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Research Article

Demographics and Predictive Clinical-Laboratory Parameters of Systemic, Renal and Cardiac AA Amyloidosis - A Postmortem Clinicopathologic Study of 161 Rheumatoid Arthritis Patients

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Abstract

Aim: The aim of this study has been to determine the prevalence of systemic, renal and cardiac AA amyloidosis (sAAa, rAAa and cAAa) in rheumatoid arthritis (RA), and to assess the predictive clinical laboratory parameters for sAAa, rAAa and cAAa.

Patients and Methods: One hundred sixty one (161) non- selected autopsy patients with RA were studied. RA was confirmed clinically according to the criteria of the American College of Rheumatology. sAAa was specified histologically, based on evaluation of 5 organs (heart, lung, liver, kidney and pancreas) in each of 161 patients. Amyloid A deposition was diagnosed according to Romhányi by modified (more sensitive) Congo red staining, and confirmed in serial sections by immunohistochemical and histochemical methods. The prevalence (existence) of amyloid A deposition were evaluated microscopically with an Olympus BX51 polarizing microscope.

The correlations were determined by the Student (Welch) t-probe, comparing the age, sex of patients, onset of RA, duration of disease, and laboratory parameters at the last hospitalization: with and without sAAa, rAAa or cAAa, and between sAAa, rAAa and cAAa.

Results and Discussion: sAAa complicated RA in 34 (21.12%) of 161 patients; in 127 (78.88%) of 161 patients amyloid A deposits were not seen.

Amyloid A deposits were found in 29 (87.88%) kidneys and in 29 (87.88%) heart of 33 RA patients with sAAa. The kidneys and heart were negative for amyloid in 4 (12.12%) of 33 cases (the kidneys and heart of one-one patients with sAAa were not available)

There was no significant difference in survival time, onset or duration of RA between patients with and without sAAa, rAAa or cAAa, and between sAAa, rAAa and cAAa. Amyloidosis developed in both sexes, and any time in the course of the disease.

The discussed clinical-laboratory parameters allude to the existence of sAAa only, and more or less referred of amyloid A deposition in the kidneys. None of them (no one) was specific to or unique for amyloid itself. The patients with sAAa, rAAa or cAAa were anemic, with significantly lower levels of hemoglobin, and serum bilirubin in comparison without sAAa.

They had lower levels of serum albumin and higher levels of alpha2-globulin in comparison without sAAa. The patients with sAAa, rAAa or cAAa showed decreased renal function: they had higher levels of, blood urea nitrogen (BUN), higher levels of serum creatinine, and serum potassium, lower levels of serum sodium and proteinuria. Urine specific gravity was significantly lower in patients with sAAa, rAAa, or cAAa in comparison without sAAa.

The early diagnosis of AA amyloidosis is important. Therefore examination of all available surgical or biopsy material for amyloid is suggested in all hospitalised RA patients, with or without clinical evidence of amyloidosis.

When clinical suspicion of amyloidosis is raised in case of unexplained weight loss, fatigue, anemia, impaired renal function, restrictive cardiomyopathy, hepatomegaly, or gastrointestinal complaints (malabsorption, malnutrition, diarrhea, obstruction, disturbed motility, bleeding) or reduced respiratory capacity, a biopsy is needed. We suggest gingival or rectal biopsies in any suspected cases, stained with Congo red according to Romhány, using an appropriate polarizing microscope with high brightness.

Keywords: *Rheumatoid Arthritis; Systemic; Renal and Cardiac AA Amyloidosis; Clinical-Laboratory Parameters*

Abbreviations

RA: Rheumatoid Arthritis; ARA: American College of Rheumatology; sAAa: Systemic AA Amyloidosis; cAAa: Cardiac AA Amyloidosis; rAAa: Renal AA Amyloidosis; CoD: Cause of Death; U: Uremia; Cl+: Clinically Diagnosed; Cl-: Clinically not Diagnosed; ND: No Data Available; ESR: Erythrocyte Sedimentation Rate (); CRP: C Reactive Protein; BUN: Blood Urea Nitrogen; RBC: Red Blood Cells; WBC: White Blood Cells; LDH: Lactate Dehydrogenase; GPT: Glutamat-Piruvat Aminotransferase; gamma GT: Gamma-Glutamyl Transferase; SAA: Serum Amyloid A; SAP: Serum Amyloid P Component

Introduction

Systemic (generalized) AA amyloidosis (sAAa) is one of the main and most insidious complications of rheumatoid arthritis (RA) [1]. The serum amyloid A proteins are produced by the liver, are spread via the bloodstream and are deposited throughout the body. sAAa is related to the cardiovascular system [2,3], and amyloid A deposition in the kidneys (renal AAa - rAAa), and in the heart (cardiac AAa - cAAa) is connected with it. rAAa leads to death by uremie in half of RA patients with sAAa [1]. cAAa causes or contributes to the lethal outcome by heart failure in nearly one quarter of cases [4].

The aim of this study has been to determine the prevalence and severity of sAAa, rAAa and cAAa in RA, and to assess the predictive clinical laboratory parameters for sAAa, rAAa and cAAa.

Patients (Autopsy Population) and Methods

At the National Institute of Rheumatology 9475 patients died between 1969 and 1992; among them 161 with RA (females 116, average age: 64.95 years, range 87 - 16, onset of RA: 50.19, average disease duration: 14.79 years; males 45, average age: 66.29 years, range 88 - 19, onset of RA: 52.57, average disease duration: 13.46 years at death); all of them were autopsied [1].

RA was confirmed clinically according to the criteria of the American College of Rheumatology (ARA) [5].

sAAa was specified histologically, based on evaluation of 5 organs (heart, lung, liver, kidney and pancreas) in each of 161 patients. Amyloid A deposition was diagnosed according to Romhányi [6] by modified (more sensitive) Congo red staining [7], and confirmed in serial sections by immunohistochemical and histochemical methods [3,8]. The prevalence (existence) of amyloid A deposition were evaluated microscopically with an Olympus BX51 polarizing microscope [4]. Selected sections were examined with a JEM 100CX electron microscope [1].

The correlations were determined by the Student (Welch) t-probe, comparing the age, sex of patients, onset of RA, duration of disease, and laboratory parameters (Latex, Waaler-Roose values, ESR, CRP, albumin/globulin ratio, serum electrophoresis (albumin, alpha-1-globulin, alpha-2-globulin, beta-globulin, gamma-globulin), RBC, hemoglobin, WBC, systolic and diastolic blood pressure, blood urea nitrogen (BUN), serum creatinine, serum potassium and sodium values, urine specific gravity, proteinuria, urine sediment (RBC, WBC), serum bilirubin, LDH, GPT, gamma GT, blood sugar, and diastase values) at the last hospitalization: with and without sAAa, rAAa or cAAa, and between sAAa, rAAa and cAAa.

Results

sAAa complicated RA in 34 (21.12%) of 161 patients; in 127 (78.88%) of 161 patients amyloid A deposits were not seen.

Amyloid A deposits were found in 29 (87.88 %) kidneys of 33 RA patients with sAAa. Kidneys were negative for amyloid in 4 (12.12%) of 33 patients (in 1 of 34 patients with sAAa tissue blocks of kidneys were not available).

