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Clinical Research

Risk Factors for Developing Renal Amyloidosis in Patients with Rheumatoid Arthritis

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Abstract

Background. Secondary or acquired amyloidosis (AA) develops in patients with chronic infections and inflammatory diseases such as rheumatoid arthritis (RA). The detection of risk factors is one of the most important objectives that will help to improve the patient's survival. **Methods and results.** In this study, we observed 104 patients: 45 (1st group) with RA complicated by renal amyloidosis (histologically confirmed) and 59 RA patients without this complication (2nd group). All patients were Belarusian citizens. Patient's previous medical history and data were analyzed. All patients had undergone tests for the detection of *C. trachomatis* infection. Urethra or cervical scrapes were analyzed by polymerase chain reaction (PCR) method and/or by cultural method; the presence of *C. trachomatis* G and M antibodies was detected by immunoenzyme assay. Further, we compared the influence of the SAA1 gene polymorphism in AA-positive RA patients with those in AA-negative RA. A statistical analysis was conducted to detect possible risk factors for developing renal amyloidosis secondary to RA. The odds ratio (OR) calculated for the SAA1 α/α genotype was 45.26, and the 95% confidence interval (CI) was – 95%CI (9.9–206.8). It was shown that the SAA1 α/α genotype dominated in both groups and consisted 95.6% (1st group) and 32.2% (2nd group), respectively. The OR for *C. trachomatis* infection was 26.6; 95%CI (9.26–76.37). Further, we created a prognostic model to determine the risk factors for developing renal amyloidosis in patients with RA. **Conclusions.** The risk of developing secondary amyloidosis in RA patients significantly depends on SAA1 genotype and the presence of *C. trachomatis* infection and can be evaluated using the prognostic model. IJBM 2011; 1(2):87-96.
Key words: *secondary amyloidosis, rheumatoid arthritis, risk factors.*

Introduction

Rheumatoid arthritis (RA) is characterized by progressive joint damage, and it affects internal organs and leads to patient's disability. Besides the persistent joint pain, visceral lesion shortens patient's life span considered one of the most severe complications, approximately for three years [1]. Renal involvement is defining clinical course and prognosis of the disease. Secondary amyloidosis often leads to renal failure and fatal outcome in patients with RA [2]. The treatment strategy in renal amyloidosis demands close collaboration between nephrologists and rheumatologists. The decrease in glomerular filtration rate is accompanied due to the limitation of therapeutic possibilities; life prognosis depends on rapidity of renal failure progressing. The risk of developing renal amyloidosis remains uncertain and needs discussion. AA amyloidosis, acquired or secondary amyloidosis, is a relatively rare disease which may complicate serious chronic infections and chronic inflammatory diseases.

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Precursors of AA amyloid are acute-phase proteins produced by different cell types, mainly neutrophils and fibroblasts in liver [3]. Nowadays, the most common underlying process is system connective tissue diseases, including RA. Thus far, up to 60% of secondary amyloidosis develops in RA patients [4, 5, 6], while 10 years ago it was just 35% [7]. Renal amyloidosis is considered to develop in 10-15% of RA patients [8]. Kobayashi et al. revealed that rectal mucosal biopsy showed amyloid deposits in 13.3% RA patients but clinical presentations had just 4.4% [9]. During 1979–1988, different types of amyloidosis were revealed in 19% of renal biopsy conducted in Japan.

It is well known that there is a gene localized on a short shoulder of chromosome 11 encoding precursor protein – Serum Amyloid A (SAA). It relates to acute-phase proteins, close to C-reactive protein (CRP). SAA is synthesized by liver in response to inflammatory cytokines, hence its level significantly increases in inflammation [10]. SAA is considered to be more sensitive acute-phase protein than CRP [11, 12, 13]. Proinflammatory cytokines (interleukin-1, interleukin-6, tumor necrosis factor-α, etc.) induce the activity of serum amyloid A (SAA) gene and consequently SAA synthesis. Under normal conditions, SAA level is less than 10 μg/ml [14, 15, 16]. The correlation between SAA levels and risk of developing secondary amyloidosis is contradictory [17, 18, 19]. The deposition of AA amyloid is considered to slow down when SAA concentration is less than 3 μg/ml.

