31 patients with giAAa; 4*: Tissue blocks of large intestine were available only in 24 of 31 patients with giAAa.*

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 patients with sAAa (n = 34) and total population (n = 161) ($p < 0.321$, $p < 0.376$, $p < 0.554$), neither between

females (p < 0.800, p < 0.631, p < 0.682) and males (p < 0.348, p < 0.565, p < 0.688), between patients with sAAa (n = 34) and without sAAa (n = 127) (p < 0.213, p < 0.262, p < 0.462), neither between females (p < 0.745, p < 0.528, p < 0.593) and males (p < 0.297, p < 0.521, p < 0.662), furthermore between patients with sAAa (n = 34) and with giAAa (n = 31) (p < 0.811, p < 0.883, p < 0.867), neither between females (p < 0,623, p < 0.820, p < 0.861) and males (p < 1.00, p < 1.00, p < 1.00).

The difference was not significant in in survival time (mean age) at death between patient cohorts with giAAa (n = 31) and gastric AAa (n = 28) (p < 0.808), with giAAa (n = 31) and small intestine AAa (n = 23) (p < 0.963) or with giAAa (n = 31) and large intestine AAa (n = 24) (p < 0.846), neither between females (p < 0.990, p < 0.686, p < 0.800) and males (p < 0.835, p < 0.835, p < 0.722).

Comparing onset of RA, and duration of disease at the time of death there was no significant difference in onset or duration of RA between patients with giAAa (n = 31) and gastric AAa (n = 28) (p < 0.851, p < 0.700), with giAAa (n = 31) and small intestine AAa (n = 23) (p < 0.599, p < 0.658) or with giAAa (n = 31) and large intestine AAa (n = 24) (p < 0,759, p < 0.741), neither between females (p < 0.762, p < 0.490, p < 0.870) and males (p < 1.00, p < 1.00, p < 0.819).

Amyloidosis developed in both sexes, at any time in the course of the disease, and in any section of the gastrointestinal tract (Table 1 and 2).

Patient cohorts with RA	Age	Onset of RA	Duration of RA
RA patients n = 161 (total) versus pts. with sAAa n = 34 of 161	**0,321**	**0,376**	**0,554**
Female n = 116 of 161 versus n = 29 of 34	0,800	0,631	0,682
Male n = 45 of 161 versus n = 5 of 34	0,348	0,565	0,688
With sAAa n = 34 of 161 vs without sAAa n = 127 of 161	**0,213**	**0,262**	**0,462**
Female n = 29 of 34 versus n = 87 of 127	0,745	0,528	0,593
Male n = 5 of 34 versus n = 40 of 127	0,297	0,521	0,662
With sAAa n = 34 of 161 vs with giAAa n = 31 of 34	**0,811**	**0,883**	**0,867**
Female n = 29 of 34 versus n = 26 of 31	0,623	0,820	0,861
Male n = 5 of 34 versus n = 5 of 31	1,000	1,000	1,000
With giAAa n = 31 of 34 vs with gastric AAa n = 28 of 31	**0,808**	**0,851**	**0,700**
Female n = 26 of 31 versus n = 24 of 28	0,990	0,762	0,699
Male n = 5 of 31 versus n = 4 of 28	0,835	1,000	1,000
With giAAa n = 31 of 34 vs with small intestine AAa n = 23 of 31	**0,963**	**0,599**	**0,658**
Female n = 26 of 31 versus n = 19 of 23	0,686	0,490	0,632
Male n = 5 of 31 versus n = 4 of 23	0,835	1,000	1,000
With giAAa n = 31 of 161 vs with large AAa n = 24 of 31	**0,846**	**0,759**	**0,741**
Female n = 26 of 31 versus n = 20 of 24	0,800	0,870	0,671
Male n = 5 of 31 versus n = 4 of 24	0,722	0,819	0,656

Table 2: *The statistical correlations ("p" values of significance) between female and male RA patients with and without sAAa, and with giAAa.*

Glossary to table 2: RA: Rheumatoid Arthritis; sAAa: Systemic AA amyloidosis; giAAa: Gastrointestinal AA amyloidosis.

In table 2 is summarized the relationship ("p" values of correlation) of demographics, onset and duration of disease between RA patients (n = 161), with sAAa (n = 34 of 161) and without sAAa (n = 127 of 161), with giAAa (n = 31 of 34), including the stomach (n = 28), small (n = 23), and large intestine (n = 24).

Characteristics of gastrointestinal AA amyloidosis

Tissue blocks of GI tract were available in 31 of 34 patients with sAAa, and were not in 3 patients.

The accumulation of amyloid A deposits in patients with the giAAa was a progressive and basically linear process (with exponential increment in the end stage).

In 2 (6,45%) of 31 RA patients with giAAa there was no AA deposition in the GI tract; this was considered a "latent" stage of gastrointestinal amyloidosis (the amount of AA deposits was: 0,00).

In the gastrointestinal tract the arterioles, small arteries and interstitial collagen fibers (collagen I) were affected most commonly and had massive AA deposits; these may be the sites of early amyloid deposition.

The amount of AA was less frequent and moderate on reticular fibers (collagen III), medium size veins, medium size arteries, basement membranes of gastrointestinal glands, furthermore on small veins, representing an advanced stage of amyloid deposition.

Rare and minimal AA deposits occurred in the myocytes, venules and nerves; the involvement of these structures corresponded to the late (terminal) stage of amyloidosis (see vertical green lines, Table 3).

The quantitative differences of AA deposits on different tissue structures of the GI tract (n = 31) of RA patients are summarized in table 3 and figure 1.1-1.4.

	Pr. n^0/y	female / male	a	A	I	ret	VV	AA	BM	V	Myo	v	n	Avg	Cause of Death	Cl+ Cl- Dg	Prevalence / Pts. in % of "+" cases	Amount / Pts. in % of max "3"
1	237/70	f	0	0	0	0	0	0	0	0	0	0	0	0,00	rAAa-U	Cl-	0,00	0,00
2	90/85	f	0	0	0	0	0	0	0	0	0	0	0	0,00			0,00	0,00
3	155/87	f	0,5	0,5	0	0	0	0	0	0	0	0	0	0,09			18,18	3,03
4	243/87	f	0,67	0,33	0	0	0	0	0	0	0	0	0	0,09	cAAa	Cl-	18,18	3,03
5	322/81	f	1	0,67	0	0	0	0	0	0	0	0	0	0,15	cAAa	Cl-	18,18	5,06
6	183/92	f	1	1	1	0	0	0	0	0	0	0	0	0,27			27,27	9,09
7	240/88	f	0,67	0,33	0,33	1,33	0,33	0,17	0	0,33	0	0,33	0	0,35			72,73	11,58
8	287/91	f	2	1,33	0	0	0,33	0,67	0	0	0	0	0	0,39	cAAa	Cl-	36,36	13,12
9	52/92	f	2	1	0,5	0,5	0	0,5	0	0	0	0	0	0,41			45,45	13,64
10	232/74	m	2	1	1	1	0	0	0	0	0	0	0	0,45	rAAa-U	Cl+	36,36	15,15
11	76/79	f	2	1	1	0	1	0	0	0	0	0	0	0,45			36,36	15,15
12	80/80	f	3	0,75	0,5	0,5	0	0	1	0	0	0	0	0,52	rAAa-U	Cl+	45,45	17,42
13	226/85	f	2	1	1,67	0,67	0,33	0	0	0	0	0	0	0,52			45,45	17,18
14	430/80	f	2,33	1,33	1,33	0	0,67	0,33	0,33	0,33	0	0	0	0,60	cAAa	Cl-	63,64	20,15
15	39/76	f	3	2	0	0	0	1	1	0	0	0	0	0,64	rAAa-U	Cl-	36,36	21,21
16	174/88	f	2	1	1,5	0,33	0,67	0,33	1	0,33	0	0	0	0,65	rAAa-U	Cl-	72,73	21,70
17	137/76	f	3	2	1	0,5	0	1	0	0	0	0	0	0,68	rAAa-U	Cl+	45,45	22,73
18	203/88	f	2	1,33	1,5	1	0,67	0,33	0	0,33	0,5	0	0	0,70	rAAa-U	Cl-	72,73	23,21
19	245/88	f	2,67	2	0,33	0	0,33	1	0	0	1,67	0	0,33	0,76	cAAa	Cl-	63,64	25,24
20	45/74	f	3	2	2	0,67	1	0,33	0	0,33	0	0	0	0,85	cAAa	ND	63,64	28,27
21	342/86	m	2	1,33	2	1	1,33	0	1,67	0	0	0	0	0,85	rAAa-U	Cl-	54,55	28,27
22	73/87	f	3	2,67	2	0,33	0,83	1,33	0,33	0,17	0,67	0	0,33	1,06	rAAa-U	Cl+	90,91	35,33
23	395/76	f	2,33	1,17	3	3	1,33	0	0	1	0	0	0	1,08	cAAa	Cl-	54,55	35,85
24	367/75	f	3	2,5	2	1	1	1,5	1,5	0,25	0	0	0	1,16	cAAa	Cl-	72,73	38,64
25	162/78	f	3	2	2	1	0	1	0	1	2	1	0	1,18			72,73	39,39
26	43/85	m	3	2,5	1,5	0,75	1,5	1,5	0,5	1	1,5	0,5	0,5	1,34	rAAa-U	Cl-	100,00	44,70

27	181/80	m	3	2	2,67	2,67	0,67	0,33	1,33	0,17	0,67	0	1,67	1,38	rAAa-U	Cl+	90,91	46,00
28	255/83	f	3	2,33	2	0,67	2	1,33	2,67	1	0	0,33	0,33	1,42	rAAa-U	Cl+	90,91	47,45
29	101/90	f	2,67	2,33	1,67	2	2,33	1	1,33	1,67	0,67	1,17	0	1,53	rAAa-U	Cl-	90,91	51,03
30	265/80	f	3	2	2,67	2,67	3	1	0,67	2	0	1	0	1,64	rAAa-U	Cl+	81,82	54,58
31	53/87	m	3	2,33	2,33	2,33	2,33	1,67	2,33	1,33	1,33	0,33	0,83	1,83	rAAa-U	Cl+	100,00	61,03
32	266/78	f																
33	V/T	f													rAAa-U	Cl-		
34	306/90	f													rAAa-U	Cl+		
	Statistics																	
31	Count		31	31	31	31	31	31	31	31	31	31	31	31	23	Cl+		
	Sum		65,84	43,73	37,50	23,92	21,65	16,32	15,66	11,24	9,01	4,66	3,99	23,05		8		
	Avg		2,124	1,411	1,210	0,772	0,698	0,526	0,505	0,363	0,291	0,150	0,129	0,743	0,7435	Cl-		
	SD		0,97	0,78	0,93	0,89	0,83	0,56	0,76	0,55	0,57	0,33	0,34	0,51		14		
	"0" n		2	2	7	11	12	12	18	16	23	24	25	2		ND		
	"+" n		29	29	24	20	19	19	13	15	8	7	6	29		1		
	Prev. %		93,55	93,55	77,45	64,55	61,29	61,29	41,95	48,39	25,81	22,58	19,36	93,56				
	Sev. %		70,79	47,02	40,32	25,72	23,28	17,55	16,84	12,09	9,69	5,01	4,29	24,78				
	GI tract																	
			1	2	3	4	5	6	7	8	9	10	11					
			a	A	I	ret	VV	AA	BM	V	Myo	v	n	Avg				

Table 3: *Prevalence and amount of AA deposits in RA patients with giAAa, and on different tissue structures of the GI tract arranged according to the increasing average amounts of AA deposits /patient (vertical column of average amount /patients), and the decreasing amount of AA deposits /structure (horizontal line in %).*

Remarks to table 3: Pr. n^0/y - Protocol number / year; Prevalence / Pts. in %: Positive cases in % of 11 gastrointestinal tissue structures. Amount / Pts. in %: Average amount of amyloid A in % of maximal value of severity ("3") certified (detected) on 11 gastrointestinal tissue structure.

Tissue blocks of GI tract were not available in 3 (266/78, V/T, 360/90) of 34 patients with sAAa.

In 2 (6.45%) of 31 RA patients with giAAa amyloid A deposits were not found in the GI tract, these represented a latent stage of gastrointestinal amyloidosis (the amount of AA deposits was: 0.00).

sAAa led to death in 23 of 31 patients with giAAa; 8 of these 23 were diagnosed clinically, and 14 were not (in one case clinical data were not available).

CoD - Cause of death:

Uremia led to death in 15 of 31 patients due to massive AA deposition in the kidneys (rAAa-U) with consecutive renal insufficiency.

Cardiac insufficiency with lethal outcome led to death in 3 (322/81, 430/80, 45/74) of 31 patients exclusively caused by cardiac amyloidosis (cAAa).

Cardiac amyloidosis (cAAa - in combination with other reasons) contributed to the death in additional 5 (243/87, 287/91, 245/88, 395/76, 367/75) of 31 patients.

Gastrointestinal amyloidosis did not play a direct role in mortality.

Cl+: Clinically recognized (n = 8) - Cl-: Clinically not recognized (n = 14). ND - No Data (n = 1). f: Female, m: Male. Avg: Average. SD: Standard Deviation.

Prev. % - Prevalence in %: prevalence of giAAa in % of 31 patients conveys the presence of AA on different tissue structures of GI tract.

Sev. % - Severity in %: severity of AA in % of 31 patients designates the average AA deposits on different tissue structures of GI tract in % of maximal value ("3").

Abbreviations: Arteriole (a), Small Artery (A), Medium Size Artery (AA), Venule (v), Small Vein (V), Medium Size Vein (VV) [22], Interstitial Collagen Fiber (I), Reticular Fiber (Collagen III) (ret), Basement Membrane of Intestinal Gland (BM), Smooth Muscle Cells of Tunica Muscularis (Myo), nerve (n).

The prevalence and amount of AA deposits of patients with giAAa run basically parallel to each other (see trend lines of increment in figure 1.1).

Prevalence and severity of gastrointestinal AA deposits showed a more or less pronounced difference in some patents (Figure 1.1).

Figure 1.1 demonstrates the prevalence and amount of amyloid A deposits in 31 RA patients with giAAa.