Amyloid A deposits were found in 29 (87.88 %) heart of 33 RA patients with sAAa. The heart was negative for amyloid in 4 (12.12%) of 33 cases (the heart of one patient with sAAa was not available).

Demographics, onset and duration of disease of female and male RA patients with sAAa (n = 34 of 161), rAAa (n = 29 of 33) or cAAa (n = 29 of 33), and without sAAa (n = 127 of 161) are summarized in tables 1.1 - 1.2 and figures 1.1 - 1.5.

	Number of patients	Average age in years at death	Range (in years)	Age at onset of disease	Disease duration (in years)
With sAAa	34 of 161	62.41	88 - 19	47.61	15.58
Female	29	64.34	83 - 32	48.56	15.70
Male	5	51.20	88 - 19	41.25	14.75
With rAAa	29 of 33	62.03	88 - 19	47.81	15.12
Female	24	64.29	83 - 44	49.00	15.18
Male	5	51.20	88 - 19	41.25	14.75
With cAAa	29 of 33	60.97	88 - 19	46.93	15.00
Female	24	63.00	83 - 32	47.91	15.04
Male	5	51.20	88 - 19	41.25	14.75
Without sAAa	127 of 161	66.10	87 - 16	51.77	14.09
Female	87	65.15	87 - 16	50.79	14.45
Male	40	68.15	87 - 20	53.94	13.30

Table 1.1: *Sex, average age (range), onset and duration of RA in patients with sAAa, rAAa, cAAa and without sAAa.*

The relationship ("p" values of correlation) of demographics, onset and duration of disease between female and male RA patients with sAAa (n = 34 of 161), rAAa (n = 29 of 33) or cAAa (n = 29 of 33), and without sAAa (n = 127 of 161), furthermore between sAAa (n = 34 of 161), rAAa (n = 29 of 33), and cAAa (n = 29 of 33) patients is summarized in table 1.2.

There was no significant difference in survival time, onset or duration of RA between patients with and without sAAa ($p < 0.21$, $p < 0.46$, $p < 0.26$), neither between female ($p < 0.74$, $p < 0.59$, $p < 0.52$) nor between male ($p < 0.29$, $p < 0.66$, $p < 0.52$).

Comparing the age, sex, onset of RA, and duration of disease at the time of death there was no significant difference between female ($p < 0.70$, $p < 0.78$, $p < 0.62$) and male ($p < 0.29$, $p < 0.66$, $p < 0.52$) RA patients with rAAa ($p < 0.19$, $p < 0.62$, $p < 0.31$) and without sAAa, furthermore between female ($p < 0.41$, $p < 0.81$, $p < 0.46$) and male ($p < 0.29$, $p < 0.66$, $p < 0.52$) RA patients with cAAa ($p < 0.12$, $p < 0.67$, $p < 0.23$) and without sAAa.

There was no significant difference in survival time, onset or duration of RA patients between patients with cardiac or renal amyloid A deposits ($p < 0.79$, $p < 0.96$, $p < 0.86$), neither between females ($p < 0.66$, $p < 0.96$, $p < 0.81$) and males ($p < 1.00$, $p < 1.00$, $p < 1.00$).

	Total (Females and Males)			Females			Males		
RA patients – p <	Avg Age	Disease duration	Onset of disease	Avg Age	Disease duration	Onset of disease	Avg Age	Disease duration	Onset of disease
With sAAa (n = 34) versuss without sAAa (n = 127)	0,213	0,462	0,262	0,745	0,593	0,528	0,297	0,662	0,521
With rAAa (n = 29) versus without sAAa (n = 127)	0,189	0,652	0,316	0,705	0,784	0,621	0,297	0,662	0,521
With cAAa (n = 29) versus without sAAa (n = 127)	0,117	0,676	0,236	0,415	0,816	0,461	0,297	0,662	0,521
With sAAa (n = 34) versus rAAa (n = 29)	0,924	0,860	0,968	0,985	0,863	0,918	1,000	1,000	1,000
With sAAa (n = 34) versus cAAa (n = 29)	0,723	0,820	0,890	0,664	0,822	0,888	1,000	1,000	1,000
With rAAa (n = 29) versus cAAa (n = 29)	0,797	0,966	0,864	0,660	0,965	0,814	1,000	1,000	1,000

Table 1.2

Amyloidosis developed in both sexes, and any time in the course of the disease (Tables 1.1 and 1.2 and Figures 1.1-1.5).

Demographics, onset and duration of RA in patients with sAAa (n = 34 of 161), rAAa (n = 29 of 33) or cAAa (n = 29 of 33) and without sAAa (n = 127 of 161).

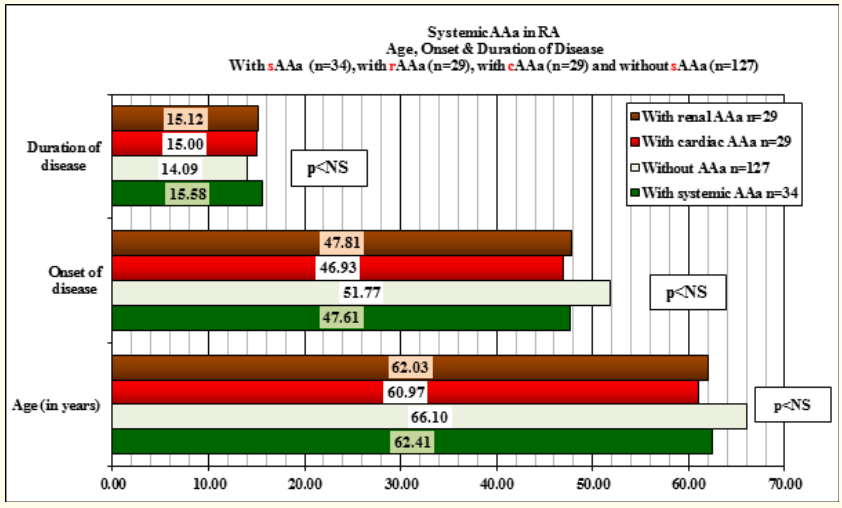


Figure 1.1: *Comparing the age, sex, onset of RA, and duration of disease at the time of death there was no significant difference in survival time, onset and duration of disease between patients with sAAa, rAAa or cAAa and without sAAa, furthermore between sAAa, rAAa and cAAa patients, neither females nor males.*

Demographics, onset and duration of disease of RA patients with sAAa (n = 34 of 161), rAAa (n = 29 of 33) or cAAa (n = 29 of 33) and without sAAa (n = 127 of 161) are summarized in figures 1.2a-c - 1.5a-c.