Baba et al. demonstrated the linkage between SAA1 gene polymorphisms and susceptibility to amyloidosis [20]. Recently described -13T/C single nucleotide polymorphism in 5′-flanking region of SAA1 gene showed a positive association with AA-amyloidosis development in Japanese and Caucasian patients with RA [21]. A risk factor for developing secondary amyloidosis was -13T allele variant.

A patient's survival in secondary amyloidosis strongly depends on the nature of the underlying process. According to the published data, one third of patients with AA-amyloidosis suffer from chronic renal failure (CRF) in five years after proteinuria onset [22, 23, 24]. The unfavorable prognosis of renal amyloidosis is associated with the previous diagnostic difficulties of organ failure. Besides, there is no effective way for treatment and prophylaxis of this complication. Further, delayed verification of diagnosis and delayed referral to nephrologists are associated with poor prognosis of chronic kidney disease (CKD) progression. Sasatomi et al. [23] showed that 2-year survival rate of patients with secondary amyloidosis was 55%, 5-year survival rate was 30%, and 10-year survival rate was only 20%. Joss et al. examined 43 patients with RA; average survival rate after the manifestation of AA-amyloidosis was 52.9 months. 42% of patients died because of infectious complications, and 12.5% because of end-stage renal disease (ESRD). Factors correlating with poor prognosis were low serum albumin level and high level of proteinuria. In this study, 5-year survival rate was 43% [25].

The progressive renal function decline in secondary amyloidosis shows possible risk factors that are extremely important. In some published data, these factors are male sex, earlier onset of RA, positive rheumatoid factor (RF), severe RA course, other extra-articular RA manifestations, poor RA activity control, high persistent CRP level serum [26, 27]. Immonen et al. reported that prednisolone monotherapy in RA onset is associated with high mortality rate in patients with amyloidosis secondary to juvenile arthritis (compared to Disease-Modifying Antirheumatic Drugs (DMARDs) and/or cytostatic agent) [28].

Therefore, one can suppose that SAA level increases not only in connective tissue diseases, but also in longstanding inflammatory processes, including chronic infections. In clinical practice, we commonly observe the combination of rheumatoid arthritis, mostly seronegative, with persistent *Chlamydia trachomatis* infection [29]. Therefore, the presence of persistent *C. trachomatis* infection in patients with rheumatoid arthritis may act as a supplementary stimulus for the development of secondary amyloidosis.

Objective

To determine the risk factors for developing secondary amyloidosis in RA patients and to create a prognostic model for risk evaluation.

Patients and Methods

In this study, we analyzed 104 RA patients from Belarusian Rheumatology center. All patients met the 1987 revised RA criteria of the American Rheumatism Association (ARA) [30]. 45 patients had secondary amyloidosis, which was confirmed by renal biopsy, rectal mucous biopsy, or gingival biopsy. The morphological method was used in the case of the following scenario:

- Persistent proteinuria in two urine samples;
- ESR elevation in two blood samples, not associated with other clinical and laboratory signs of RA activity;
- Elevation of urea and creatinine serum level in two blood samples;
- Persistent constipation/diarrhea in RA patients.

Tissue sections were stained with Congo red and analyzed in polarized light. Bright green birefringence was taken as evidence of amyloid.

All patients were divided into two groups:
1st group: AA-positive RA patients (n=45);
2nd group: AA-negative RA patients (n=59).

All patients had undergone the following tests:

- Clarification regarding medical history and previous medical data to investigate possible predictors of secondary amyloidosis in RA.
- Physical examination with visual analogue scale assessing pain, swollen joint count (SJK) and tender joint count (TJC) to asses SDAI.
- Blood analysis.
- Biochemical blood analysis (total protein, albumin, CRP, RF, urea, creatinine, potassium).
- Urine analysis.
- 24-hours protein excretion.
- Determination of glomerular filtration rate using

Cockcroft-Gault equation and Modification of Diet in Renal Disease (MDRD) Study equations.

- Blood lipid analysis.
- Coagulogram.
- Tests for *C. trachomatis* infection with obligate polymerase chain reaction (PCR) or cultural method (using McCoy medium). Further, we took into consideration the previous medical data about *C. trachomatis* infection.
- SAA1 genotyping by three polymorphic sites −13T/C, 2995C/T, and 3010C/T.
- Radiographic joint examination to determine RA stage according to Steinbrocker's criteria.