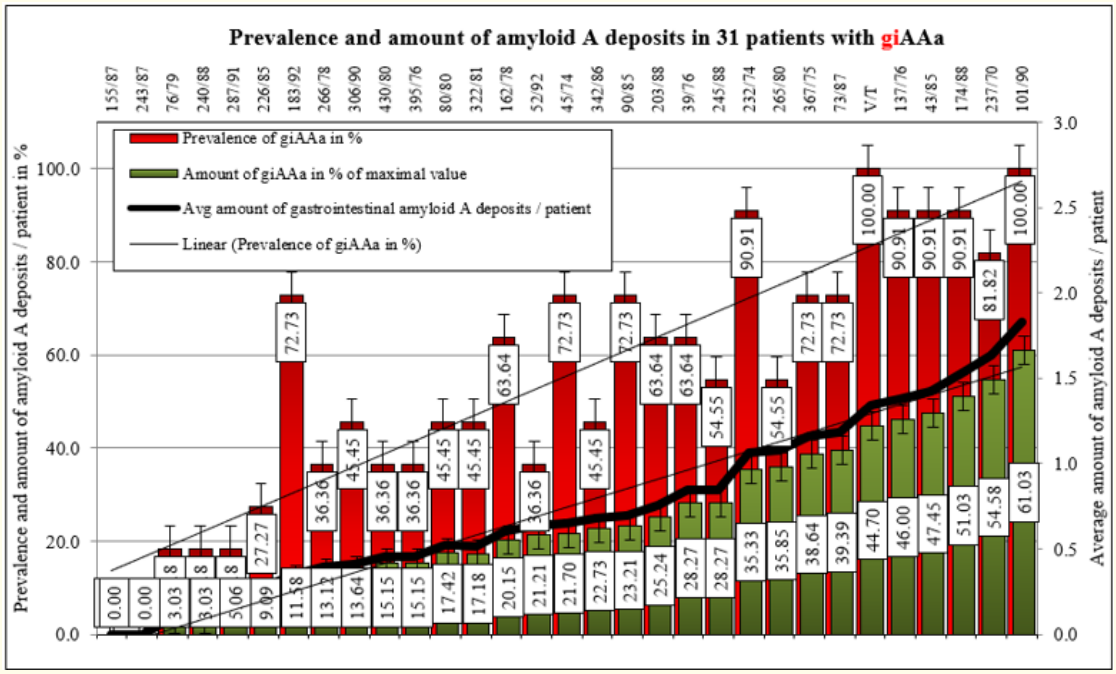

Figure 1.1: *Prevalence and amount of amyloid A deposits in 31 (19.14%) of 161 patients with giAAa arranged according to the increasing average amount of amyloid A deposits /patient (vertical column of table 3).*

Legend to figure 1.1: The prevalence and amount of amyloid A deposits in RA patients with giAAa changed basically parallel (see trend lines) with considerable individual variations (Table 3).

The individual differences were mainly due to the contingency of sampling and the limitations of the semi-objective evaluation method.

The prevalence and amount of AA deposits on different tissue structures of the GI tract ran parallel to each other. The frequently involved tissue structures showed marked deposits of amyloid. Deposits were less striking in less frequently involved tissue structures.

Difference was only found for basement membranes and small veins (V), in which the difference was inverse (Table 3 and figure 1.2).

Quantitative differences (prevalence and amount) of AA deposits on different tissue structures of GI tract with giAAa are summarized in figure 1.2.

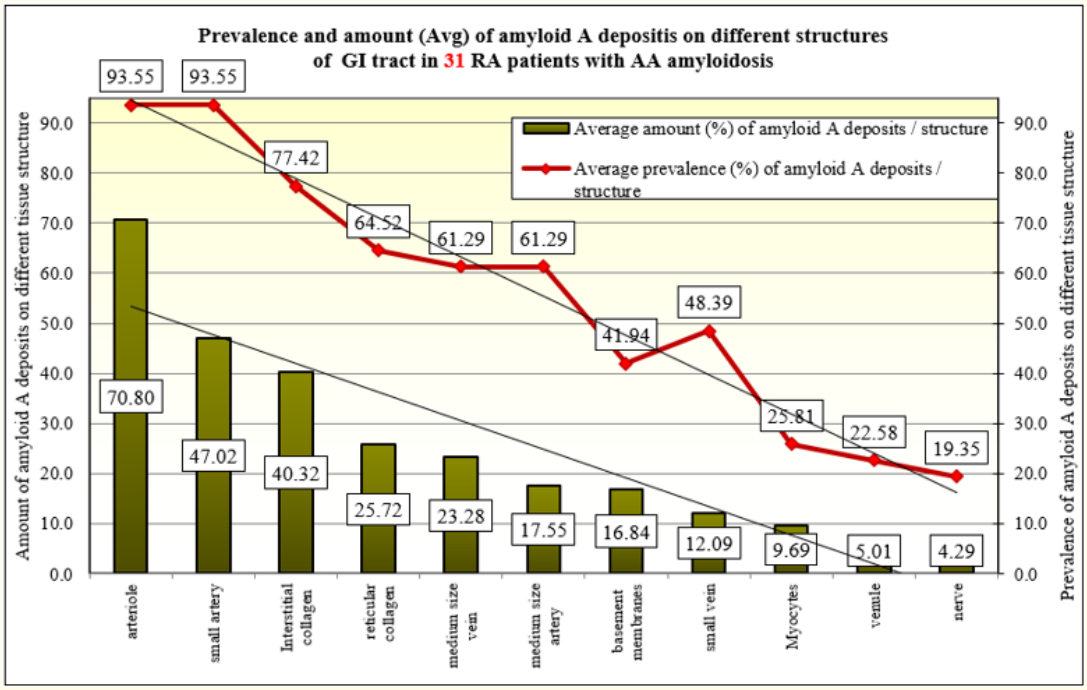

Figure 1.2: *Prevalence and amount of AA deposits on different tissue structures of the GI tract.*

Legend to figure 1.2: Average prevalence and amount of AA deposits on different tissue structures of the GI tract changed basically parallel.

Difference was only found for basement membranes and small veins (V), in which deposition was inverse (Table 3 and figure 1.2).

Detectable amounts of AA deposits on different tissue structures of the GI tract did not appear simultaneously.

In the early stage of giAAa there were histologically detectable amyloid deposits only on a few structures (arterioles, small arteries, interstitial collagen fibers), in advanced stages more structures were involved (reticular fibers, medium size veins and arteries, basement membranes of gastrointestinal glands or small veins. On other gastrointestinal structures (smooth muscle cells, venules, and nerves) AA deposits were seen only in late stages of giAAa (with massive involvement of the former (Figure 1.3).

The amount of deposited AA was different on tissue structures, and increased simultaneously, but the proportion of deposited AA was nearly constant and independent of the stage of amyloidosis.

The amount of AA protein deposits on different tissue structures of the GI tract is demonstrated in figure 1.3.

Characteristics of gastric AA amyloidosis

Tissue blocks of the stomach were available in 28 of 31 patients with giAAa.

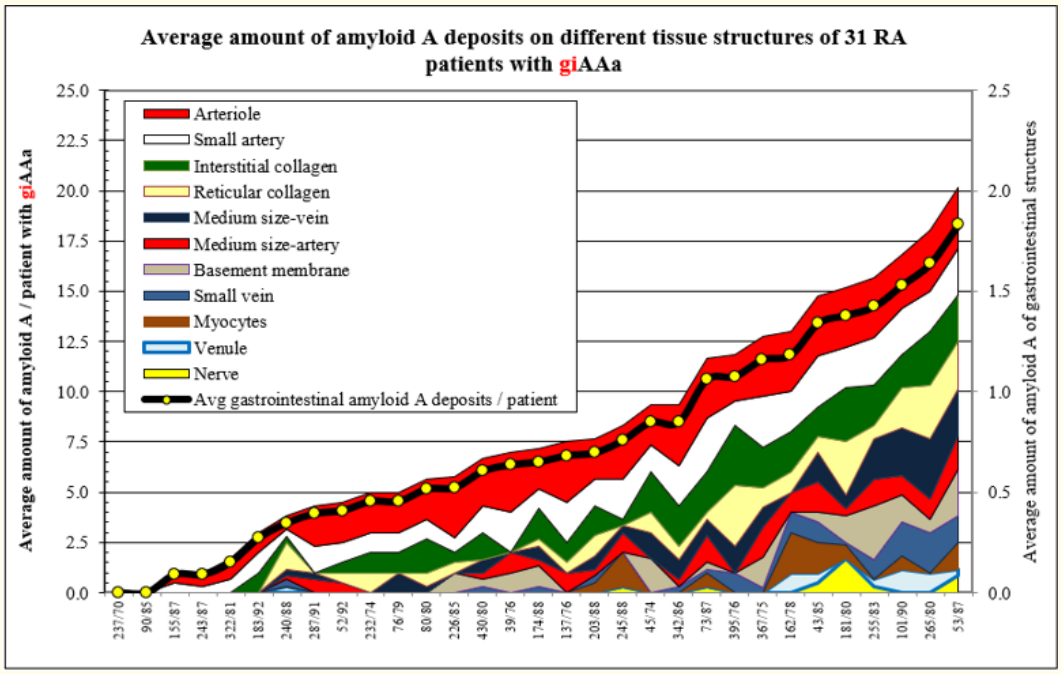

Figure 1.3: *Chronology (development) of AA deposition on different structures of the GI tract in 31 RA patients with giAAa arranged according to the decreasing amount of AA deposits/structure.*

Legend to figure 1.3: The amount of AA deposits on different tissue structures of the GI tract is arranged according to their decreasing severity (Table 3).

The amount of gastrointestinal amyloid A deposits /patient (black line) shows the average cumulative amount of amyloid A deposits on different tissue structures; the increment is nearly linear, constant and continuous.

AA deposition did not start at the same time on different tissue structures of the GI tract.

The amount of AA deposits on different tissues increased simultaneously, the rate was nearly constant on different tissue structures and independent of the stage of amyloidosis.

The differences of individual patients were mainly due to the contingency of sampling and the limitations of the semi-objective evaluation method.

In 4 (14.29%) of 28 RA patients complicated with giAAa there was no AA deposition in the stomach; this was considered a "latent" stage of gastric amyloid deposition (the amount of AA deposits was: 0.00).

The accumulation of AA deposits in the stomach was also a progressive and basically linear process (with an abrupt start and exponential increment at the end stage).

In the stomach - similarly to other locations in the gastrointestinal tract - the arterioles, small arteries and interstitial collagen fibers (collagen I) were affected most commonly and had massive AA deposits; these were the early sites of gastric amyloid deposition.

On reticular fibers (collagen III), basement membranes, medium size arteries and veins the amount of AA was less frequent and moderate, representing an advanced stage of gastric amyloid deposition.

Rare and minimal AA deposits occurred on myocytes, nerves, small veins, and venules; the involvement of these structures corresponded the late premortem (terminal) stage of amyloidosis (Table 4).

The sequence of amyloid A deposition in the stomach differed from that of the general gastrointestinal, small or large intestine amyloid A deposition at advanced and late stage of amyloidosis (at in the early stage the sequence was the same).

The quantitative differences of AA deposits on different tissue structures of the stomach in 28 RA patients are summarized in table 4 and figure 2.1-2.3.

The prevalence and amount of AA deposits in patients with gastric AAa run basically parallel to each other (See table 4 and the trend lines of increment (Figure 2.1), except in the early and end stages of amyloid deposition.

In the early stage of the gastric AAa the AA deposition started abruptly, and in the terminal stage AA deposition progressed again rapidly and the growth curve showed an exponential increment figure 2.1).

In some patients there was a considerable difference between prevalence and severity of gastric AAa due to the contingency of sampling and the limitations of the semi-objective evaluation method (Figure 2.1).0

Figure 2.1 demonstrates the prevalence and amount of AA deposits in the stomach of 28 RA patients with gastric AAa.

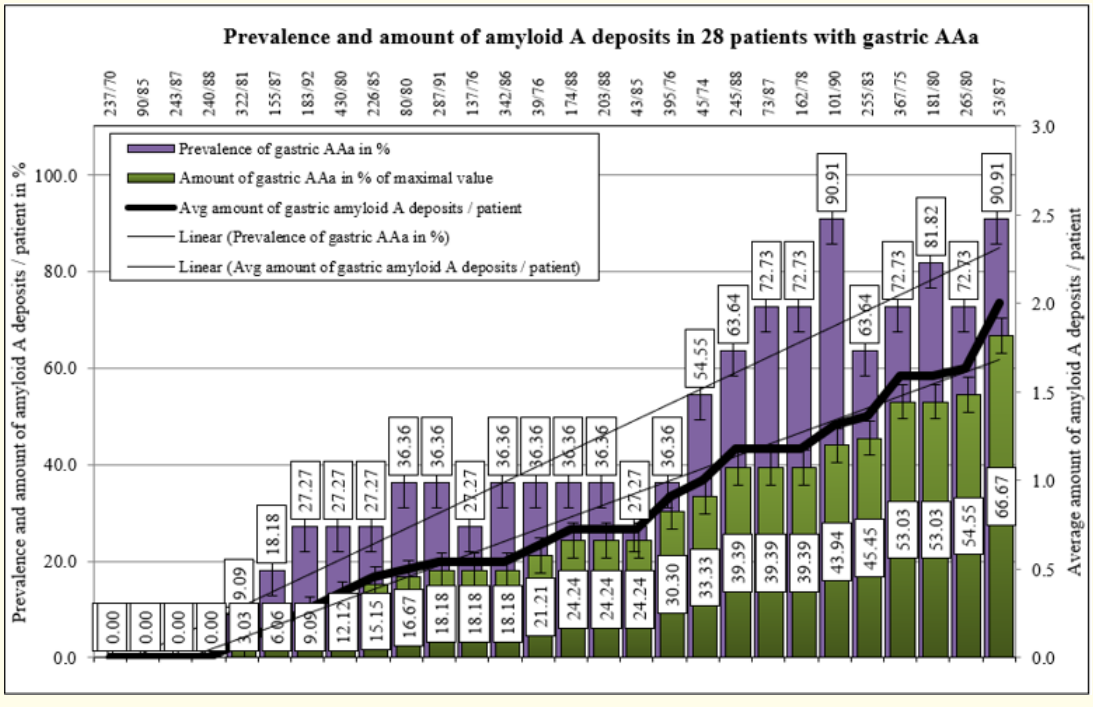

Figure 2.1: *Prevalence and amount of AA deposits in 28 RA patients with gastric Aaa arranged according to the increasing Avg amount of AA deposits/patient.*

Legend to figure 2.1: The prevalence and amount of amyloid A deposits in the stomach changed parallel (see trend lines) with considerable individual differences (Table 4).

The prevalence and amount of AA deposits on different tissue structures of the stomach ran 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 reticular collagen (III) and medium size arteries (AA), basement membranes and medium size veins (VV), furthermore nerves and small veins (V), in which the differences were inverse (Table 4 and figure 2.2).

Quantitative differences (prevalence and amount) of AA deposits on different tissue structures of gastric AAa are summarized in figure 2.2.