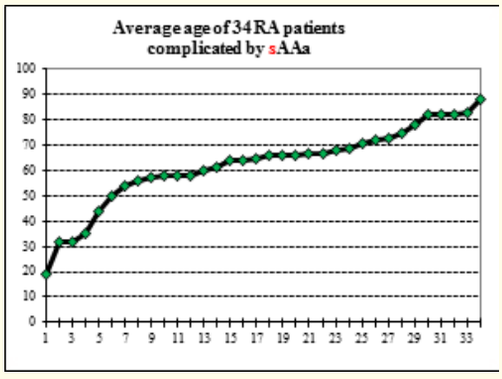


Figure 1.2a

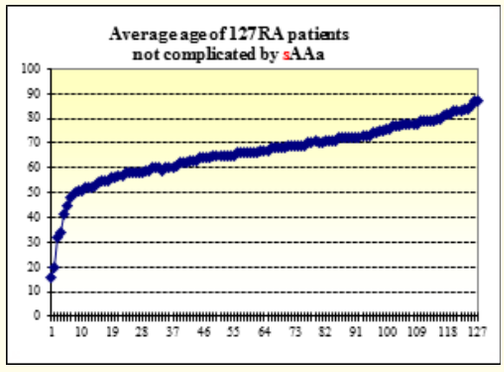


Figure 1.3a

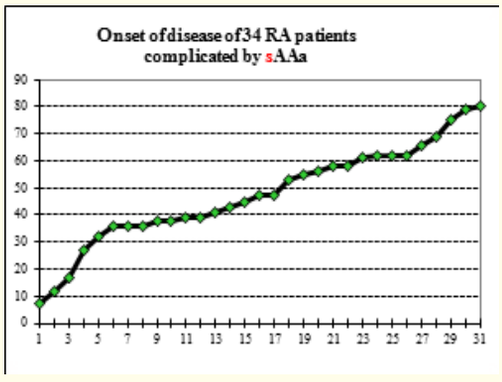


Figure 1.2b

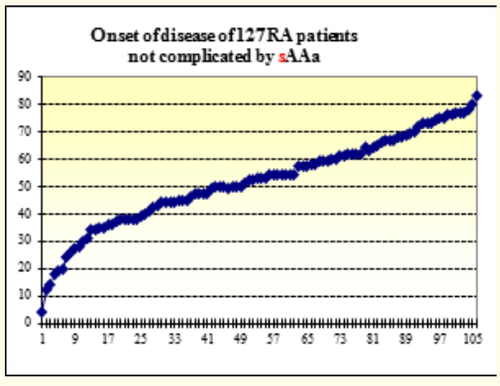


Figure 1.3b

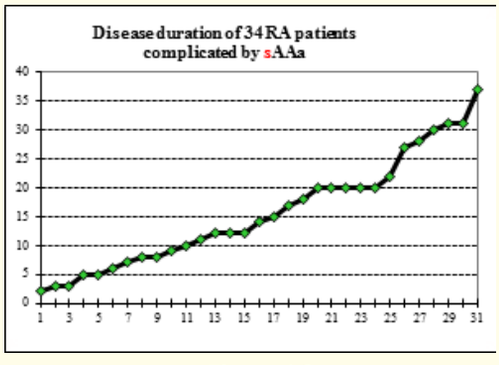


Figure 1.2c

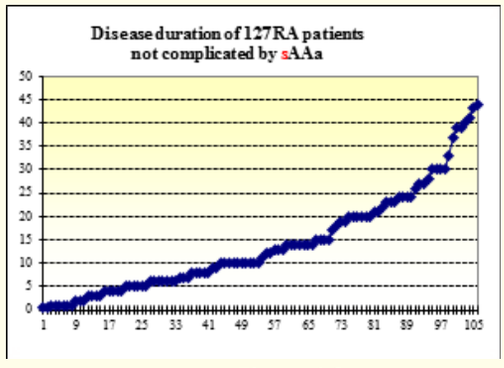


Figure 1.3c

Figure 1.2a-c – 1.3a-c: *There was no significant difference in survival time, onset and duration of disease between RA patients with sAAa and without sAAa, neither females nor males.*

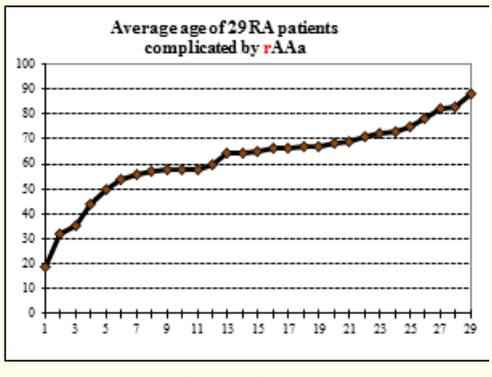


Figure 1.4a

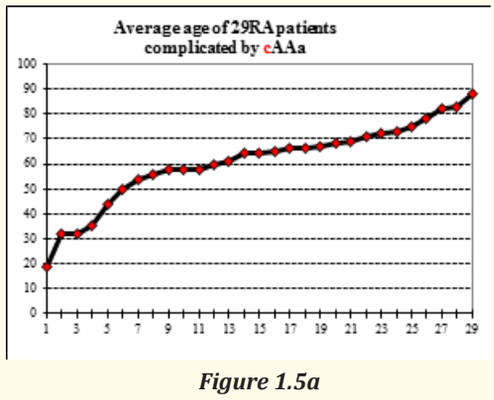


Figure 1.5a

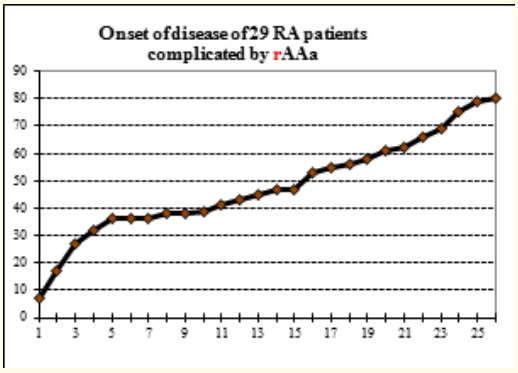


Figure 1.4b

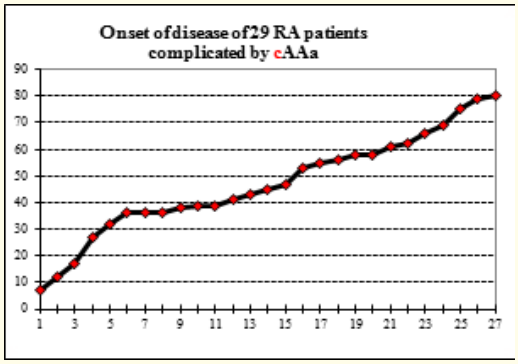


Figure 1.5b

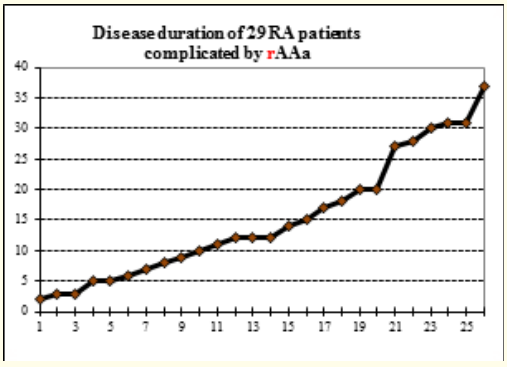


Figure 1.4c

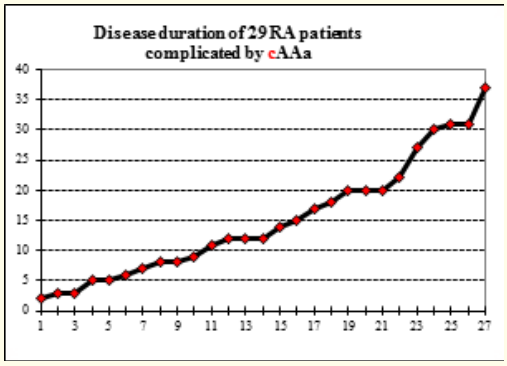


Figure 1.5c

Figure 1.4a-c – 1.5a-c: *There was no significant difference in survival time, onset and duration of disease between rAAa and cAAa patients, neither females nor males.*

There was no significant difference in clinical laboratory parameters between RA patients with sAAa, rAAa or cAAa (Tables 2.1 - 2.2 and Figures 2.1 - 2.2).