Patient's group characteristics. There were 37 female and 8 male patients in the 1st group, and 50 female and 9 male in the 2nd group (Figure 1). The difference between the groups were not significant (P=0.83). The average age of patients in the 1st group and 2nd group was 58 (40; 62) years, and 52 (42; 57), respectively (P=0.14).

There were no significant differences in RA activity (SDAI), radiographic stage and RF positivity/negativity between the two groups (Table 1). Simplified Disease Activity Index (SDAI) was suggested by Professor J. Smolen. In this method, he used CRP serum level in mg/dL (0-10) instead LnESR (as used in all DAS modifications).

$$\boldsymbol{SDAI = SJC + TJC + Global\ disease\ assessment_{patient} + Global\ disease\ assessment_{physician} + CRP(mg/dL)}$$

In AA-positive RA patients, 97.8% presented proteinuria (1 patient (2.2%) – had renal function impairment without proteinuria).

Determination of SAA1 genotype. Native DNA was extracted from leucocytes of blood samples obtained from 45 AA-positive RA patients (1st group) and 59 AA-negative RA patients (2nd group). To amplify a segment of the SAA1 gene including the polymorphic sites −13T/C, 2995C/T, and 3010C/T, it was genotyped by polymerase chain reaction (PCR) with subsequent restriction enzyme digest analysis. PCR was conducted with the help of MyCyclerTM Thermal cycler (BIORAD) amplifier.

−13T/C gene SAA1 polymorphism was detected by amplification method with subsequent restriction enzyme digest analysis with AciI endonuclease ("Fermentas",

Fig. 1
Patients sex distribution

Table 1
Description of patient's groups

		1st group (AA-positive RA patients)	2nd group (AA-negative RA patients)	p
RF n (%)	RF +	36 (80)	43 (72.9)	*0.54*
	RF -	9 (20)	16 (27.1)	
Activity n (%) (SDAI)	1	0	0	*0.8*
	2	13 (28.9)	27 (45.8)	
	3	32 (71.1)	32 (54.2)	
Radiologic stage n (%)	I	1 (2.2)	1 (1.7)	*0.19*
	II	3 (6.6)	12 (20.3)	
	III	21 (46.7)	25 (42.4)	
	IV	20 (44.4)	21 (35.6)	

Lithuania) and electrophoretic separation in 8% polyacrylamide gel. Restriction analysis was conducted strictly according to the instruction of MBI Fermentas. 2995 C/T and 3010 C/T gene SAA1 polymorphism was detected by the amplification method with subsequent restriction enzyme digest analysis with BanI (2995) or BclI (3010) endonuclease. Every polymorphism was detected separately. The detection of electrophoretic separation was conducted in 2% agarose gel under ultraviolet (UV) light by Vilber Lourmat transilluminator (France), and the results were fixed by Nikon 2100 digital camera.

Statistical methods. The obtained data were located in a database Microsoft®Office Excel 2002. Statistical analysis was conducted by means of STATISTICA 6.0 package (StatSoft. Inc., USA). The distribution of studied parameters was assessed by Shapiro–Wilk's W test. The results were expressed as median (Me), upper and lower quartiles (25; 75%). Non-parametric Mann–Whitney U rank test was employed for the comparison of two independent groups. The statistical analyses of genotype and allele frequency comparisons of the various single nucleotide polymorphisms between groups were performed using the chi-square test. Further, the correlation between two groups was assessed by Gamma method for non-parametric data. We used odds ratio (OR) to assess the risk factors for developing secondary amyloidosis. The prognostic factors predicting renal amyloidosis in RA patients was analyzed using logit regression method. P-values less than 0.05 were considered statistically significant.

Results

Genotyping

−13T/C gene SAA1 polymorphism.

−13T/C gene SAA1 polymorphism was established by heavy fragments – 212 and 190 nucleotide pairs (Figure 2). If the patient's genotype was homozygous, we observed fragments with one molecular weight on the electrophoregramm. Conversely, in the case of heterozygous genotype, two fragments with various molecular weights were observed.