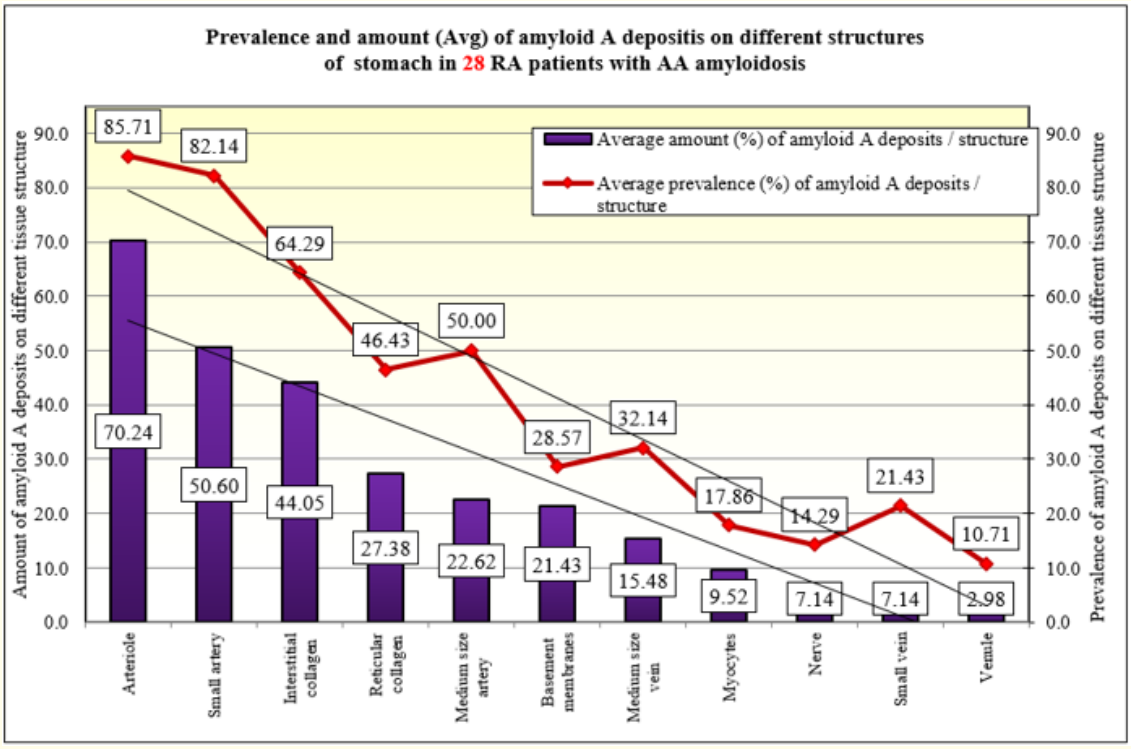

Figure 2.2: *Prevalence and amount of AA deposits on different tissue structures of the stomach.*

Legend to figure 2.2: Average prevalence and amount of AA deposits on different tissue structures of the stomach changed basically parallel.

Differences were found only for reticular collagen (III) and medium size arteries (AA), basement membranes and medium size veins (VV), furthermore nerves and small veins (V), in which depositions were inverse (Table 3).

Detectable amounts of AA deposits on different tissue structures of the stomach did not appear simultaneously.

In the early stage of gastric AAa there were histologically detectable amyloid deposits only on a few structures (arterioles, small arteries, interstitial collagen fibers (I). In advanced stages more structures were involved (reticular fibers, medium size arteries, basement membranes of gastric glands, and medium size veins veins). On other structures (smooth muscle cells, nerves, small veins, and venules) AA deposits were seen only in late stages of gastric AAa, with massive involvement of the former (Figure 2.3).

The amount of deposited AA was different on tissue structures, and increased simultaneously, but the proportion of deposited AA was nearly constant and independent of the stage of amyloidosis.

The amount of AA protein deposits on different tissue structures of the stomach is demonstrated in figure 2.3.

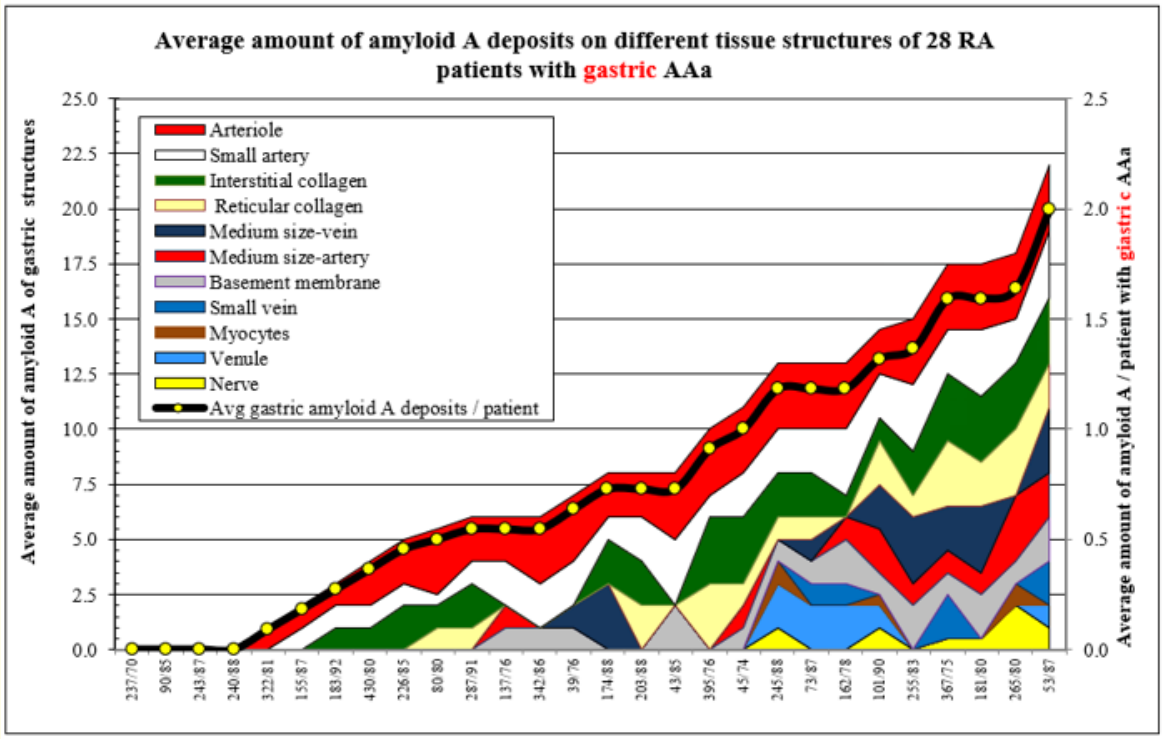

Figure 2.3: *Chronology (development) of AA deposition on different structures of the stomach in 28 RA patients with gastric AA arranged according to the decreasing amount of AA deposits/structure.*

Legend to figure 2.3: The amount of AA deposits on different tissue structures of the stomach is arranged according to their decreasing severity (Table 4).

The amount of gastric amyloid A deposits /patient (black line) shows the average of the cumulative amount of amyloid A deposits on different tissue structures; the increment is nearly linear, constant and continuous, except the end stage of the gastric AAa.

AA deposition not started on different tissue structures of the GI tract at the same time.

The amount of AA deposits on different tissues increased simultaneously, the rate was nearly constant and independent of the stage of amyloidosis.

The differences of individual patients were mainly due to the contingency of sampling and the limitations of the semi-objective evaluation method.

AA deposits in the stomach are demonstrated on figure 2.4 and 2.5.

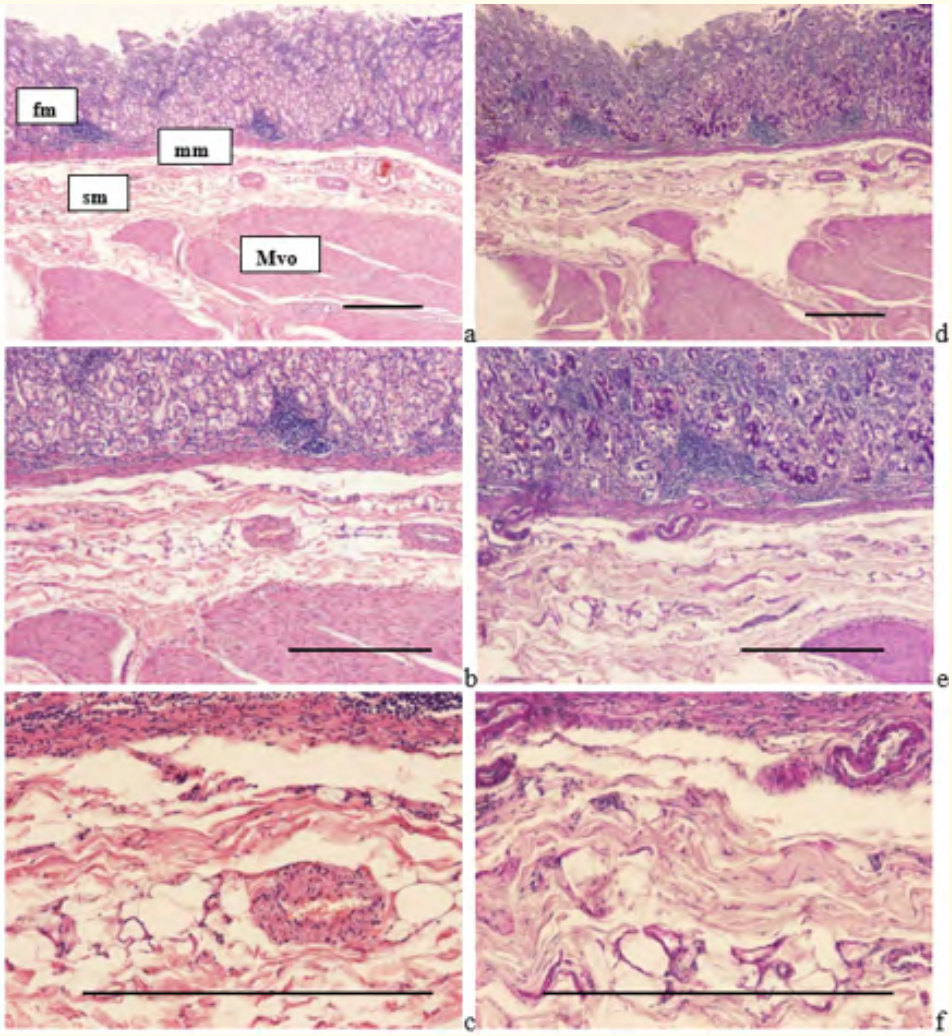

Figure 2.4a-2.4f: *Rheumatoid arthritis, systemic secondary AA amyloidosis, gastrointestinal AA deposits with erosive gastritis.*

Gastric fundic mucosa (fm), muscularis mucosae (mm), submucosa (sm), and muscularis propria (Myo).

AA deposition in the submucosa, within the walls of arterioles (a) and small arteries (A), and interstitial collagen fibers.

(a) HE, scale bar: 1000 [µm], magnification: ×20; (b) same as (a) scale bar: 1000 [µm], magnification: ×40; (c) same as (a) scale bar: 1000 [µm], magnification: ×100; (d) PAS, same as (a) scale bar: 1000 [µm], magnification: ×20; (e) same as (c) scale bar: 1000 [µm], magnification: ×40; (f) same as (a) scale bar: 1000 [µm], magnification: ×100.

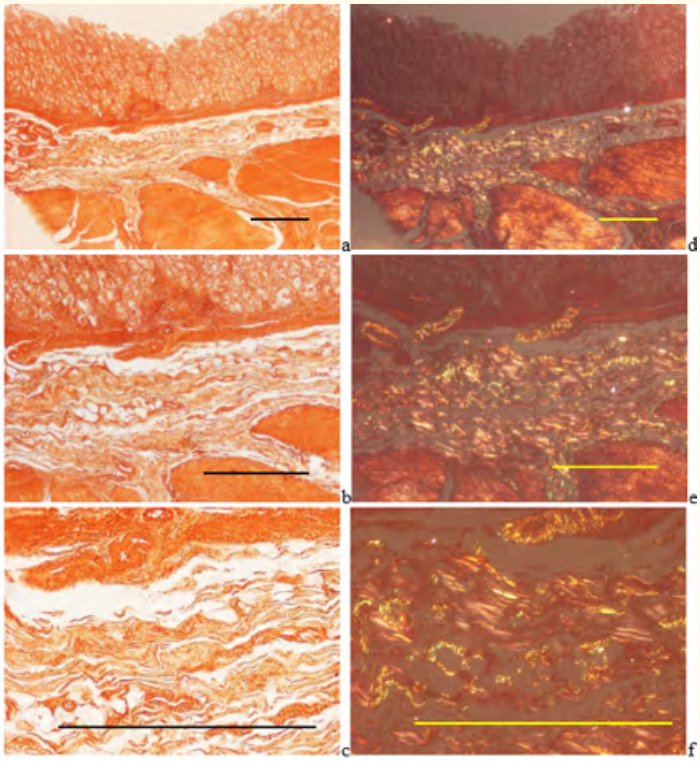

Figure 2.5a-2.5f: *Rheumatoid arthritis, systemic secondary AA amyloidosis, gastrointestinal AA deposits with erosive gastritis.*

Gastric fundic mucosa (fm), muscularis mucosae (mm), submucosa (sm), and muscularis propria (Myo), same microscopical fields as figure 2.4a-2.4f

AA deposition in the submucosa, within the walls of arterioles (a) and small arteries (A), and interstitial collagen fibers.

(a) Congo red staining, without alcoholic differentiation, covered with gum Arabic, scale bar: 1000 [µm], magnification: ×20; (b) same as (a) scale bar: 1000 [µm], magnification: ×40; (c) same as (a) scale bar: 1000 [µm], magnification: ×100; (d) Congo red staining, without alcoholic differentiation, covered with gum Arabic, and viewed under polarized light, same as (a) scale bar: 1000 [µm], magnification: ×20; (e) same as (d) scale bar: 1000 [µm], magnification: ×40; (f) same as (d) scale bar: 1000 [µm], magnification: ×100.

Characteristics of amyloid A deposition of the small intestine

Tissue blocks of the small intestine were available in 23 of 31 patients with giAAa.

In 1 (4.35%) of 23 RA patients with giAAa there was no AA deposition on the tissue structures of the small intestine; this was considered a "latent" stage of small intestine amyloid deposition (the amount of AA deposits was: 0.00).

The accumulation of AA deposits in the small intestine was also a progressive and basically linear process (with an abrupt start and exponential increment at the end stage).

In the small intestine - similarly to the amount of total (general) gastrointestinal amyloid A deposit - the arterioles, small arteries, and interstitial collagen fibers (collagen I), were affected most commonly and had massive AA deposits, which represented the early sites of amyloid deposition.