The patients with sAAa, rAAa or cAAa were anemic ($p < 0.03$), with significantly lower levels of hemoglobin ($p < 0.01 - 0.04$), and serum bilirubin ($p < 0.001 - 0.008$) in comparison without sAAa (Table 2.1 and Figure 2.1).

They had lower levels of serum albumin ($p < 0.02 - 0.04$), and higher levels of alpha2-globulin ($p < 0.02 - 0.05$) in comparison without sAAa (Table 2.1 and Figure 2.2).

The patients with sAAa, rAAa or cAAa showed decreased renal function: they had higher levels of, blood urea nitrogen (BUN) ($p < 0.02 - 0.03$), higher levels of serum creatinine ($p < 0.002 - 0.004$), and serum potassium ($p <$ NS), lower levels of serum sodium ($p < 0.01 - 0.02$) and proteinuria ($p < 0.001 - 0.001$). Urine specific gravity was significantly lower in patients with sAAa ($p < 0.007$), rAAa ($p < 0.002$), or cAAa ($p < 0.004$) in comparison without sAAa (Tables 2.2 and Figure 2.2).

The pertinent clinical laboratory parameters with sAAa, rAAa or cAAa and without sAAa at death are shown in tables 2.1-2.2 and figures 2.1-2.2.

Figures 3-13 represents different stages of sAAa and rAA.

	With sAAa n = 34	With rAAa n = 29	With cAAa n = 29	Without sAAa n = 127
RBC - T/l	3.54 ± 0.64	3.53 ± 0.68	3.55 ± 0.67	3.87 ± 0.69
Hemoglobin - g/dl	7.31 ± 2.39	7.35 ± 2.55	7.15 ± 2.32	8.54 ± 2.49
Albumin - g/L	42.87 ± 6.47	42.31 ± 6.65	42.04 ± 6.49	45.85 ± 6.01
Alpha2-globulin - %	14.75 ± 3.65	15.23 ± 3.74	15.07 ± 3.87	13.14 ± 3.15
BUN - mmol/l	17.29 ± 16.03	17.85 ± 17.09	18.21 ± 16.97	9.70 ± 6.66
Creatinine - µmol/l	191.62 ± 148.72	199.21 ± 144.59	212.12 ± 151.47	95.73 ± 47.97
Serum Potassium -mmol/l	4.85 ± 0.79	4.93 ± 0.81	4.90 ± 0.83	4.59 ± 3.24
Serum Sodium - mmol/l	137.85 ± 4.93	137.86 ± 4.92	137.87 ± 4.99	140.60 ± 4.18
Urine specific gravity	1011.30 ± 5.55	1010.07± 5.24	1011.00± 5.12	1015.69 ± 6.61
Proteinuria - 0-4+	1.90 ± 1.54	1.92 ± 1.55	2.00 ± 1.58	0.82 ± 0.96
Serum bilirubin - µmol/l	7.57±3.90	5.00 ± 4.74	7.63 ± 3.92	10.96 ± 6.18

Table 2.1: *Significantly differemt or characteristic clinical laboratory parameters of RA patients with sAAa, rAAa or cAAa and without sAAa.*

Relationship between laboratory parameters - p <0.05 (moved)	RBC	Hemoglobin	Albumin	Alpha2-globulin	BUN	Creatinine	Serum Potassium	Serum Sodium	Urine specific gravity	Proteinuria	Serum bilirubin
With sAAa (n = 34) versus without sAAa (n = 127)	0,038	0,021	0,047	0,053	0,020	0,004	0,506	0,015	0,007	0,001	0,001
With rAAa (n = 29) versus without sAAa (n = 127)	0,057	0,047	0,034	0,025	0,030	0,004	0,397	0,027	0,002	0,001	0,008
With cAAa (n = 29) versus without sAAa (n = 127)	0,068	0,013	0,021	0,043	0,023	0,002	0,430	0,025	0,004	0,001	0,002
With sAAa (n = 34) versus with rAAa (n = 29)	0,955	0,952	0,776	0,665	0,904	0,862	0,740	0,994	0,724	0,481	0,706
With sAAa (n = 34) versus with cAAa (n = 29)	0,947	0,805	0,666	0,774	0,842	0,647	0,814	0,990	0,867	0,407	0,962
With rAAa (n = 29) versus with cAAa (n = 29)	0,908	0,773	0,892	0,896	0,941	0,779	0,931	0,997	0,852	0,859	0,751

Table 2.2: *The link between RA patients with sAAa, rAAa or cAAa and without sAAa with significantly different (moved) or characteristic clinical laboratory parameters.*

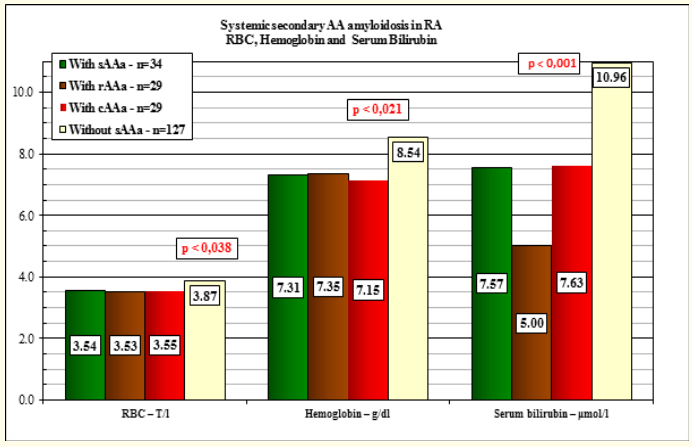


Figure 2.1*: *Significantly different clinical laboratory parameters of female and male RA patients with sAAa, rAAa or cAAa and without sAAa*

There was no significant difference in clinical laboratory parameters between RA patients with sAAa (n = 34), rAAa (n = 29) or cAAa (n = 29). There was a significant relationship between RA patients with sAAa, rAAa or cAAa and without sAAa. The RA patients with sAAa, rAAa or cAAa were anemic (p < 0.038), with significantly lower levels of hemoglobin (p < 0.02 - 0.04), and serum bilirubin (p < 0.001 - 0.008).