Results of-13T/C gene SAA1 polymorphism detection are presented in Table 2.–13T risk allele in the investigated samples meets only in heterozygotic genotypes in all patients.Thus, no significant differences between two patient's groups were found. Therefore,–13T allele is not required for developing AA-amyloidosis in Belarusian patients with RA.

2995 C/T and 3010 C/T SAA1 gene polymorphism.

Electrophoregramm of 2995 C/T SAA1 gene polymorphism after BanI processing is shown in Figure 3.

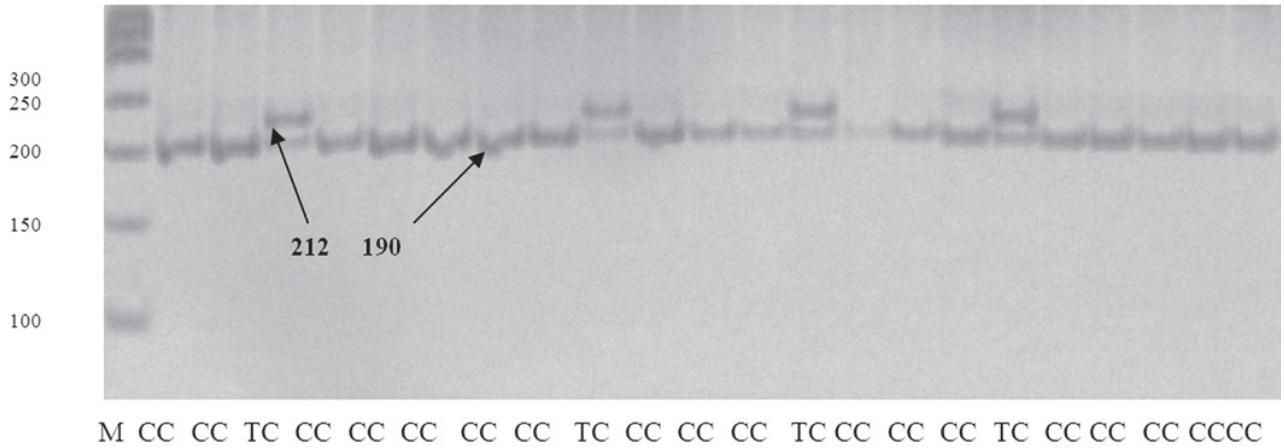

Fig. 2
Electophoregramm of -13T/C fragments

Table 2
-13T/C SAA1 gene polymorphism detection

	1st group (AA-positive RA patients)	2nd group (AA-negative RA patients)	p
n	45	59	
-13T/C gene SAA1 polymorphism	**n (%)**		
TT	0 (0.0)	0 (0.0)	
TC	10 (22.2)	12 (20.3)	0.5
CC	35 (77.8)	47 (79.7)	0.5
Alleles			
-13T	10 (11.1)	12 (10.2)	0.5
-13C	80 (88.9)	106 (89.8)	0.5