On the medium size veins, reticular fibers (collagen III), small veins, medium size arteries and the basement membranes the amount of AA was less frequent and moderate, representing an advanced stage of small intestine amyloid deposition.

Rare and minimal AA deposits occurred on the smooth muscle myocytes, venules, and nerves (similarly to the sequence of the total gastrointestinal deposits); the involvement of these structures corresponded to a late (terminal) stage of amyloidosis (Table 5).

The quantitative differences of AA deposits on different tissue structures of the small intestine in 23 RA patients are summarized in table 5 and figure 3.1-3.3.

	Pr. n^0/y	female / male	A	A	I	VV	Ret	V	AA	BM	Myo	V	N	Avg	Cause of Death	Cl+ Cl- Dg	Prevalence / Pts. in %	Amount / Pts. in % of max "3"
1	237/70	f	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	rAAa-U		0,00	0,00
2	243/87	f	2,00	1,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,27	cAAa	Cl-	18,18	9,09
3	322/81	f	1,00	2,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,27	cAAa	Cl-	18,18	9,09
4	76/79	f	2,00	1,00	1,00	1,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,45			36,36	15,15
5	287/91	f	3,00	2,00	0,00	0,00	0,00	0,00	1,00	0,00	0,00	0,00	0,00	0,55	cAAa	Cl-	27,27	18,18
6	226/85	f	2,00	1,00	2,00	1,00	1,00	0,00	0,00	0,00	0,00	0,00	0,00	0,64			45,45	21,21
7	430/80	f	3,00	2,00	1,00	0,00	0,00	0,00	1,00	0,00	0,00	0,00	0,00	0,64	cAAa	Cl-	36,36	21,21
8	52/92	f	3,00	2,00	1,00	0,00	1,00	0,00	1,00	0,00	0,00	0,00	0,00	0,73			45,45	24,24
9	137/76	f	3,00	2,00	2,00	0,00	1,00	0,00	1,00	0,00	0,00	0,00	0,00	0,82	rAAa-U	Cl+	45,45	27,27
10	245/88	f	3,00	2,00	0,00	0,00	0,00	0,00	1,00	0,00	3,00	0,00	0,00	0,82	cAAa	Cl-	36,36	27,27
11	45/74	f	3,00	2,00	1,00	1,00	1,00	1,00	0,00	0,00	0,00	0,00	0,00	0,82	cAAa	ND	54,55	27,27
12	240/88	f	1,00	1,00	1,00	1,00	3,00	1,00	0,50	0,00	0,00	1,00	0,00	0,86			72,73	28,79
13	174/88	f	3,00	2,00	1,00	2,00	0,00	1,00	1,00	0,00	0,00	0,00	0,00	0,91	rAAa-U	Cl-	54,55	30,30
14	73/87	f	3,00	3,00	1,00	1,50	0,00	0,50	2,00	0,00	0,00	0,00	0,00	1,00	rAAa-U	Cl+	54,55	33,33
15	203/88	f	3,00	2,00	1,00	2,00	0,00	1,00	1,00	0,00	1,50	0,00	0,00	1,05	rAAa-U	Cl-	63,64	34,85
16	342/86	m	3,00	2,00	2,00	3,00	0,00	0,00	0,00	3,00	0,00	0,00	0,00	1,18	rAAa-U	Cl-	45,45	39,39
17	181/80	m	3,00	3,00	3,00	1,00	3,00	0,00	0,00	1,00	0,00	0,00	0,00	1,27	rAAa-U	Cl+	54,55	42,42
18	395/76	f	3,00	2,00	3,00	3,00	3,00	2,00	0,00	0,00	0,00	0,00	0,00	1,45	cAAa	Cl-	54,55	48,48
19	265/80	f	3,00	2,00	2,00	3,00	2,00	2,00	1,00	0,00	0,00	1,00	0,00	1,45	rAAa-U	Cl+	72,73	48,48
20	255/83	f	3,00	2,00	2,00	3,00	1,00	2,00	1,00	3,00	0,00	1,00	1,00	1,73	rAAa-U	Cl+	90,91	57,58
21	43/85	m	3,00	2,00	3,00	3,00	1,50	2,00	1,00	1,00	3,00	1,00	1,00	1,95	rAAa-U	Cl-	100,0	65,15
22	53/87	m	3,00	2,00	3,00	3,00	2,00	2,00	2,00	3,00	1,00	1,00	0,50	2,05	rAAa-U	Cl+	100,0	68,18
23	101/90	f	3,00	3,00	2,00	3,00	3,00	3,00	2,00	1,00	1,00	2,00	0,00	2,09	rAAa-U	Cl-	90,91	69,70
24	90/85	f																
25	155/87	f																
26	183/92	f																
27	232/74	m													rAAa-U	Cl+		
28	80/80	f													rAAa-U	Cl+		

29	39/76	f												rAAa-U	Cl-		
30	367/75	f												cAAa	Cl-		
31	162/78	f															
23	Small intestine																
	Count		23	23	23	23	23	23	23	23	23	23	23	19	Cl+		
	Sum		59,00	43	32	31,5	22,5	17,5	16,5	12	9,5	7	2,5	23	6		
	Avg		2,565	1,870	1,391	1,370	0,978	0,761	0,717	0,522	0,413	0,304	0,109	1,000	Cl-		
	SD		0,84	0,69	1,03	1,26	1,15	0,95	0,69	1,04	0,91	0,56	0,30	0,57	12		
	"0" n		1	1	5	8	11	12	9	17	18	17	20	1	ND		
	"+" n		22	22	18	15	12	11	14	6	5	6	3	22	1		
	Prev. %		95,65	95,65	78,26	65,22	52,17	47,83	60,87	26,09	21,74	26,09	13,04	95,65			
	Sev. %		85,51	62,32	46,38	45,65	32,61	25,36	23,91	17,39	13,77	10,14	3,623	33,33			
			1	2	3	4	5	6	7	8	9	10	11				
			A	A	I	VV	ret	V	AA	BM	Myo	v	n	Avg			

Table 5: *Prevalence and amount of AA deposits on different tissue structures of the small intestine arranged according to the increasing average amounts of AA deposits/ patient (vertical column of average amount /patients), and the decreasing amount of AA deposits /structure (horizontal line in %).*

Remarks to table 5: Pr. n^0/y - Protocol number / year. Prevalence / Pts. in %: Positive cases in % of the total count of 11 tissue structures of the small intestine. Amount / Pts. in %: Average amount of amyloid A in % of maximal value of severity ("3") certified on 11 tissue structures of the small intestine.

Tissue blocks of small intestine were not available in 8 (90/89, 155/87, 183/92, 232/74, 80/80, 39/76, 367/75, 162/78) of 31 patients with giAAa.

In one (4.35%) of 23 RA patients amyloid A deposits were not found in the small intestine, this represented a latent stage of small intestine amyloidosis (the amount of AA deposits was: 0.00).

Cl+: Clinically recognized amyloid A deposits in small intestine (n = 6) - Cl-: Clinically not recognized (n = 12). ND: No Data (n = 1). f: Female, m: Male. Avg: Average. SD: Standard Deviation.

Prev. % - Prevalence in %: Prevalence of AAa in % of 23 patients conveys the presence of AA on different tissue structures of small intestine.

Sev. % - Severity in %: Severity of AA in % of 23 patients designates the average AA deposits on different tissue structures of small intestine in % of maximal value ("3").

The prevalence and amount of the small intestinal amyloid A deposition run basically parallel to each other, except in the early and end stages of amyloid deposition (see the trend lines of increment figure 3.1 and table 5).

In the early stage of small intestinal AAa the amyloid deposition started abruptly, and in the terminal stage AA deposition progressed again rapidly and the growth curve showed an exponential increment (Figure 3.1).

In some patients there was a considerable difference between prevalence and amount of amyloid A deposits in patients, due to the contingency of sampling, and the limitations of the semi-objective evaluation method (Figure 3.1).

Figure 3.1 demonstrates the prevalence and amount of AA deposits in the small intestine of 23 RA patients with AAa.

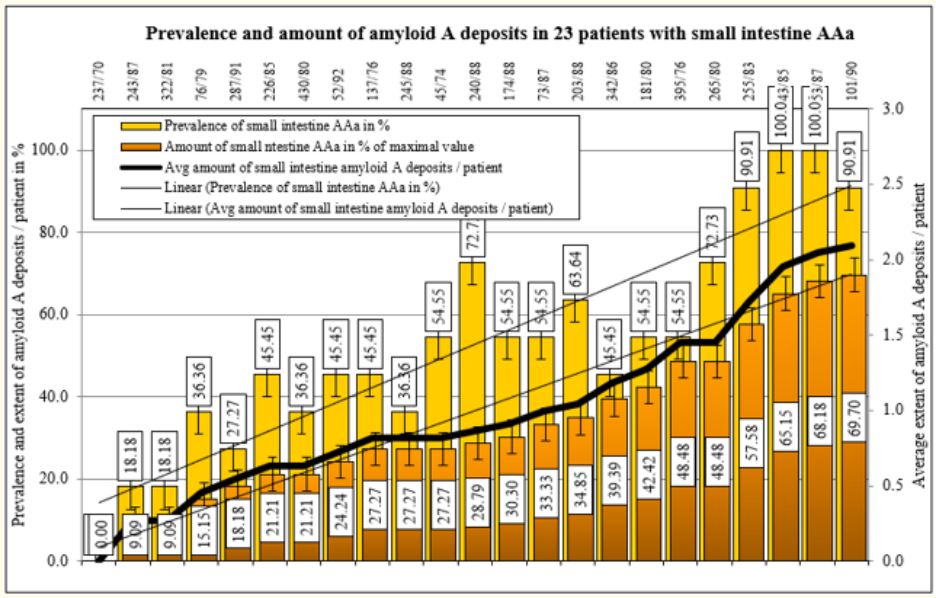

Figure 3.1: *Prevalence and amount of AA deposits of patients in the small intestine arranged according to the increasing Avg amount of AA deposits/patient.*

Legend to figure 3.1: The prevalence and amount of amyloid A deposits in the small intestine changed basically parallel, except in the early and end stage (see trend lines and table 5).

There were considerable individual differences between prevalence and amount in some patients, due to the contingency of sampling, and limitations of the semi-objective evaluation method.

The prevalence and amount of AA deposits on different tissue structures of the small intestine ran 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 the small veins (V) and medium size arteries (AA) or for myocytes and venules (v) of the small intestine, in which the relationships (relations) were inverse (Table 5 and figure 3.2).

Quantitative differences (prevalence and amount) of AA deposits on different tissue structures of small intestine AAa are summarized in figure 3.2.

Detectable amounts of AA deposits on different tissue structures of the small intestine did not appear simultaneously.

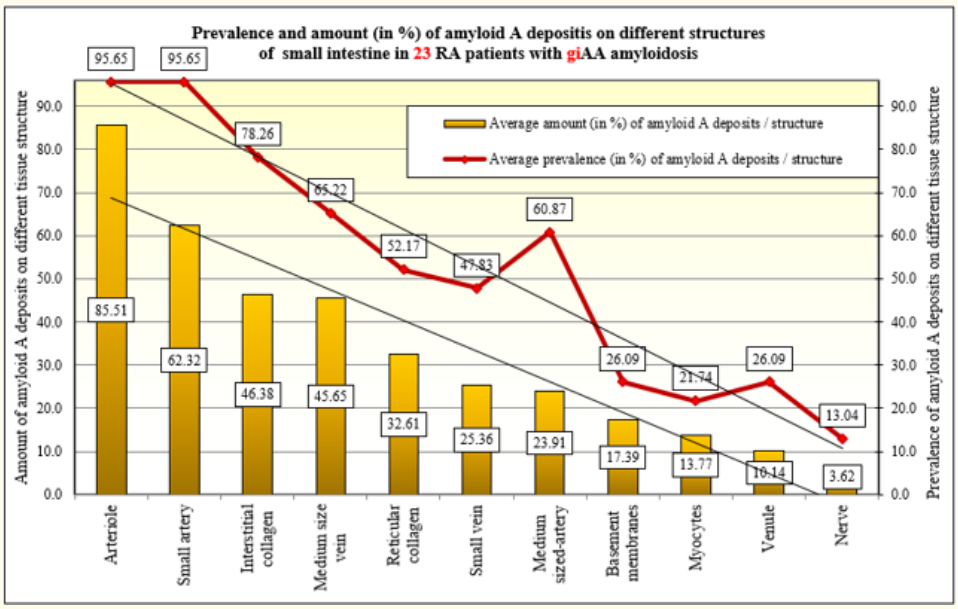

Figure 3.2: *Prevalence and amount of AA deposits on different tissue structures of the small intestine.*

Legend to figure 3.2: Average prevalence and amount of AA deposits on different tissue structures of the small intestine changed basically parallel.

Differences were found for the small veins (V) and medium size arteries (AA), furthermore for myocytes and venules (v), in which depositions were inverse (Table 5).

In the early stage of small intestine AAa there were histologically detectable amyloid deposits only on a few structures (arterioles, small arteries, interstitial collagen fibers (I), and medium size veins. In advanced stages more structures were involved (reticular fibers, small veins, and medium size arteries). On other structures (basement membranes, smooth muscle cells, venules, and nerves) AA deposits were seen only in the late stages of small intestine AAa, with massive involvement of the former ones (Figure 3.3).

The amount of deposited AA was different on tissue structures, and increased simultaneously, but the proportion of deposited AA was nearly constant and independent of the stage of amyloidosis.

The amount of AA protein deposits on different tissue structures of the small intestine is demonstrated in figure 3.3.