**Figure 2.1 indicates only the "p" values between sAAa and without sAAa.*

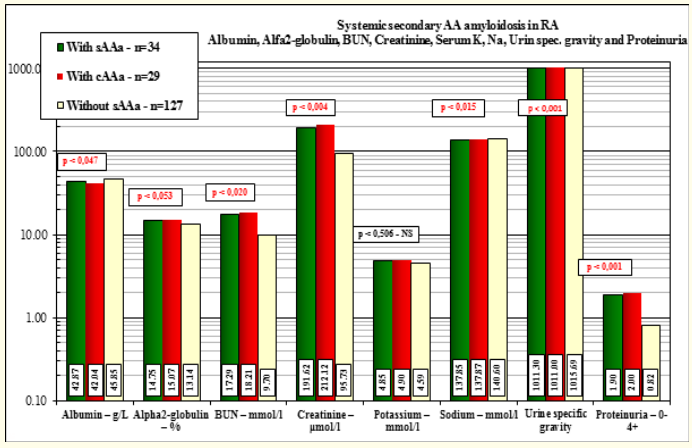
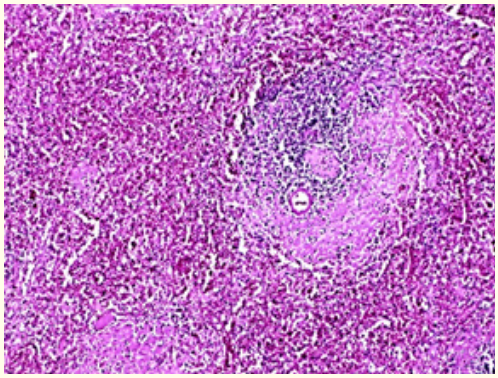


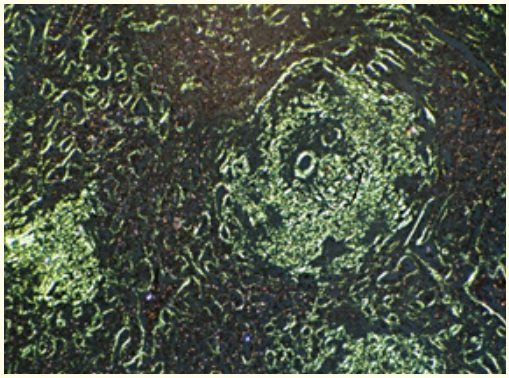
Figure 2.2*: *Significantly different (or characteristic) clinical laboratory parameters with sAAa, rAAa or cAAa and without sAAa*

There was no significant difference in clinical laboratory parameters between RA patients with sAAa (n = 34), rAAa (n = 29) or cAAa (n = 29). There were significant differences between RA patients with sAAa, rAAa or cAAa and without sAAa. The patients with sAAa (n = 34), rAAa (n = 29) or cAAa (n = 29) showed decreased renal function: they had higher levels of alpha2-globulin (p < 0.02 - 0.05), blood urea nitrogen (BUN) (p < 0.02 - 0.03), higher levels of serum creatinine (p < 0.002 - 0.004), serum potassium (p < 0.3 - 0.5; NS), and proteinuria (p < 0.001 - 0.001), lower levels serum sodium (p < 0.015 - 0.027), and albumin (p < 0.02 - 0.04), and urine specific gravity (p < 0.002 - 0.007) in comparison with patients without sAAa (n = 127).

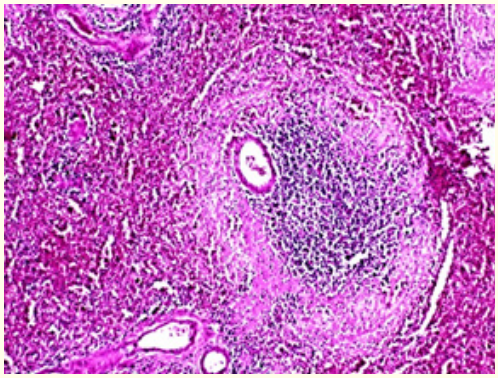
**Figure 2.2 indicates only the "p" values between sAAa (n = 34), and without sAAa (n = 127).*



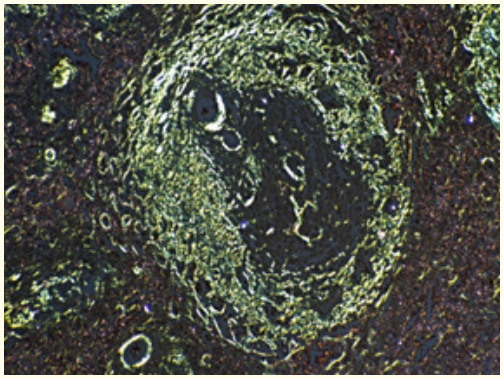
3a



4a



3b



4b

Figures 3a-b and 4a-b: *RA, sAAa, Spleen, with characteristic nodular and diffuse (mixed) ("sago" and "lardaceous") type of amyloid A deposition. Deposits are present in the wall of arterioles venous sinuses and in reticulin fibers (collagen IV). Amyloid A deposits are mainly nodular and only moderately diffuse.*

(3a) HE, x50 (3b) HE, x125

(4a) same field as (3a), stained with Congo red according to Romhányi, without alcoholic differentiation, covered with gum arabic, and viewed under polarized light, x50 (4b) same field as (3b), stained with Congo red according to Romhányi, without alcoholic differentiation, covered with gum arabic, and viewed under polarized light, x125

(The original magnification corresponds to the 24x36 mm transparency slide - the correct hight: weigth ratio is 2:3).

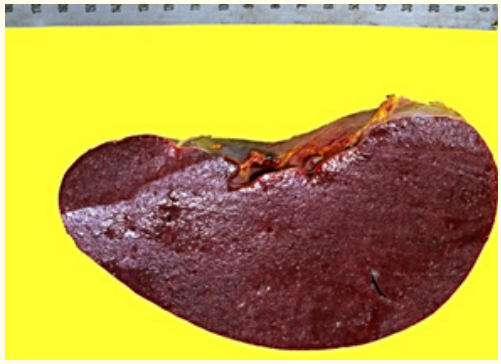


Figure 5: *Spleen, RA complicated by sAAa.*

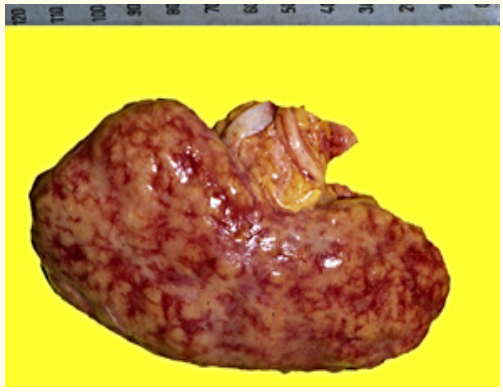
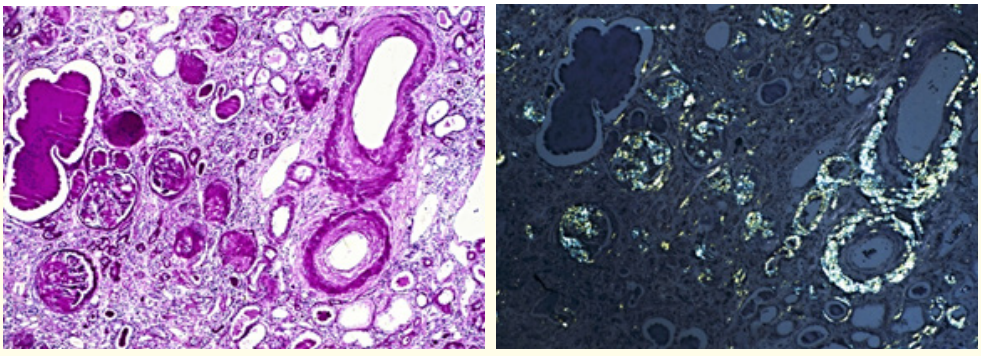
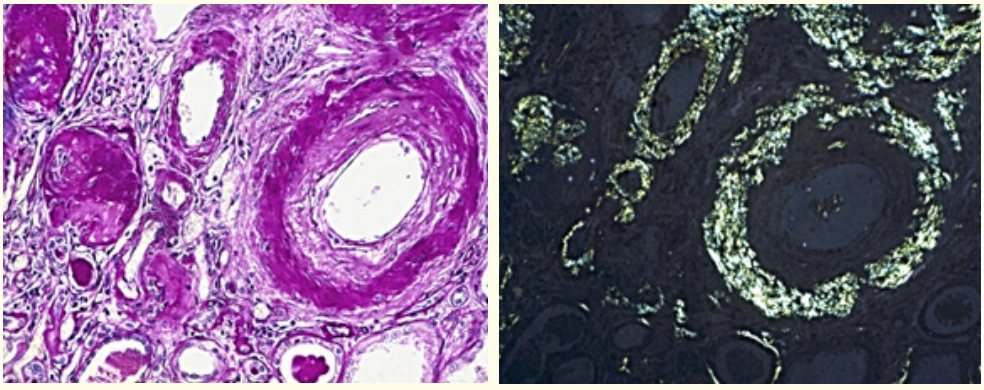