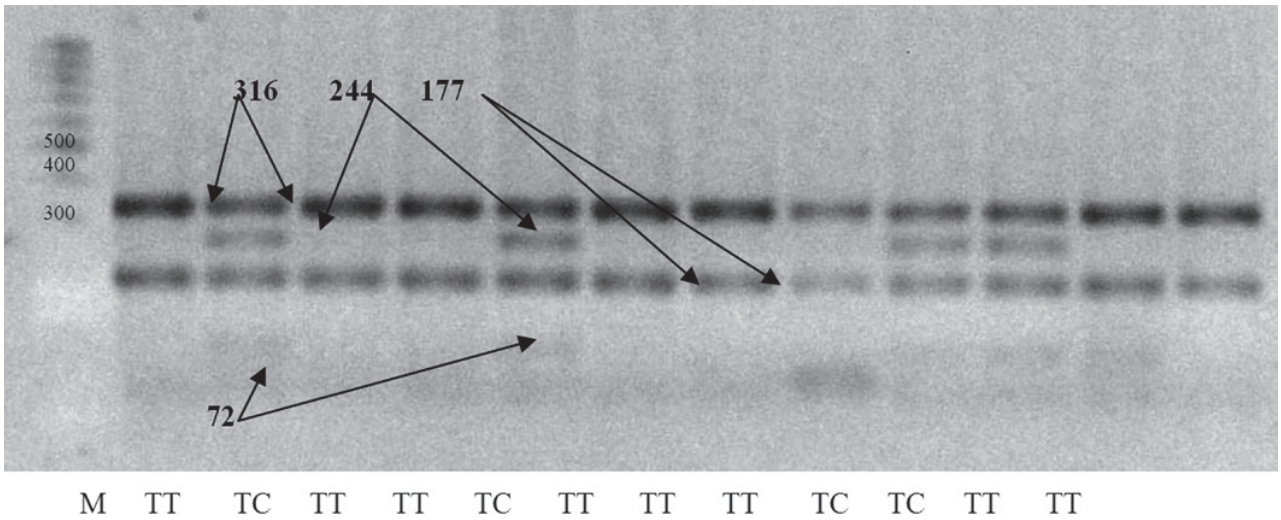

Fig. 3
Electophoregramm picture of BanI fragments 2995C/T locus

In the case of single nucleotide replacement in position 3010, restrictase BclI cut an amplified fragment (518 nucleotide pairs) in 427 and 91 nucleotide pairs.

Patient's genotypes on two polymorphic loci of SAA1 gene are presented in Table 3. Moreover, we observed strong correlation between genotype variant and presence of secondary amyloidosis: R=0.93, P<0.0001 (Figure 4).

The comparison of groups on genotype and three allele frequencies showed statistically significant differences. The most notable differences were observed in α/α genotype – χ^2=31.1; P<0.001. Forty-three out of 45 AA-positive RA patients had α/α genotype, while only 32.2% AA-negative RA patients showed this genotype. The similar tendency was observed on the frequency of α allele : χ^2=47.01(P<0.001).

It is remarkable that γ/γ genotype was not found in both the groups. This genotype variant is considered a risk factor for developing secondary amyloidosis in Japanese RA patients. Furthermore, in AA-positive patients, γ allele was not present. Thus, one can consider the presence of γ allele to be "protective" as it was defined only in AA-negative group.

The same conclusion concerns β allele, as it was present only in 2 AA-positive RA patients (χ^2=25.79, P<0.001).

An "odds ratio" (OR) for α/α genotype was 45.26 with confidence interval (CI) – CI (9.9–206.8) (Table 4). Thus, the relative risk of developing secondary amyloidosis significantly increases in the case of α/α genotype.Therefore, this genotype variant is a genetic risk factor for developing secondary amyloidosis in Belarusian patients with RA.

Statistical analysis showed strong correlation between α allele and secondary amyloidosis (R=1) (P<0.001). At the same time, the presence of γ allele reduces theprobability of this RA complication (R=–1) (P<0.001). β allele also acts similarly(R=–0.91) (P<0.001). Hence, α allele is the significant risk factor for developing secondary amyloidosis in RA patients, while β and γ alleles have protective influence.

−13T allele of SAA1 gene (−13T/C locus) presented in 10.2% AA-positive and in 11.1% in AA-negative RA patients (P=0.5). There were no homozygous −13T/T patients in both the groups. Thus,−13T allele is not a risk factor for developing renal amyloidosis in Belarusian patients with RA.

Chlamydia trachomatis as a risk factor for developing of secondary amyloidosis

Chronic infection increases serum SAA level. However, in turn, SAA induces the formation of insoluble derivatives in tissue that leads to progression of AA amyloidosis. Therefore, we decided to assess the possible influence of chronic *C. trachomatis* infection on secondary amyloidosis development.

38 (84.4%) out of 45 patients with AA-positive RA patients had concomitant *C. trachomatis* infection during all course of RA. In the 2nd group, association of RA with *C. trachomatis* infection was revealed only in 10 (16.9%) out of 59 patients.

During the medical history analysis and from the data of patient's objective examination, special attention was paid to the clinical signs of *C. trachomatis* infection. 28 (62.2%) patients in the 1st group presented an unusual onset of RA: knee swelling and synoviitis, achillitis, asymmetric joint involvement (for example, right or left knee joint). At different stages of RA in 9 (20%) patients of 1st group, it was observed that they had joint swelling with skin hyperemia. In 2 (4.4%) AA-positive RA had atypical joint involvement in disease onset – I metacarpophalangeal articulation and V proximal interphalangeal joint. 8 (17.8%) patients from the 1st group presented longstanding hyperthermia during disease course that can be a sign of chronic *C. trachomatis* infection. However, it was observed that 3 (6.7%) patients receiving DMARDs had hyperthermal reaction. Thus, antibacterial therapy normalized the temperature. Almost all patients of the 1st group had persistent high disease activity. Probably, it is connected with irregular receiving DMARDs, which was mentioned by the majority of AA-positive patients. Besides, latent *C. trachomatis* infection supported persistent RA activity.