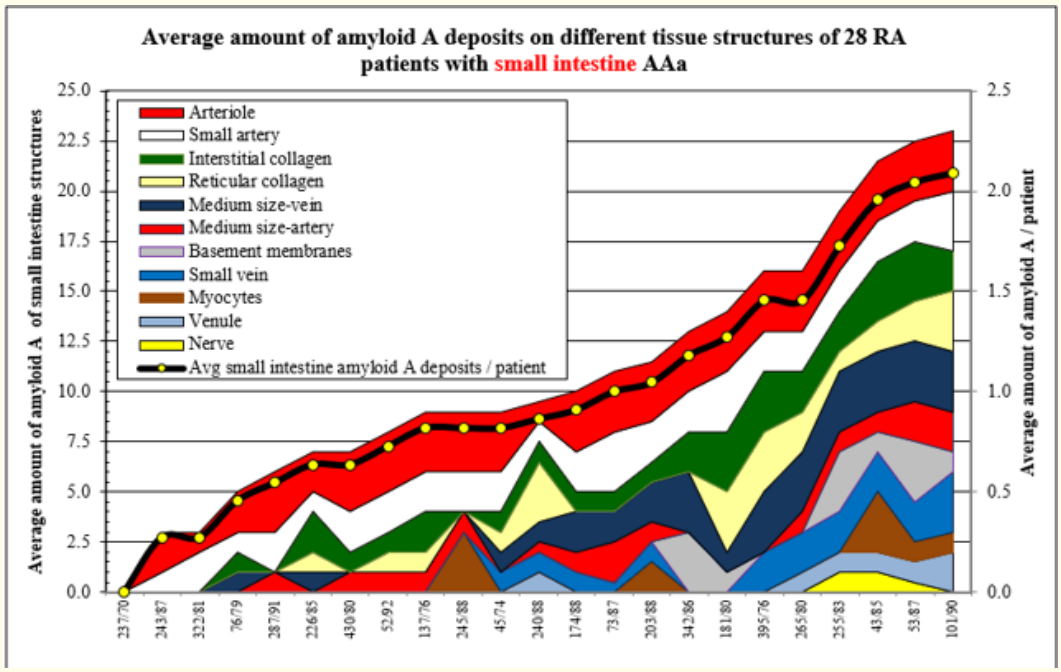

Figure 3.3: *Chronology (development) of AA deposition on different structures of the small intestine in 23 RA patients according to the decreasing amount of AA deposits/structure.*

Legend to figure 3.3: The amount of AA deposits on different tissue structures of the small intestine is arranged according to their decreasing severity (Table 5).

The amount of small intestinal amyloid A deposits /patient (black line) shows the average of the cumulative amount of amyloid A deposits on different tissue structures; the increment is nearly linear, constant and continuous, except the end stage of the small intestine AAa.

AA deposition did not start at the same time on different tissue structures of the small intestine.

The amount of AA deposits on different tissues increased simultaneously, the rate was nearly constant and independent of the stage of amyloidosis.

The differences of individual patients were mainly due to the contingency of sampling and the limitations of the semi-objective evaluation method.

AA deposits in the small intestine (jejunum) are demonstrated on figure 3.4 and 3.5.

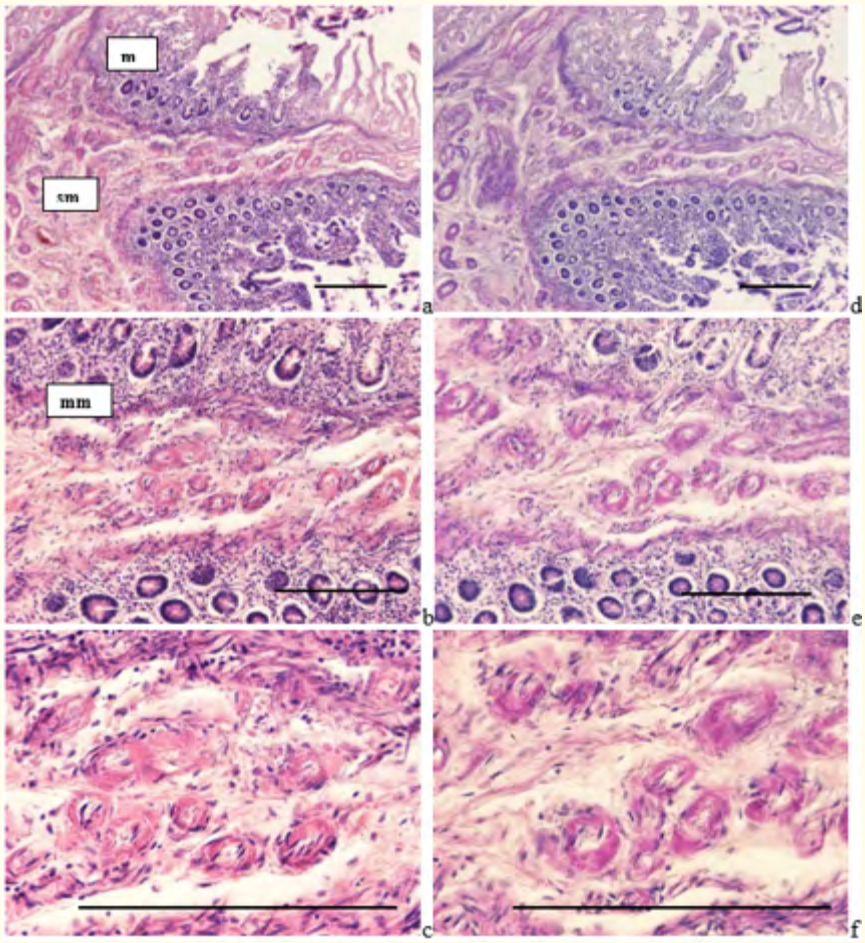

Figure 3.4a-3.4f: *Rheumatoid arthritis, systemic secondary AA amyloidosis, gastrointestinal amyloid A deposits, small intestine, jejunum.*

Mucosa (m), muscularis mucosae (mm), and submucosa (sm).

AA deposition in the submucosa, within the walls of arterioles (a), venules (v) are spared.

(a) HE, scale bar: 1000 [µm], magnification: ×20; (b) same as (a) scale bar: 1000 [µm], magnification: ×40; (c) same as (a) scale bar: 1000 [µm], magnification: ×100; (d) PAS, same as (a) scale bar: 1000 [µm], magnification: ×20; (e) same as (c) scale bar: 1000 [µm], magnification: ×40; (f) same as (a) scale bar: 1000 [µm], magnification: ×100.

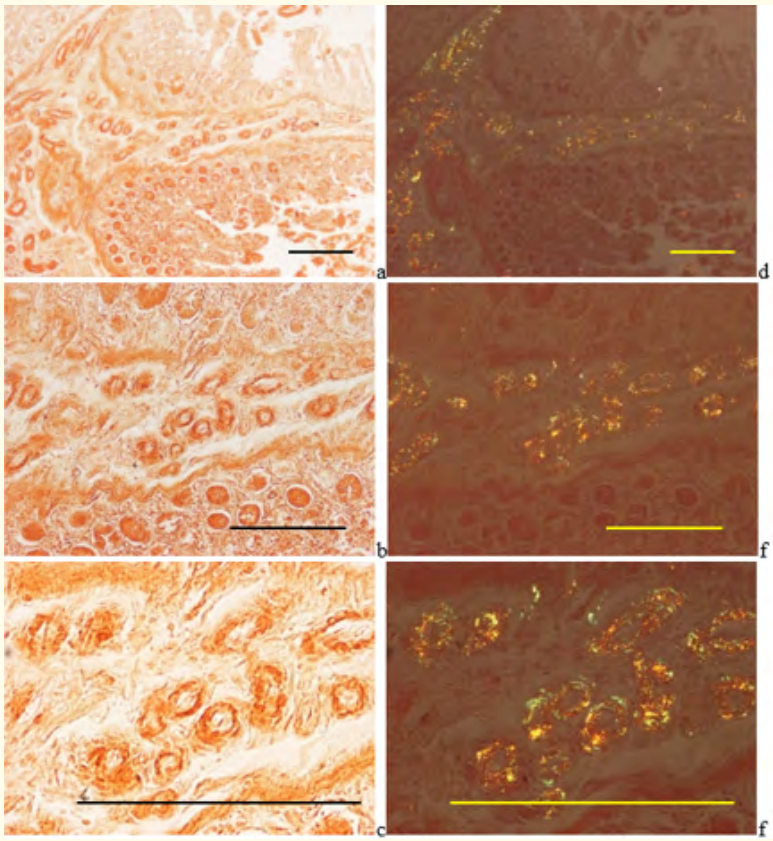

Figure 3.5a-3.5f: *Rheumatoid arthritis, systemic secondary AA amyloidosis, gastrointestinal amyloid A deposits, small intestine, jejunum.*

Mucosa (m), muscularis mucosae (mm), and submucosa (sm), same microscopical fields as figure 3.4.

AA deposition in the submucosa, within the walls of arterioles (a), venules (v) are spared.

(a) Congo red staining, without alcoholic differentiation, covered with gum Arabic, scale bar: 1000 [µm], magnification: ×20; (b) same as (a) scale bar: 1000 [µm], magnification: ×40; (c) same as (a) scale bar: 1000 [µm], magnification: ×100; (d) Congo red staining, without alcoholic differentiation, covered with gum Arabic, and viewed under polarized light, same as (a) scale bar: 1000 [µm], magnification: ×20; (e) same as (d) scale bar: 1000 [µm], magnification: ×40; (f) same as (d) scale bar: 1000 [µm], magnification: ×100.

Characteristics of amyloid A deposition of the large intestine

Tissue blocks of the large intestine were available in 24 of 31 patients with giAAa.

In 4 (16.66%) of 24 RA patients there was no AA deposition on tissue structures of the large intestine; this was considered a "latent" stage of large intestine amyloid deposition (the amount of AA deposits was: 0.00).

The accumulation of AA deposits of the large intestine was also a progressive and basically linear process (with an abrupt start and exponential increment at the end stage).

In the large intestine- similarly to the amount of total gastrointestinal amyloid A deposition - the arterioles, small arteries, and interstitial collagen fibers (collagen I) were affected most commonly and had massive AA deposits, which corresponded to the early sites of amyloid deposition.

On the medium size veins, reticular fibers (collagen III), small veins, medium size arteries and the basement membranes the amount of AA was less frequent and moderate, representing an advanced stage of the large intestine amyloid deposition.

Rare and minimal AA deposits occurred on smooth muscle myocytes, venules, and nerves; the involvement of these structures corresponded to a late premortem (terminal) stage of amyloidosis (Table 6).

The sequence of amyloid A deposition was the same on different tissue structures of the small and large intestine at early, advanced, and late stages of gastrointestinal amyloidosis.

The quantitative differences of AA deposits on different tissue structures of the large intestine in 24 RA patients are summarized in table 6 and figure 4.1-4.3.

															Cause of Death	Cl+ Cl- Dg	Prevalence / Pts. in %	Amount / Pts. in % of max "3"
			a	A	I	VV	Ret	V	AA	BM	Myo	v	n	Avg				
1	237/70	f	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	rAAa-U		0,00	0,00
2	90/85	f	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00			0,00	0,00
3	243/87	f	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	cAAa		0,00	0,00
4	240/88	f	1,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,09	cAAa		0,00	0,00
5	322/81	f	1,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,09	cAAa		9,09	3,03
6	155/87	f	1,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,09			18,18	6,06
7	183/92	f	1,00	0,00	0,00	1,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,18			27,27	9,09
8	430/80	f	2,00	0,00	1,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,27	cAAa		27,27	12,12
9	226/85	f	1,00	1,50	0,00	1,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,32	rAAa-U		27,27	15,15
10	80/80	f	1,00	1,50	0,00	1,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,32	rAAa-U		36,36	16,67
11	287/91	f	2,00	1,00	1,00	1,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,45	rAAa-U	Cl+	36,36	18,18
12	137/76	f	2,00	1,00	1,00	1,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,45			27,27	18,18
13	342/86	m	3,00	0,00	1,00	0,00	0,00	2,00	0,00	0,00	0,00	0,00	0,00	0,55	rAAa-U	Cl+	36,36	18,18
14	39/76	f	3,00	2,00	2,00	0,00	1,00	0,00	0,00	0,00	0,00	0,00	0,00	0,73	cAAa	ND	36,36	21,21
15	174/88	f	3,00	1,00	2,00	0,00	1,00	0,00	0,00	1,00	0,00	0,00	0,00	0,73	cAAa		36,36	24,24
16	203/88	f	2,00	2,00	1,00	0,00	2,00	1,00	1,00	0,00	0,00	0,00	0,00	0,82	cAAa		36,36	24,24
17	43/85	m	1,00	2,00	1,00	2,00	1,00	2,00	0,00	0,00	0,00	0,00	0,00	0,82	rAAa-U		27,27	24,24
18	395/76	f	1,00	3,00	0,50	3,00	1,00	0,00	1,00	0,00	0,00	0,00	0,00	0,86	cAAa		36,36	30,30
19	45/74	f	3,00	3,00	3,00	0,00	1,00	0,00	0,00	1,00	0,00	0,00	0,00	1,00	rAAa-U	Cl+	54,55	33,33

20	245/88	f	3,00	2,00	2,00	0,00	2,00	2,00	1,00	1,00	0,00	0,00	0,00	1,18	rAAa-U	Cl+	63,64	39,39
21	73/87	f	3,00	2,00	2,00	1,00	2,00	1,00	1,00	0,00	0,00	0,00	1,00	1,18	rAAa-U		72,73	39,39
22	162/78	f	3,00	2,00	1,00	2,00	0,00	1,00	0,00	0,00	2,00	3,00	0,00	1,27	rAAa-U	Cl+	72,73	39,39
23	101/90	f	3,00	1,00	2,00	3,00	2,00	1,00	1,00	1,00	2,00	0,00	0,00	1,45	rAAa-U	Cl+	90,91	43,94
25	90/85	f																
26	183/92	f																
27	76/79	f																
28	39/76	f																
29	137/76	f														Cl+		
30	162/78	f																
31	43/85	m																
	Count		24	24	24	24	24	24	24	24	24	24	24	24	20	Cl+		
	Sum		43	28	22,5	19	16	10	7	5	4	3	2	14,5		7		
	Avg		1,792	1,167	0,938	0,792	0,667	0,417	0,292	0,208	0,167	0,125	0,083	0,604		Cl-		
	SD		1,10	1,08	0,91	1,06	0,92	0,72	0,55	0,41	0,56	0,61	0,28	0,49		12		
	"0" n		3	9	9	13	14	17	18	19	22	23	22	3		ND		
	"+" n		21	15	15	11	10	7	6	5	2	1	2	21		1		
	Prev. %		87,50	62,50	62,50	45,83	41,67	29,17	25,00	20,83	8,33	4,17	8,33	87,50				
	Sev. %		59,72	38,89	31,25	26,39	22,22	13,89	9,72	6,94	5,56	4,17	2,78	20,14				
			1	2	3	4	5	6	7	8	9	10	11					
			a	A	I	VV	ret	V	AA	BM	Myo	v	n	Avg				

Table 6: *Prevalence and amount of AA deposits on different tissue structures of the large intestine arranged according to the increasing average amounts of AA deposits /patient (vertical column of average amount /patients), and the decreasing amount of AA deposits /structure (horizontal line in %).*

Remarks to table 6: Pr. n^0/y - Protocol number / year. Prevalence /Pts. in %: Positive cases in % of the total count of 11 tissue structures of the large intestine. Amount / Pts. in %: Average amount of amyloid A in % of maximal value of severity ("3") certified on 11 tissue structures of the large intestine.

Tissue blocks of large intestine were not available in 7 (90/89, 183/92, 76/79, 39/76, 137/76, 162/78, 43/45) of 31 patients with giAAa.