Figure 6: *Kidney (Preceding stage of classical "Waxy kidney"), RA complicated by sAAa.*



7a ***8a***



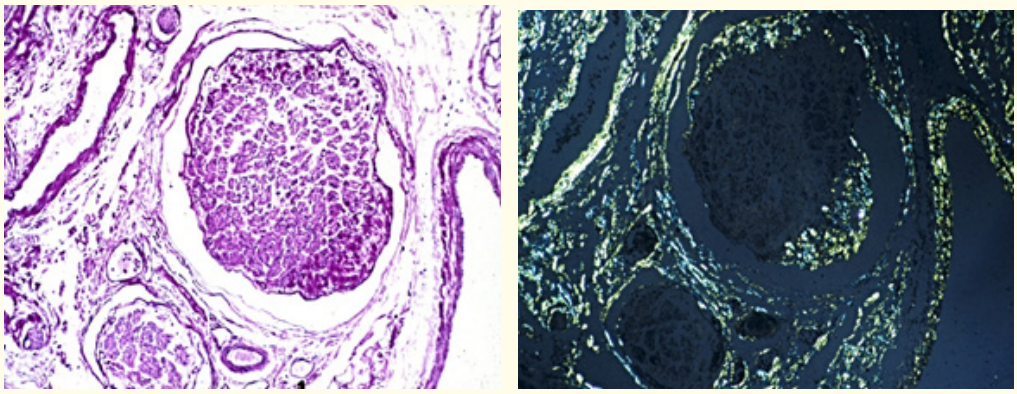
7b ***8b***

Figures 7a-b and 8a-b: *RA, Kidney, advanced stage of sAAa.*

Massive Amyloid A deposits are present in the wall of arterioles, small arteries, basement membranes of cortical convoluted tubules, glomeruli, furthermore in the wall of venules and small veins.

(7a) HE-PAS, x50 (7b) same field as (7a) HE-PAS, x125

(8a) same field as (7a), stained with Congo red according to Romhányi, without alcoholic differentiation, covered with gum arabic, and viewed under polarized light, x50 (8b) same field as (7b and 8a), stained with Congo red according to Romhányi, without alcoholic differentiation, covered with gum arabic, and viewed under polarized light, x125



9a ***9b***

Figures 9a-b: *RA, Kidney, peripelvic region, late stage of sAAa.*

Massive Amyloid A deposits are present in interstitial collagen and reticulin fibers (collagen IV), in the wall of venules and small veins, furthermore in the nerves with peri- and endoneural deposits.

(9a) HE, x200 (9b) same field as (9a), stained with Congo red according to Romhányi, without alcoholic differentiation, covered with gum arabic, and viewed under polarized light, x125

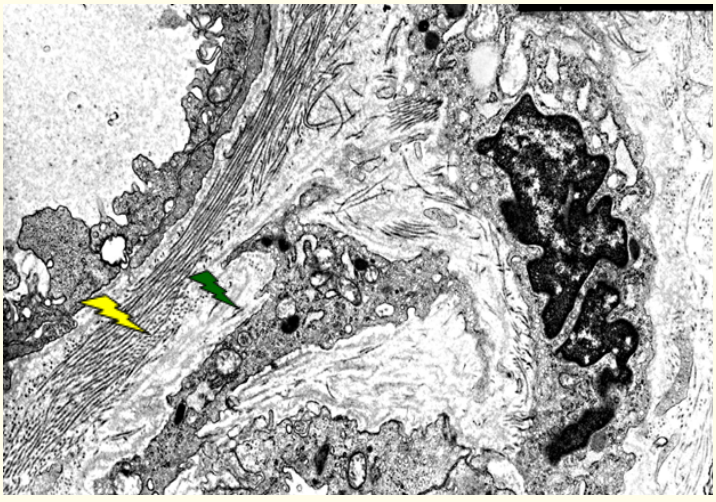


Figure 10: *Electron micrograph.*

RA complicated by sAAa, colon, dilated capillary lined by damaged endothelial cells. Notice a second (outer) reparative layer of endothelial cells (white arrow). The amyloid filaments and fibrils are arranged parallel with the subendothelial basal lamina and collagen fibers (200 nm in diameter - yellow sign). Amyloid filaments (7-10 nm thick - green sign) are located in condensed clusters near the vessel wall between processes of fibroblast and macrophages,

Original magnification (O) corresponds to the 60x90mm negative: x6600

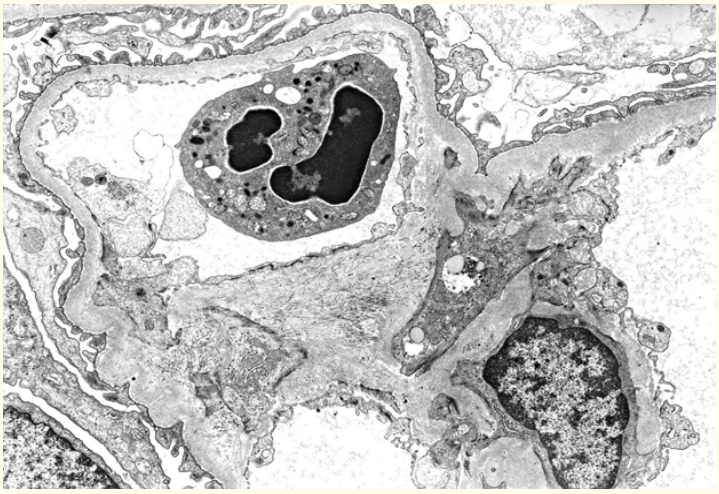


Figure 11: *Electron micrograph.*

RA, kidney, early stage of rAAa,

Dilated glomerular capillary lined by damaged endothelial cells. The amyloid filaments and fibrils are arranged parallel to the subendothelial basal lamina.