Table 3
2995C/T and 3010C/T gene SAA1 polymorphism detection

Genotype			1st group (AA-positive RA patients) n=45	2nd group (AA-negative RA patients) n=59	χ^2	p
2995C/T	3010 C/T	α, β, γ	n (%)	n (%)		
TT	CC	α/α	43 (95.6)	19 (32.2)	31.2	<0.001
TC	CT	α/β	2 (4.4)	16 (27.1)	10.3	<0.001
TC	CC	α/γ	0 (0.0)	10 (16.9)	6.6	0.005
CC	TT	β/β	0 (0.0)	9 (15.3)	5.71	0.005
CC	TC	β/γ	0 (0.0)	5 (8.5)	2.37	0.07
CC	CC	γ/γ	0 (0.0)	0 (0.0)		
α			88 (97.8%)	64 (54.2)	47.01	<0.001
β			2 (2.2%)	39 (33.1)	25.79	
γ			0 (0.0)	15 (12.7)	10.5	

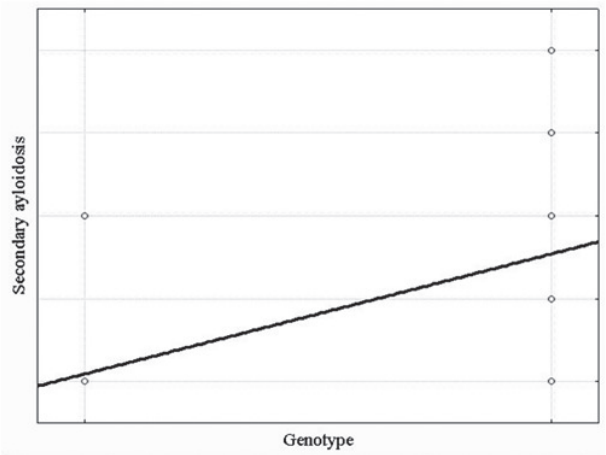

Fig. 4
Correlation between a genotype and secondary amyloidosis (direct linear dependence)

Table 4
Odds ratio for different variant of SAA1genotype.

	OR	CI
Genotype		
α/α	45.26	9.9-206.8
α/β	37.13	8.7-157.9
α/γ	0	-
β/β	0	-
β/γ	0	-
Allele		
α	37,13	8,7-157,9
β	0,05	0,01 – 0,2
γ	0	-

Case report

A 30-year-old woman suffered from pain and swelling of I metacarpophalangeal articulation on the right hand, V proximal interphalangeal joint, deformation of left elbow joint, pain in radiocarpal joint, knees, morning stiffness lasting an hour and foot edema.

Anamnesis morbi. In 1997, she developed swelling in the right knee. The patient was referred to general practitioner, and was receiving nonsteroidal anti-inflammatory drug and physiotherapeutic procedures for five years. In 1999, she underwent arthroscopy and partial synovectomy. In 2001, C. trachomatis infection was diagnosed by PCR-method; patient underwent five courses of antibiotics (without effect). During these five years, she developed swelling in the left knee and elbow as well as symmetric V proximal interphalangeal joint swelling. In 2006, the patient developed morning joint stiffness, pain and symmetric swelling of radiocarpal joints; on radiographic data - joint space narrowing and erosions. She was diagnosed with rheumatoid arthritis associated with C. trachomatis infection. She was prescribed sulfasalazine (2 g/day). Two months later, she discontinued the treatment because of the adverse effect (gastrointestinal disorder). In August 2006, she was prescribed leflunomide (20 mg/day). In spring 2007, proteinuria was detected in the urine analysis(0.62 g/L), anemia (hemoglobin level 90 g/L) and increased ESR (73 mm/h) were also found. In June 2007, AA-amyloidosis was histologically confirmed by nephrobiopsy. Renal function progressively declined (urea 23 mM/L, creatinine 450 μM/L, GFR 23 mL/min; proteinuria 3 g/L). On September 2008, the patient was on hemodialysis.