In 4 (16.66%) of 24 RA patients amyloid A deposits were not found in the large intestine, this represented a latent stage of large intestine amyloidosis (the amount of AA deposits was: 0.00).

Cl+: Clinically recognized amyloid A deposits in small intestine (n = 7) - Cl-: Clinically not recognized (n = 12). ND - No Data (n = 1). f: female, m: male. Avg – Average. SD - Standard Deviation.

Prev. % - Prevalence in %: Prevalence of AAa in % of 24 patients conveys the presence of AA on different tissue structures of the large intestine.

Sev. % - Severity in %: Severity of AA in % of 24 patients designates the average AA deposits on different tissue structures of the large intestine in % of maximal value ("3").

The prevalence and amount of the large intestinal amyloid A deposition run basically parallel to each other, except in the early and end stages of amyloid deposition (see the trend lines of increment figure 4.1 and table 6).

In the early stage of large intestinal AAa the amyloid deposition started abruptly, and in the terminal stage AA deposition progressed again rapidly and the growth curve showed an exponential increment (Figure 4.1).

In some patients there was a considerable difference between prevalence and amount of amyloid A deposits due to the contingency of sampling, and the limitations of the semi-objective evaluation method (Figure 4.1).

Figure 4.1 demonstrates the prevalence and amount of AA deposits in the large intestine of 24 RA patients with AAa.

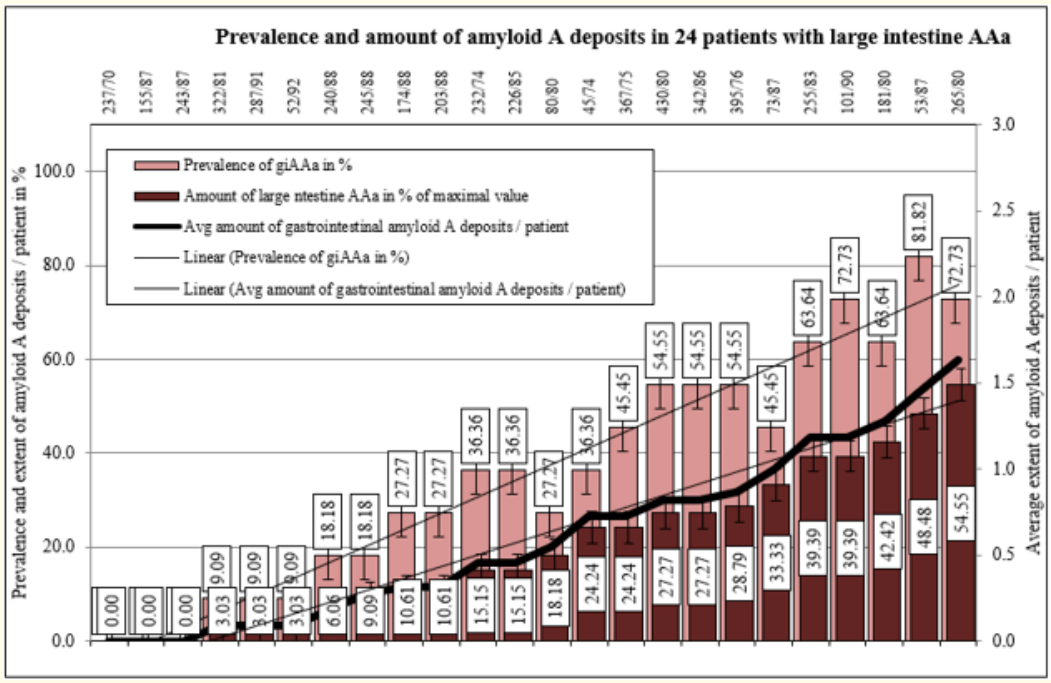

Figure 4.1: *Prevalence and amount of AA deposits in patients with large intestine AAa arranged according to the increasing average amount of AA deposits/patient.*

Legend to figure 4.1: The prevalence and amount of amyloid A deposits in the large intestine changed basically parallel, except in the early and end stage (see trend lines and table 6).

There were considerable individual differences between prevalence and amount in some patients, due to the contingency of sampling, and the limitations of the semi-objective evaluation method.

The prevalence and amount of AA deposits on different tissue structures of the large intestine ran parallel to each other.

Frequently involved tissue structures had marked deposits of amyloid.

Deposits were less striking in less frequently involved tissue structures.

Difference was only found for the venules (**v**) and nerves (**n**) of the large intestine, in which deposition was inverse (Table 6 and figure 4.2).

Quantitative differences (prevalence and amount) of AA deposits on different tissue structures of large intestine AAa are summarized in figure 4.2.

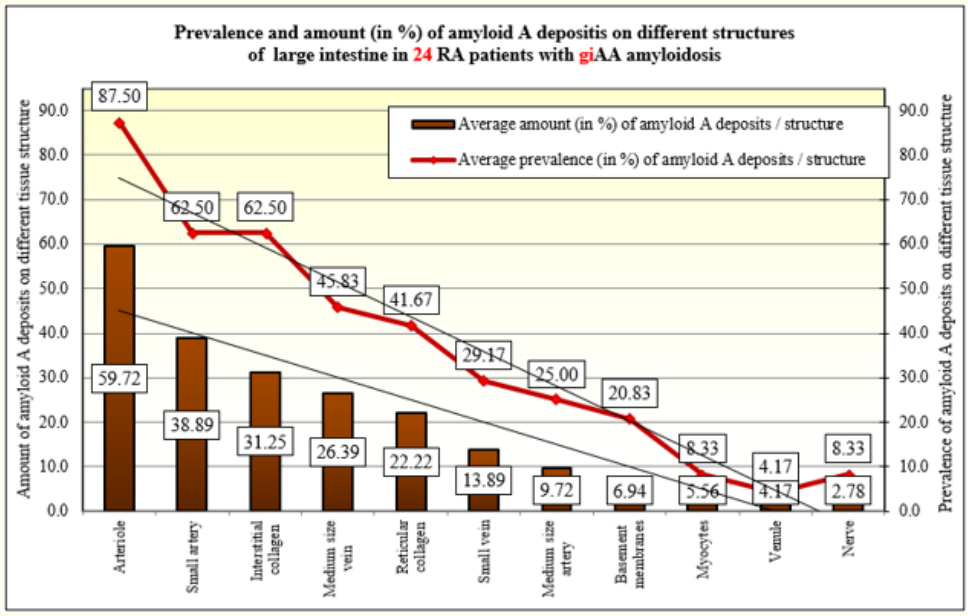

Figure 4.2: *Prevalence and amount of AA deposits on different tissue structures of the large intestine.*

Legend to figure 4.2: Average prevalence and amount of AA deposits on different tissue structures of the small intestine changed basically parallel.

Difference was found only for venules (v) and nerves (n) in which deposition was inverse (Table 6).

Detectable amounts of AA deposits on different tissue structures of the large intestine did not appear simultaneously.

In the early stage of AAa in the large intestine there were histologically detectable amyloid deposits only on a few structures (arterioles, small arteries, interstitial collagen fibers (I).

In advanced stages of AAa more structures were involved (medium size veins, reticular fibers, small veins, medium size arteries and basement membranes).

On other gastrointestinal structures (smooth muscle cells, venules, and nerves) AA deposits were seen only in late stages of AAa in the large intestine (with massive involvement of the former (Figure 4.3).

The amount of deposited AA was different on tissue structures of the large intestine, it increased simultaneously, but the proportion of deposited AA was constant and independent of the stage of amyloidosis.

The amount of AA protein deposits on different tissue structures of the large intestine is demonstrated in figure 4.3.

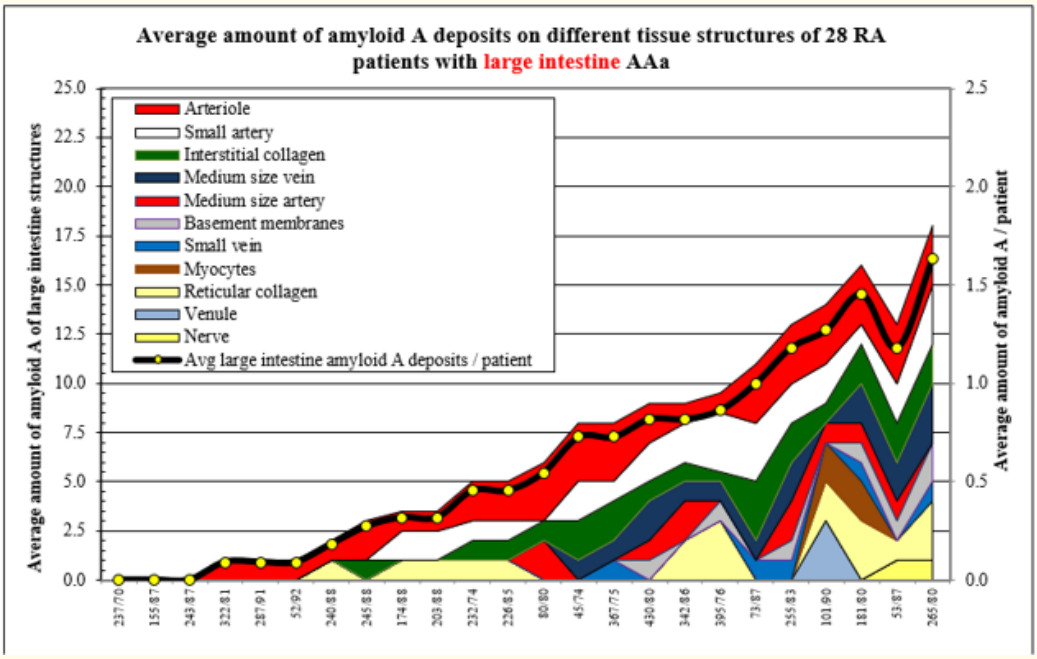

Figure 4.3: *Chronology (development) of AA deposition on different structures of the large intestine in 24 RA patients arranged according to the decreasing amount of AA deposits/structure.*

Legend to figure 4.3: The amount of AA deposits in the large intestine is arranged according to their decreasing severity on different tissue structures (Table 6).

The amount of large intestinal amyloid A deposits /patient (black line) shows the average cumulative amount of amyloid A deposits on different tissue structures; the increment is nearly linear, constant and continuous, except in the end stage of the large intestine AAa.

AA deposition did not start at the same time on different tissue structures of the large intestine.

The amount of AA deposits on different tissues increased simultaneously, the rate was nearly constant and independent of the stage of amyloidosis.

The differences of individual patients were mainly due to the contingency of sampling and the limitations of the semi-objective evaluation method.

AA deposits in the large intestine (cecum) are demonstrated on figure 4.4 and 4.5.

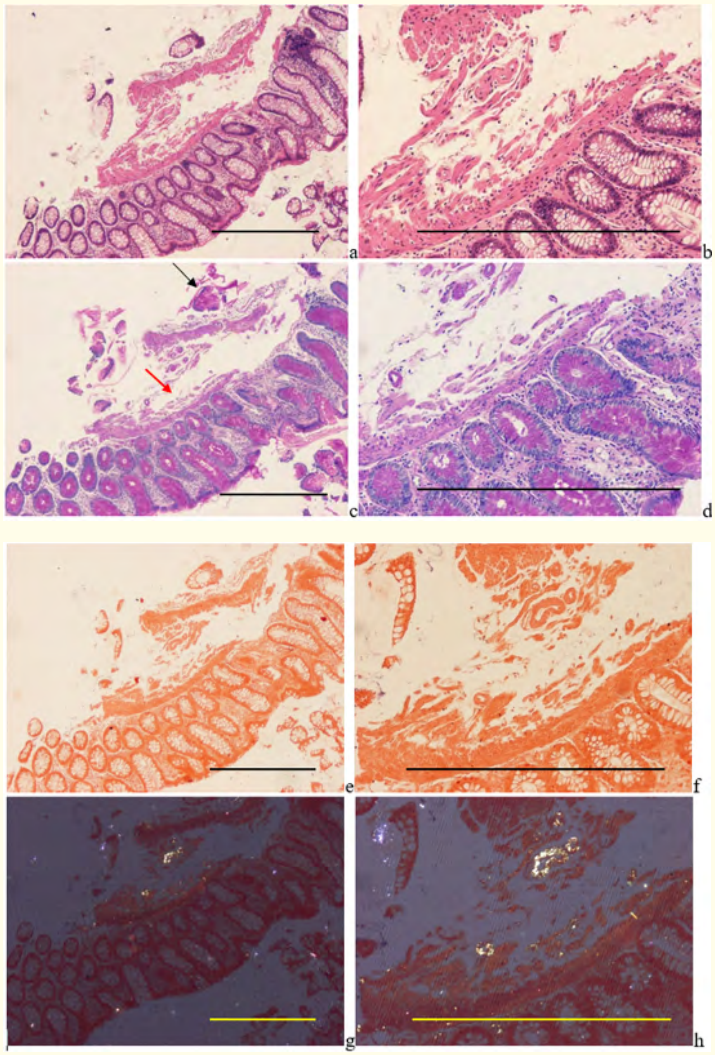

Figure 4.4a-4.4h: *Rheumatoid arthritis, systemic secondary AA amyloidosis, gastrointestinal AA deposits, large intestine, cecum.*

Mucosa (m), muscularis mucosae (mm), and submucosa (sm).

AA deposition in the submucosa, within the walls of arterioles (black arrow), the venules (red arrow) is spared.

(a) HE, scale bar: 1000 [μm], magnification: ×40; (b) same as (a) scale bar: 1000 [μm], magnification: ×100; (c) PAS, same as (a) scale bar: 1000 [μm], magnification: ×40; (d) same as (c) scale bar: 1000 [μm], magnification: ×100; (e) Congo red staining, without alcoholic differentiation, covered with gum Arabic, same as (a) scale bar: 1000 [μm], magnification: ×40; (f) same as (e) scale bar: 1000 [μm], magnification: ×100; (g) Congo red staining, without alcoholic differentiation, covered with gum Arabic, and viewed under polarized light, same as (a) scale bar: 1000 [μm], magnification: ×40; (h) same as (g) scale bar: 1000 [μm], magnification: ×100.