O: x3300

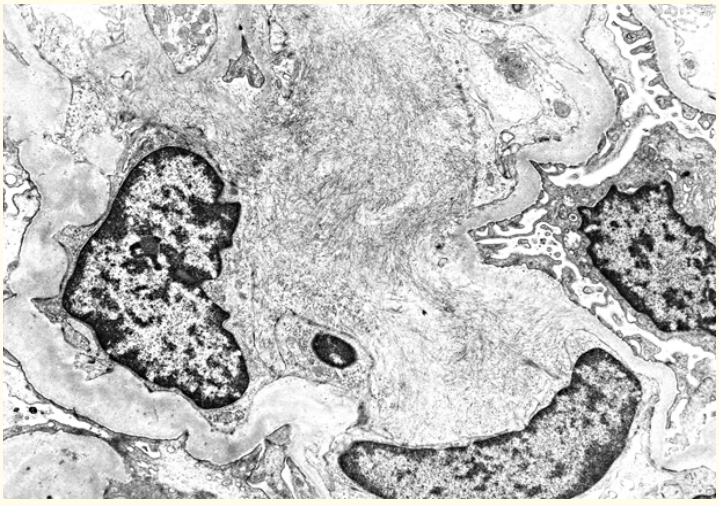


Figure 12: *Electron micrograph.*

RA, kidney, advanced stage of rAAa,

Dilated glomerular capillary lined by normal and damaged endothelial cells. Massive amyloid A deposits. The amyloid filaments and fibrils are arranged in parallel and fragmented clusters within the mesangial matrix, O: x3300

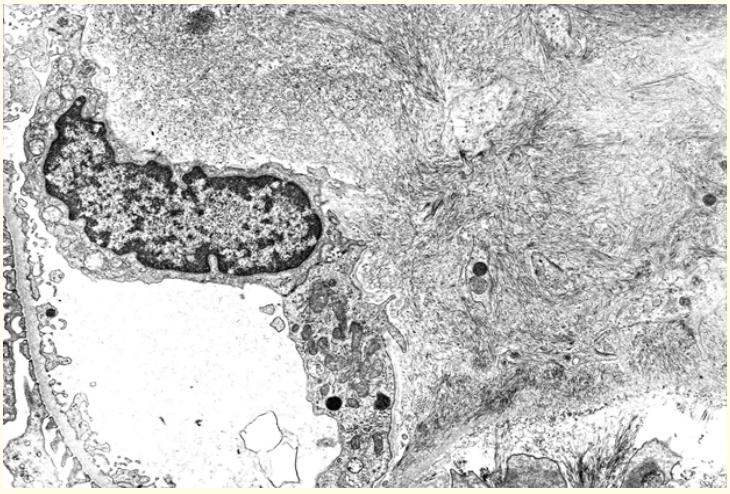


Figure 13: *Electron micrograph.*

RA, kidney, late stage of rAAa,

Dilated glomerular capillary lined by damaged endothelial cells

Massive amyloid A deposits. The amyloid filaments and fibrils are arranged in parallel and fragmented bundles within the mesangial matrix, O: x3300

Discussion

In our cohort of RA patients there was no significant difference in survival time (age at death), onset and duration of disease between females and males or between patients with sAAa, rAAa, cAAa and without sAAa. Amyloidosis may develop as a slowly progressive chronic process in both sexes and at any time of the disease [4].

No blood test is specific for amyloidosis [9]. Significant differences of laboratory parameters between RA patients with sAAa, rAAa or cAAa and without sAAa are not pathognomonic for amyloidosis and the diagnostic values of discussed laboratory parameters are limited.

Anemia may be regarded as a kind of non-hemolytic anemia only [10] accompanied by lower levels of serum bilirubin. Low serum bilirubin levels may prove to be a significant marker for the general anti-oxidant status [10], which shows the general condition of RA patients complicated by sAAa only. Low levels of serum albumin [11] and high values of alpha2-globulin are related to the basic inflammatory processes of the disease, and not to amyloid A deposition [12]. Serum levels of albumin may decrease in any inflammation and should only be regarded as a "negative" acute-phase protein [11].

The significant differences between RA patients with sAAa, rAAa or cAAa and without sAAa are connected with decreased renal function; the higher levels of blood urea nitrogen (BUN) ($p < 0.02$ - 0.03), serum creatinine ($p < 0.002$ - 0.004), serum potassium ($p < 0.3$ - 0.5; NS), and proteinuria ($p < 0.001$ - 0.001), with lower levels serum sodium ($p < 0.015$ - 0.027), and urine specific gravity ($p < 0.002$ - 0.007) show the impaired function of the kidneys only, like other causes of nephrotic syndrome. The mentioned clinical-laboratory parameters are not specific for sAAa, rAAa or cAAa they only imply more or less renal impairment.

Progression of renal amyloidosis is associated with severe proteinuria or nephrotic syndrome [13]. Indeed, several authors call attention the importance of proteinuria in the early literature [14-16].

The excess of proteinuria in our autopsy population may indicate the severity of sAAa, and rAAa; the excess of proteinuria correlated with the severity sAAa (severe vs mild sAAa: 0.004; severe sAAa vs without sAAa: 0.0003), and rAAa (severe vs mild rAAa: 0.0002; severe rAAa vs without rAAa: 0.00001) based on the increasing levels of significance [unpublished data]. The elevated serum potassium level may also indicate progression of renal amyloidosis (severe vs mild rAAa: 0.005; severe vs mild and latent rAAa: 0.003; severe rAAa vs without rAAa: 0.027) [unpublished data].

In some cases the values of the mentioned clinical-laboratory parameters remained in the normal range, for example serum albumin (35 - 50 g/l), total serum bilirubin (5.0 - 17.1 µmol/l), serum potassium (3.70 - 5.10 mmol/l), sodium (135 - 145 mmol/l), urine specific gravity between 1000 and 1030, etc., which reduce the clinical significance of these parameters, and only their graduall impairment may suggest a trend. Since the mentioned clinical-laboratory parameters indicate an advanced or late stage of sAAa [1], therefore their value is reduced from a therapeutic viewpoint.

The early diagnosis of AA amyloidosis is important. Therefore examination of all available surgical or biopsy material for amyloid is suggested in all hospitalised RA patients, with or without clinical evidence of amyloidosis.

When clinical suspicion of amyloidosis is raised in case of unexplained weight loss, fatigue, anemia, impaired renal function, restrictive cardiomyopathy, hepatomegaly, or gastrointestinal complaints (malabsorption, malnutrition, diarrhea, obstruction, disturbed motility, bleeding) or reduced respiratory capacity [18], a biopsy is needed. We suggest gingival or rectal biopsies, and not abdominal fat tissue, which was positive only in 25 % of our autopsied RA patients with sAAa [1].

More recent laboratory parameters such as serum amyloid A (SAA) level were not determined between 1969 and 1992. SAA and CRP are regarded now the most sensitive indicators for assessing inflammatory activity [18]. SAA proteins are produced predominantly by the liver [19]. SAA is implicated in several chronic inflammatory diseases, such as rheumatoid arthritis, amyloidosis, atherosclerosis etc [20]. Prolonged elevation of SAA is not specific for amyloidosis, and does not necessarily indicate tissue deposition of amyloid A [12].

Radioactive imaging, using radiolabeled amyloid molecules, i.e. serum amyloid P component (SAP) scintigraphy [21] or positron emission tomography (PET) [22] has not yet full-filed the hopes [23].