Thus, longstanding *C. trachomatis* infection influences on clinical manifestations and the outcome of RA causes persistent high laboratory activity (ESR, CRP, SAA) and, hence, it is a risk factor for developing secondary amyloidosis. Therefore, the clinical signs of *C. trachomatis* infection in RA patients demand PCR-verification. According to the modern principles of

secondary amyloidosis treatment, the main objective is to decrease the RA activity, which in turn decreases the SAA synthesis. Further, additional studies of antibacterial therapy completely agree with this task. Probably, these actions will allow to reduce risk for developing secondary amyloidosis and to improve life span in patients with RA.

Only 7 (11.9%) patients in the 2nd group presented asymmetric arthritis in disease onset. They had no skin hyperemia or longstanding hyperthermia. All patients regularly received DMARDs; however, low activity or disease remission was not observed in all patients.

ESR level in the 1st group was significantly higher than that in the 2nd group: 55 (40; 67) mm/h in comparison with 36 (24; 52) mm/h (P=0.003). It is connected with dysproteinemia in amyloidosis. At the same time, serum CRP levels were 30 (18; 50) and 21.5 (9; 46) for patients of 1st and 2nd group, respectively (P=0.31).

C. trachomatis infection in RA patients significantly correlates with the high probability of secondary amyloidosis (R=0.93) (P<0.0001) (Figure 5). In addition, OR for *C. trachomatis* infection was 26.6; 95%CI 9.26–76.37.

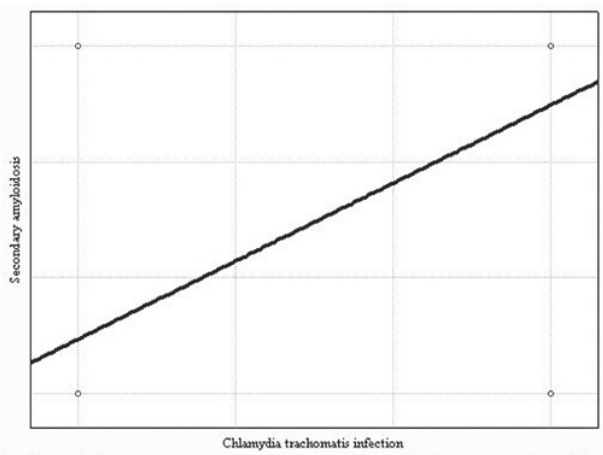

Fig. 5
Correlation between C. trachomatis infection and presence secondary amyloidosis (direct linear dependence)

Thus, the presence of *C. trachomatis* infection in RA patients is one of the risk factors for developing renal amyloidosis. All RA patients with *C. trachomatis* infection require dynamic renal function control.

Prognostic model for evaluating risk factors for developing secondary amyloidosis in patients with rheumatoid arthritis.

To reveal the predictors of secondary amyloidosis and the risk factors for its development in RA, we used logit regression analysis.

We analyzed numerous clinical and laboratory data of patients with RA (including those obtained from literature). In our study, one of the most significant clinical risk factor for developing secondary amyloidosis was the association with C. trachomatis infection. Therefore, we obtained the following equation:

$$Y = \frac{exp(-4.6159 + (3.28091) * x)}{(1 + exp(-4.6159 + (3.28091) * x))}$$

$$\chi^2 = 50.96, p < 0.000001,$$

where x – presence/absence of *C. trachomatis* infection (1 – presence or 2 – absence).

We also used a visual scale for the detection of the risk factors for developing secondary amyloidosis (Figure 6). The OR for *C. trachomatis* infection was 26.6 (95%CI – 9.26–76.37).