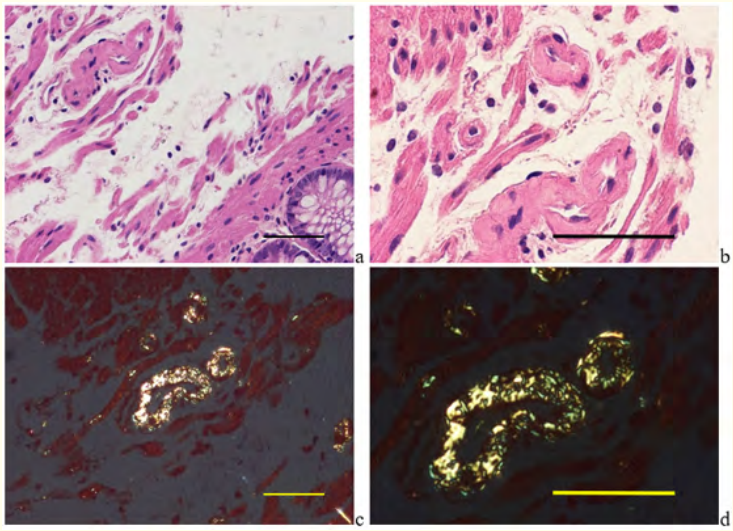

Figure 4.5a-4.5d: *Rheumatoid arthritis, systemic secondary AA amyloidosis, gastrointestinal AA deposits, large intestine, cecum.*

Mucosa (m), muscularis mucosae (mm), and submucosa (sm), same microscopical fields as figure 4.4.

AA deposition in the submucosa, within the walls of an arteriole.

In the wall of arteriole, the amyloid A deposits show crystalline transformation, indicating an old (long time existing, transformed) fibrillar structure.

(a) HE, scale bar: 100 [μm], magnification: ×200; (b) same as (a) scale bar: 100 [μm], magnification: ×400; (c) Congo red staining, without alcoholic differentiation, covered with gum Arabic, and viewed under polarized light, same as (a) scale bar: 100 [μm], magnification: ×200; (d) same as (g) scale bar: 100 [μm], magnification: ×400.

Amyloid A deposition in different sections of the GI tract - Comparison of the gastric, small, and large intestine amyloid A deposits on tissue structures of 31 patients with giAAa

The prevalence and amount of amyloid A deposits on different tissue structures of the gastrointestinal tract was the highest in the small intestine, exceeded the prevalence and amount of the gastric or large intestinal amyloid A deposits (Table 7 and figure 5.1).

The prevalence and amount of amyloid A deposition in all segments of the gastrointestinal tract was the highest on arterioles, small arteries and interstitial collagen (I) fibers, followed by reticular (collagen III) fibers and/or medium size veins (Table 7 and figure 5.2).

The sequence of the retrograde, passive accumulation of amyloid A was similar on the venous side of circulation (on medium size, small veins and venules) in the entire gastrointestinal tract, i.e. the stomach, small and large intestine.

The increment of amyloid A deposits in medium size veins was always higher than in the small veins or venules (Table 7 and figure 5.2).

Involvement of smooth muscle myocytes and nerves was rare and minimal in all segments of the gastrointestinal tract, except in the stomach, where the presence of amyloid A deposits was relatively more frequent and pronounced than in other parts of the GI tract (Table 7 and figure 5.2).

Table 7 summarizes the average prevalence and amount of amyloid A deposits on different tissue structures of the gastrointestinal tract (including the stomach, small and large intestine).

31	**GI tract**		**A**	**A**	**I**	**ret**	**VV**	**AA**	**BM**	**V**	**Myo**	**v**	**N**	**Avg**	**CoD**	**Cl+**
	Count		31	31	31	31	31	31	31	31	31	31	31	31	23	8
	Sum		65,84	43,73	37,50	23,92	21,65	16,32	15,66	11,24	9,01	4,66	3,99	23,047		Cl-
	Avg		2,12	1,41	1,21	0,77	0,70	0,53	0,51	0,36	0,29	0,15	0,13	0,74	0,74	14
	SD		0,97	0,78	0,93	0,89	0,83	0,56	0,76	0,55	0,57	0,33	0,34	0,51		ND
	“0” n		2	2	7	11	12	12	18	16	23	24	25	2		1
	“+” n		29	29	24	20	19	19	13	15	8	7	6	29		
	Prev. %		93,55	93,55	77,42	64,52	61,29	61,29	41,94	48,39	25,81	22,58	19,35	93,55		
	Sev. %		70,80	47,02	40,32	25,72	23,28	17,55	16,84	12,09	9,69	5,01	4,29	24,78		
28	**Stomach**		**a**	**A**	**I**	**ret**	**VV**	**AA**	**BM**	**V**	**Myo**	**v**	**n**	**Avg**	**CoD**	**Cl+**
	Count		28	28	28	28	28	28	28	28	28	28	28	28	22	7
	Sum		59	42,5	37	23	19	18	13	8	6	6	2,5	21,273		Cl-
	Avg		2,11	1,52	1,32	0,82	0,68	0,64	0,46	0,29	0,21	0,21	0,09	0,76	0,76	14
	SD		1,1	1,01	1,19	1,06	0,77	1,13	0,79	0,66	0,57	0,48	0,27	0,57		ND
	“0” n		4	5	10	15	14	20	19	23	24	22	25	4		1
	“+” n		24	23	18	13	14	8	9	5	4	6	3	24		
	Prev. %		85,71	82,14	64,29	46,43	50	28,57	32,14	17,86	14,29	21,43	10,71	85,71		
	Sev. %		70,24	50,6	44,05	27,38	22,62	21,43	15,48	9,52	7,14	7,14	2,98	25,32		
23	**Small intestine**		**a**	**A**	**I**	**ret**	**VV**	**AA**	**BM**	**V**	**Myo**	**v**	**n**	**Avg**	**CoD**	**Cl+**
	Count		23	23	23	23	23	23	23	23	23	23	23	23	19	6
	Sum		59	43	32	22,5	31,5	16,5	12	17,5	9,5	7	2,5	23		Cl-
	Avg		2,5652	1,8696	1,3913	0,9783	1,3696	0,7174	0,5217	0,7609	0,413	0,3043	0,1087	1	1	12
	SD		0,8435	0,6944	1,0331	1,1528	1,2633	0,688	1,0388	0,9519	0,9127	0,5588	0,2999	0,575		ND
	“0” n		1	1	5	11	8	9	17	12	18	17	20	1		1
	“+” n		22	22	18	12	15	14	6	11	5	6	3	22		
	Prev. %		95,652	95,652	78,261	52,174	65,217	60,87	26,087	47,826	21,739	26,087	13,043	95,652		
	Sev. %		85,507	62,319	46,377	32,609	45,652	23,913	17,391	25,362	13,768	10,145	3,6232	33,333		
24	**Large intestine**		**a**	**A**	**I**	**ret**	**VV**	**AA**	**BM**	**V**	**Myo**	**v**	**n**	**Avg**	**CoD**	**Cl+**
	Count		24	24	24	24	24	24	24	24	24	24	24	24	20	7
	Sum		43	28	22,5	19	16	10	7	5	4	3	2	14,5		Cl-
	Avg		1,7917	1,1667	0,9375	0,7917	0,6667	0,4167	0,2917	0,2083	0,1667	0,125	0,0833	0,6042	0,6	12
	SD		1,1025	1,0801	0,9126	1,0624	0,9168	0,7173	0,55	0,4149	0,5647	0,6124	0,2823	0,4949		ND
	“0” n		3	9	9	13	14	17	18	19	22	23	22	3		1
	“+” n		21	15	15	11	10	7	6	5	2	1	2	21		
	Prev. %		87,5	62,5	62,5	45,833	41,667	29,167	25	20,833	8,3333	4,1667	8,3333	87,5		
	Sev. %		59,722	38,889	31,25	26,389	22,222	13,889	9,7222	6,9444	5,5556	4,1667	2,7778	20,139		

Table 7: *Sequence (development) of amyloid A deposits on different tissue structures of the gastrointestinal tract (including stomach, small and large intestine) arrange according to the decreasing values of severity in % (horizontal lines).*

Amyloid A deposition did not start at the same time in different sections of the GI tract; amyloid A deposition started earlier in the small intestine, followed by the large intestine and later on in the stomach (Table 4-6 and figure 5.1).

The sequence (development) of amyloid A deposition was similar, and ran parallel in all sections of the gastrointestinal tract (Table 7 and figure 5.1).

The average prevalence of amyloid A deposits was the highest in the small intestine, accompanied with more massive amyloid A deposits (Table 7 and figure 5.1).

Figure 5.1 demonstrates the sequence (development) of amyloid A deposition on different tissue structures of the gastrointestinal tract (stomach, small and large intestine) compared with the general (total) gastrointestinal amyloid A deposition in 31 patients.

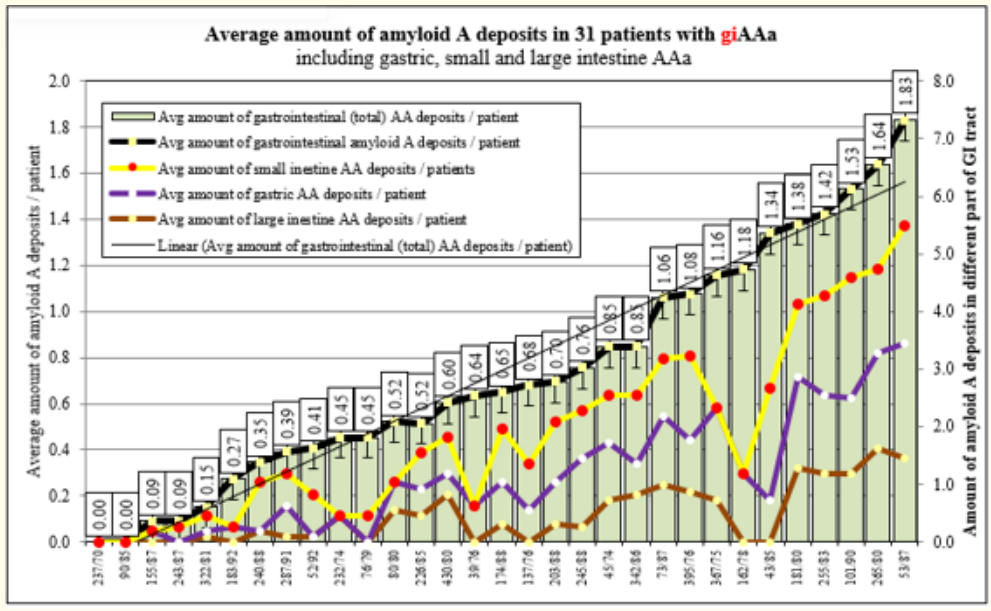

Figure 5.1: *Average amounts of gastrointestinal (gastric, small and large intestinal) amyloid A deposits in 31 RA patients with giAAa arranged according to the increasing amounts of amyloid A deposits /patients (vertical column of Table 3), and on different tissue structures (horizontal lines of table 7).*

Legend to figure 5.1: The amount of amyloid A deposits in different part of 31 RA patients with giAAa changed basically parallel, except in the end stage of amyloidosis, where the increment was exponential.

The considerable individual differences of patients were mainly due to the contingency of sampling and the limitations of the semi-objective evaluation method individual differences (Table 7).

The gastrointestinal amyloid A deposition on different tissue structures of the GI tract ran parallel to each other.

Amyloid A deposition did not start on different tissue structures at the same time; amyloid A deposition started on arterioles, small arteries and interstitial collagen (I) fibers at the earliest, and on venules, myocytes and nerves at the latest (Figure 5.2).

Figure 5.2 demonstrates the average amount of amyloid A deposits on different tissue structures of GI tract in 31 RA patients with giAAa.

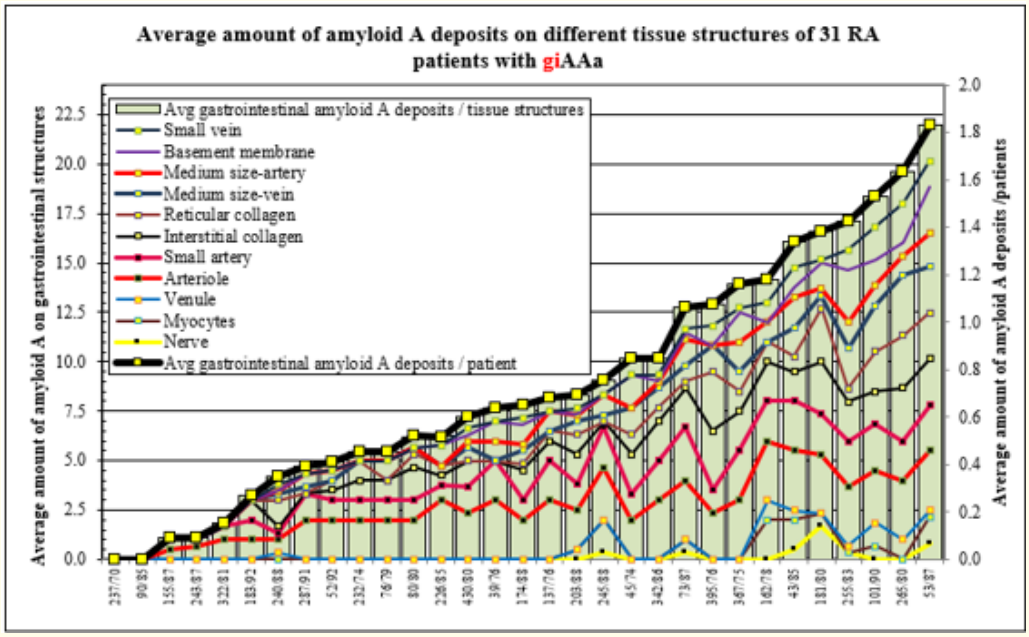

Figure 5.2: *Amount of amyloid A deposits on different tissue structures in 31 RA patients with giAAa arranged according to the increasing average amount of amyloid A deposits/patients (vertical column of table 3), and on different tissue structures (horizontal lines of table 4-6).*

Legend to figure 5.2: Amount of amyloid A deposits on different tissue structures of 31 RA patients with giAAa changed basically parallel.

Amyloid A deposition not started on different tissue structures of the GI tract at the same time; arterioles were involved at the earliest, and nerves at latest (Table 7).

The amount of AA deposits on different tissue structures of the gastrointestinal tract ran parallel to each other.

Frequently involved tissue structures showed marked deposits of amyloid.

Deposits were less striking in less frequently involved tissue structures (Table 3-7 and figure 5.3).

Figure 5.3 summarizes the amount of AA deposits on different tissue structures of the GI tract (including gastric, small intestine and large intestine amyloid A deposits).