Summarizing our clinical-laboratory parameters and the data of the pertinent literature, we cannot state more today than Alan S Cohen wrote 50 years ago: "There are no laboratory abnormalities specific to or unique for amyloid"… "There is no one finding in the blood, urine, electrocardiogram or x-ray that is specific for this disease, however", and in agreement with him, " the diagnosis should based upon a biopsy using an "appropriate staining procedure" [24].

Using a less sensitive staining method some positive cases remain undetected. A more specific method potentially detects more cases, and reveals earlier stages. Amyloidosis in most studies was diagnosed with different methods (Toluidine blue, Crystal violet, Sirius red, Congo red staining according to Romhányi, Bennhold's, Puchtler's, Bély's Congo red method etc.) of diverse specificities and sensitivities. Congo red staining according to Bennhold [25] or Puchtler is widely used [26]. Congo red staining according to Romhányi is less known and less commonly used in the Anglo-Saxon literature [6,27]. According to our experience and accurate knowledge the best method for diagnosis of amyloidosis is recently with a small modification [7] the Congo red staining according to Romhányi [6]. "Appropriate" polarizing microscope with high brightness - at least 100 watt - is indispensable.

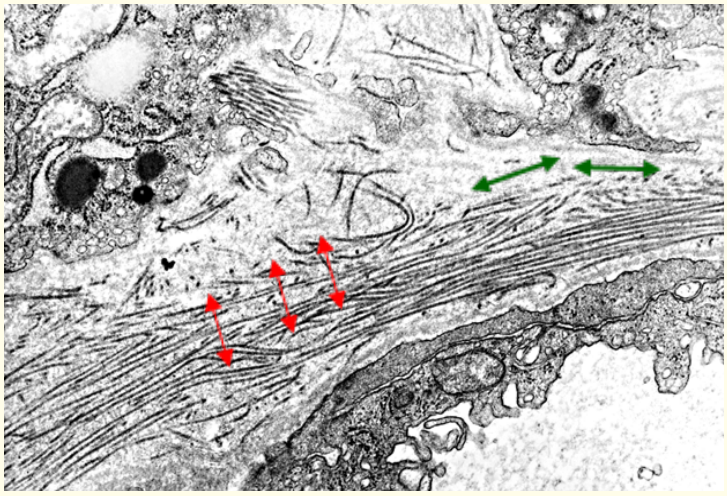
Figures 14a-b demonstrate schematically the advantage of Congo red staining according to Romhányi (without alcoholic differentiation, covered with gum arabic) [6,27], in comparison with Puchtler's Congo red staining method (with alcoholic differentiation, covered with Canada balsam) [26].

The arrangement of Congo red molecules is represented schematically on electron micrograph in relation to 200 nm collagen fibrils (red arrows), and to 7-10 nm thick amyloid A protein filaments (green arrows), in polar hydrophilic mounting medium (gum Arabic) according to Romhányi's Congo red staining [6,27].

The Congo red molecules are oriented parallel to the surface of the amyloid filaments and their birefringence - viewed under polarized light - is additive and linear (axis parallel) positive. The intensive additive linear positive birefringence is specific for amyloid filaments.

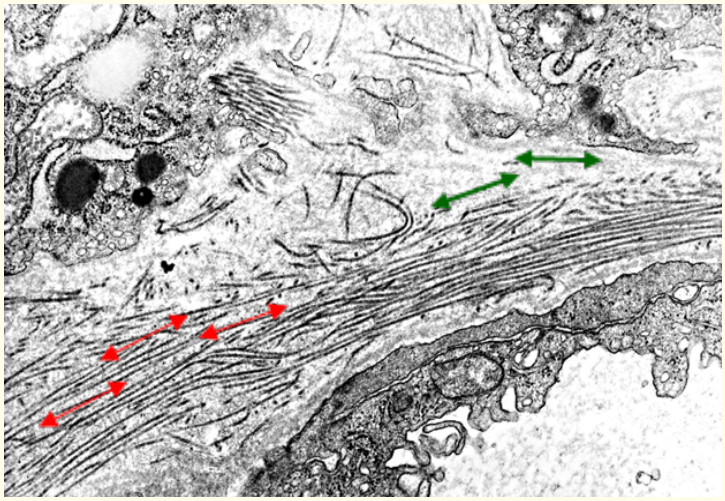
Otherwise, the Congo red molecules are oriented perpendicular to the surface of collagen, reducing the intensity of birefringence and even inducing an inversion of the original sign of the collagen birefringence, excluding the false positive diagnosis of amyloidosis ("phantom amyloidosis").

There is no alcoholic differentiation, so the minimal amyloid deposits remain detectable. Disadvantage of Romhányi's method is, that a second tissue section is needed to detect amyloid deposits, beside with the hematoxylin-eosin stained one.



Figures 14a: *Electron micrograph.*

RA complicated by sAAa, colon - Same area as Figure 10, O: x13000



Figures 14b: *Electron micrograph.*

RA complicated by sAAa, colon - Same area as Figure 10, O: x13000

The arrangement of Congo red molecules is represented schematically on the electron micrograph in relation to 200 nm collagen fibrils (red arrows) and to 7-10 nm thick amyloid A protein filaments (green arrows), in apolar hydrophobic mounting medium (Canada balsam) according to Puchtler's method [26].

The bipolar Congo red dye molecules are aligned parallel to the surface of the collagen fibrils and amyloid filaments cause an increased additive type optical reaction.

In case of less than optimal extraction of Congo red molecules, the nonspecifically bound dye molecules may cause a false positive birefringence of collagen with green polarization colors (residual Congo red dye molecules on the surface of collagen fibers may cause a false positive diagnosis of amyloidosis).

In case of more than optimal extraction of Congo red molecules, the minimal amyloid deposits may disappear: during alcoholic differentiation dye molecules may be washed off the amyloid filaments, resulting in a false negative diagnosis.

The advantage of Puchtler's method is that only one tissue section is needed for the identification of amyloid deposits, but this is inevitably associated with the risk of a false negative or false positive diagnosis of amyloidosis.

Conclusions

The discussed clinical-laboratory parameters allude to the existence of sAAa only, and more or less refer of amyloid A deposition in the kidneys. None of them (no one) is specific to or unique for amyloid itself.

Our autopsy patients with sAAa were anemic ($p < 0.03$), with significantly lower levels of hemoglobin ($p < 0.02$ - 0.04), serum bilirubin ($p < 0.001$ - 0.008), and showed decreased renal function: they had higher levels of alpha2-globulin ($p < 0.02$ - 0.05), blood urea nitrogen (BUN) ($p < 0.02$ - 0.03), creatinine ($p < 0.002$-0.004), serum potassium ($p < 0.3$ - 0.5; NS) and proteinuria ($p < 0.001$ - 0.001), with lower levels serum sodium ($p < 0.015$ - 0.027), albumin ($p < 0.02$ - 0.04) and urine specific gravity ($p < 0.002$ - 0.007).

In the proper clinical setting amyloidosis should be considered in case of unexplained weight-loss, fatigue, anemia, impaired renal function, restrictive cardiomyopathy, hepatomegaly, and gastrointestinal complaints or reduced respiratory capacity, and should indicate a biopsy. We suggest gingival or rectal biopsies in any suspected cases, stained with Congo red according to Romhány, using an appropriate polarizing microscope with high brightness.

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