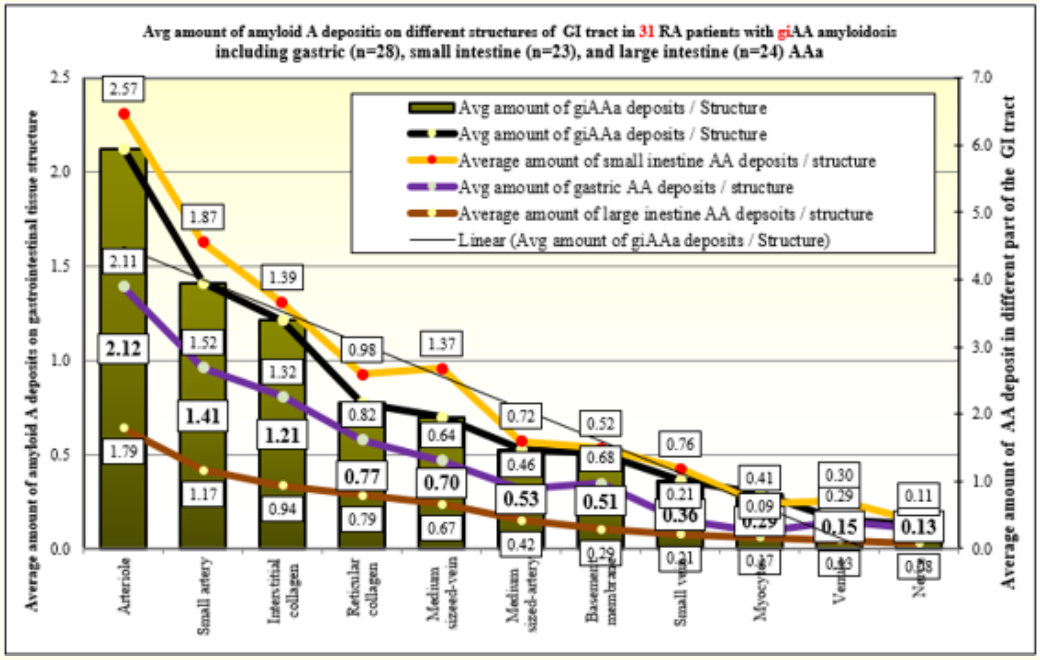

Figure 5.3: *Amount of AA deposits on different tissue structures of the GI tract comparing the amyloid A deposits of the stomach, small, and large intestine.*

Legend to figure 5.3: Average amount of AA deposits on different tissue structures of the GI tract changed basically parallel in various section of gastrointestinal tract, and was the highest in the small intestine, exceeding the amyloid deposition in the stomach or in the large intestine (Table 3-7).

Ther average amount of amyloid A deposits was the highest on the arterioles, small arteries and interstitial collagen (I) fibers, and exceeded the amount of amyloid A deposits on other structures in all segments of the GI tract (Table 3-7).

The amount of amyloid A deposits on venules and nerves were minimal in all segments of the gastrointestinal tract (Table 3-7).

Amyloid A deposits were more frequent and massive on tissue structures of the small intestine, exceeding the prevalence and amount of amyloid A deposits on tissue structures of the stomach or large intestine (Table 7 and figure 5.4).

Comparing the stomach, small and large intestine the ratio of amyloid A deposits on different tissue structures was constant and nearly the same (Figure 5.4).

Figure 5.4 summarizes the amount and ratio of AA deposits on different tissue structures of the stomach, small and large intestine.

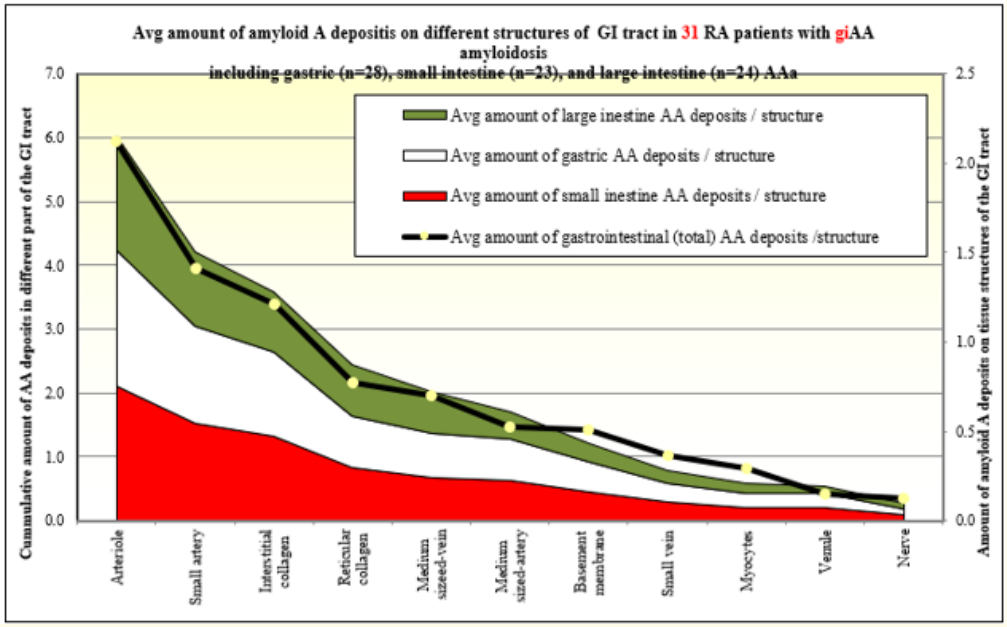

Figure 5.4: *Amount and ratio of amyloid A deposits on different tissue structures of the GI tract.*

Legend to figure 5.4: Average amount of AA deposits of the small intestine exceeded the amyloid A deposits on tissue structures of the stomach or large intestine.

The amyloid A deposition changed basically parallel on different tissue structures in various sections of the gastrointestinal tract, and the ratio of increment was constant and nearly same (Table 7).

Comparing the average prevalence of amyloid A deposits /patient there was no significant difference between stomach and small intestine ($p < 0.130$) or stomach and large intestine ($p < 0.480$); in the small intestine the average prevalence of amyloid A deposits / patient was higher than in the large intestine; the difference was significant ($p < 0.028$) (Tables 4-6),

Comparing average amount of amyloid A deposits /patient there was no significant difference between stomach and small intestine ($p < 0.143$) or stomach and large intestine ($p < 0.297$); in the small intestine the average amount of amyloid A deposits /patient was higher than in the large intestine, the difference was significant ($p < 0.015$) (Table 4-6),

Comparing average prevalence of amyloid A deposits/structure there was no significant difference between the stomach and small intestine ($p < 0337$), stomach and large intestine ($p < 0.650$) or between the small intestine and large intestine ($p < 0.170$).

Comparing average amount of amyloid A deposits /structure there was no significant difference between the stomach and small intestine ($p < 0.425$), stomach and large intestine ($p < 0.543$) or between the small intestine and large intestine ($p < 0.168$).

Table 8 summarizes the statistical correlations ("p" values of significance) of amyloid A deposits in patients and on different tissue structures of the gastrointestinal tract (including the stomach, small and large intestine).

Avg amount of amyloid A /patient	p <	**Avg amount of amyloid A /structure**	p <
Stomach vs small intestine	0,143	Stomach vs small intestine	0,425
Stomach vs large intestine	0,297	Stomach vs large intestine	0,543
Small intestine vs large intestine	**0,015**	Small intestine vs large intestine	0,168
Prevalence of amyloid A / patient	p <	**Prevalence of amyloid A / structure**	p <
Stomach vs small intestine	0,130	Stomach vs small intestine	0,337
Stomach vs large intestine	0,480	Stomach vs large intestine	0,650
Small intestine vs large intestine	**0,028**	Small intestine vs large intestine	0,170

Table 8: *Statistical correlations ("p" values of significance) between prevalence and amount of amyloid A deposit in patients and on different tissue structures of the gastrointestinal tract.*

Legend to table 8: Comparing the prevalence and amount of amyloid A deposits on different tissue structures there was no significant difference between different sections of the GI tract, except the small and the large intestine.

The prevalence and amount of amyloid A deposits on different tissue structures of the small intestine significantly exceeded the prevalence ($p < 0.028$) and amount ($p < 0.015$) of amyloid A deposits of the large intestine (Table 8).

Discussion

In our patient cohorts there was no significant difference in survival time, onset, and duration of disease, between RA patient cohorts with and without sAAa, with and without giAAa, neither in females nor in males.

Comparing the prevalence or amount of amyloid A deposits in different segment (part) of gastrointestinal tract there was no significant difference between the stomach and small intestine or stomach and large intestine, neither according to the amyloid deposits in patients nor according to the amyloid deposits on tissue structures.

Significant difference was registered only between the small and large intestine comparing the average prevalence (95.65 % versus 87.50 %; $p < 0,028$) or amount (33.333 % versus 20.139 %; $p < 0,015$) of amyloid A deposits in % according to the patients.

sAAa and giAAa developed in both sexes, and at any time in the course of the disease, and amyloid A deposition involved all parts of the gastrointestinal tract (including the stomach, small and large intestine).

Based on a national mortality statistic, amyloidosis of different types was diagnosed (without detailed histological analysis of organ involvement) in 156 (12.5%) of 1246 autopsy patients, who died in Japan between 1985-1989.

Of these 1246 autopsy patients 316 had RA, was complicated by AAa in 79 (25.2%) cases [23].

The rate of mortality due to amyloidosis was 5.8% in a nationwide series of 1666 patients with RA, who died during 1989 in Finland (in 64 patients the amyloidosis was the immediate cause of death, and in further 33 the amyloidosis existed as an intervening antecedent cause, and contributed to the death only) [24]. Likely the "amyloidosis" represented different types of fibrillar protein deposits (comment of authors).

In a large series of biopsies with various amyloid classes AAa was typed in 78 (13.4%) of 581 patients using Congo red staining and immunohistochemical methods [25].

In a cohort of 369 consecutively autopsied RA patients the prevalence of amyloidosis was 30%, the mortality 9.5% (corresponding to 66 and 35 patients respectively), and the patients died earlier, compared to 370 non-RA patients matched with the same age and sex [26]. RA was long-lasting active disease, and was characterized by higher erythrocyte sedimentation rate ($p = 0.002$), lower hemoglobin level ($p < 0.001$), accompanied more frequently with proteinuria ($p < 0.001$) and renal failure ($p < 0.001$) compared to the patients of non-RA group [26]. The authors emphasize that the amyloidosis frequently remain undetected, indeed in autopsied patients as well [27].

Suzuki., *et al*. (1994) found amyloidosis at autopsy in 17 (21.0%) of 81 RA patients, 6 (7.4%) of which led direct to death [28].

The above cited authors do not mention the type of amyloid, but they probably meant AA amyloidosis, since RA was the basic disease (comment of authors).

Chastonay and Hurlimann J (1986) identified AAa in 17 (23.94%) of 81 autopsied RA patients [29].

According to the relevant (above mentioned) literary data the prevalence of AAa in RA is between 10 - 30%, and 5 - 10% of this complication led to death [23-29].

In our autopsy population the prevalence of sAAa was (23.13%), and involved 34 of 161 patients with RA. sAAa led to death in 25 cases (73.53% of 34 and 15.53% of 161) patients.

Amyloid A deposition showed a progressive cumulative process in our RA patients; development of amyloid deposition was basically steady, continuous (consistently linear), and was related to the gradual deposition of AA on different tissue structures of various organs (Table 3-6 and figure 1.1-4.1).

The gastrointestinal tract was involved in 31 (91.18% of 34 and 19.25% of 161) patients. Gastrointestinal amyloidosis did not play a direct role in the mortality of RA patients.

There was a very strong positive relationship between sAAa (n = 34) and giAAa (n = 31) ($c = 1.0$, $\chi^2 = 137.60$, $p < 0.000$).

The strong positive relationship between sAAa and giAAa supports the assumption that the type of amyloid deposits was identical in the total patient population, i.e. the type of systemic and gastrointestinal amyloid deposits was AA in all of our patients.

We registered a delayed amyloid A deposition in GI tract compared to the systemic manifestation of amyloidosis; amyloid A deposition started later, after a latent stage in the GI tract.

Amyloid A deposition started earlier in the small intestine, where the amyloid A deposits were more frequent and massive, exceeding the prevalence and amount of amyloid A deposits in other sections of the GI tract.

There was a stage dependent amyloid A deposition on different tissue structures; the deposition started on arterioles, small arteries and interstitial collagen (I) fibers at the earliest, and on the venules, myocytes and nerves at the latest (Figure 5.2).

It is difficult to estimate the true prevalence and mortality of AAa in RA [30].

The organs are affected differently, and the site of sampling (subcutaneous fat tissue, gastrointestinal, renal, cardiac etc. biopsy) may influence the results [30].

A great variety of methods are available [31], and the detection of amyloidosis depends on the specificity and sensitivity of the demonstration technique.

Subcutaneous fat biopsy - introduced by Westermark., *et al*. (2006) - is regarded as a simple and safe method [32].

Tiitinen., *et al*. (1993) detected AAa at least in 11 (10.9%) of 102 RA patients using subcutaneous fat biopsy [33].

According to Dhawan., *et al*. (2018) abdominal subcutaneous fat biopsy results are not very sensitive for AA amyloidosis, for instance in familial Mediterranean fever (FMF); rectal biopsy is more useful than subcutaneous fat aspiration in AA amyloidosis [34].

We agree with Kobayashi., *et al*. (1996) that gastroduodenal biopsies may be useful for diagnosing secondary amyloidosis; they confirmed gastrointestinal AAa in 54 (13.3%) of 407 RA patients [35].

Dhawan., *et al*. (2018) in their review article [34] mentioned that there is no formal staging system for any of the amyloidosis.

The description of the histological development of amyloidosis shown in figure 1.1-4.1, makes possible the exact staging of this progressive process, allowing a new approach for diagnostic surgical pathology.

The nearly same ratio of increment in different sections of GI tract allows an indirect calculation (assumption) of amyloid A deposits in other, actually not analyzed sections of GI tract.

Similarly, the nearly same ratio of increment on various tissue structures of these sections allows an indirect calculation (assumption) of amyloid A deposits on other tissue structures, which are not actually present in the tissue section.

It must be kept in mind that a randomly taken tissue sample may be misleading (especially in early stage of amyloidosis).

Always remember that:

(a) The staining procedure should be appropriate [16],

(b) The illumination of a microscopic slide is crucial; for reliable demonstration of amyloid deposits a professional highly bright polarization microscope is needed,

(c) The staining intensity of Congo red may change during storage, fresh-stained, high quality tissue section are important.

Conclusion

giAAa may develop in both sexes, any time in the course of RA, and the amyloid A deposition may involve all parts of the gastrointestinal tract (including stomach, small and large intestine).

giAAa is a progressive and cumulative processes, in their early stage involve only a few structures, and increasingly more in later stages of the disease.

The chronology of AA deposition (histological description of development) on different tissue structures all parts of the gastrointestinal tract allows an indirect assessment of the stage of giAAa, which may have a prognostic value in biopsies.

giAAa does not appear to be a very serious, life-threatening complication of RA, rather it has proven to be an early complication of great clinical and pathological importance as an optimal biopsy site, especially the small intestine.

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