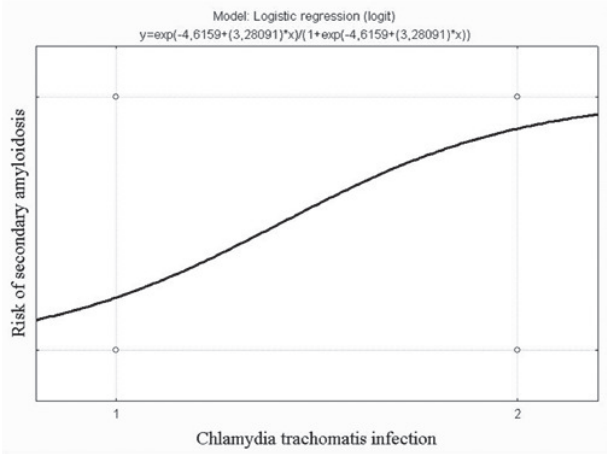

Fig. 6
Model of logit regression for prognosis of secondary amyloidosis risk in C. trachomatis infection

The probability of the right positive diagnosis (i.e., high opportunity of correct positive diagnosis in individual case) was 84.4%, and probability of the right negative conclusion (i.e. high opportunity of correct negative diagnosis in individual case) was 83.1%.

However, SAA1 genotype variant plays the most significant role in detecting the risk factors of secondary amyloidosis. Polymorphic allele variants constitute three haplotypes – α, β, and γ. All detected combinations of SAA1 allele variants are presented in Table 5.

Table 5
Possible combinations of SAA1 allele variants

Genotype	Code	1st group (AA-positive RA patients) n=45	2nd group (AA-negative RA patients) n=59
α/α	1	43 (95.6)	19 (32.2)
β/β	2	2 (4.4)	16 (27.1)
α/β	3	0 (0.0)	10 (16.9)
α/γ	4	0 (0.0)	9 (15.3)
β/γ	5	0 (0.0)	5 (8.5)

Statistical model based on SAA1 genotype is presented in the following equation:

$$Y = \frac{exp(7.36122 + (-3.2675) * x + (-1.7492) * z)}{(1 + exp(7.36122 + (-3.2675) * x + (-1.7492) * z))}$$

$$\chi^2 = 46.845, P < 0.0001,$$

where x – presence/absence of *C. trachomatis infection* (1 – presence or 2 – absence), z – SAA1 genotype variant.Visual presentation of this equation is shown in Figure 7.

The OR for genotype was 45.26 (95%CI 9.91–206.82). The probability of the right positive diagnosis in this model was 67.8% and probability of the right negative conclusion was 95.6%.

By combining the two factors i.e., C. trachomatis infection and a genotype variant, the logit regression equation is expressed as follows:

$$Y = \frac{exp(-7.3612 + (3.26752) * x + (1.74919) * z)}{(1 + exp(-7.3612 + (3.26752) * x + (1.74919) * z))}$$

$$\chi^2 = 78.999, P < 0.001,$$

where x – presence/absence of *C. trachomatis* infection (1 – presence or 2 – absence), z – SAA1 genotype variant.

Figure 8 shows the graphic representation of the developed model.The OR increases to 55.0 for the combination of two factors. The probability of the right positive diagnosis in this model was 80% and probability of the right negative conclusion was 93.2%.

Therefore, the developed model evaluates the risk factors for developing secondary amyloidosis in individual RA patient. The probability of the correct positive prognosis reaches 80% and correct negative is 93.2%. Thus, the developed model is characterized by high sensitivity.

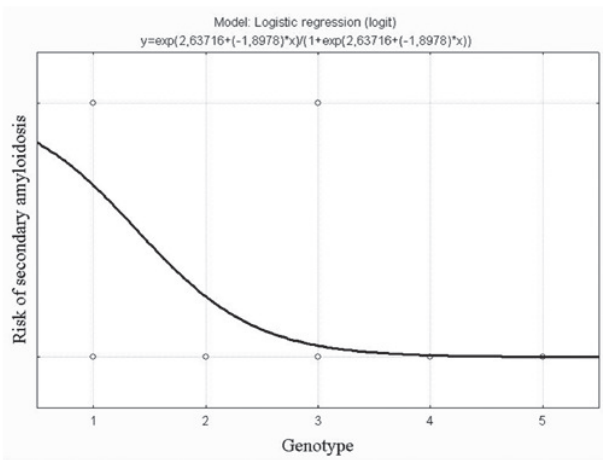

Fig. 7
Model of logit regression for prognosis of secondary amyloidosis risk according genotyping results

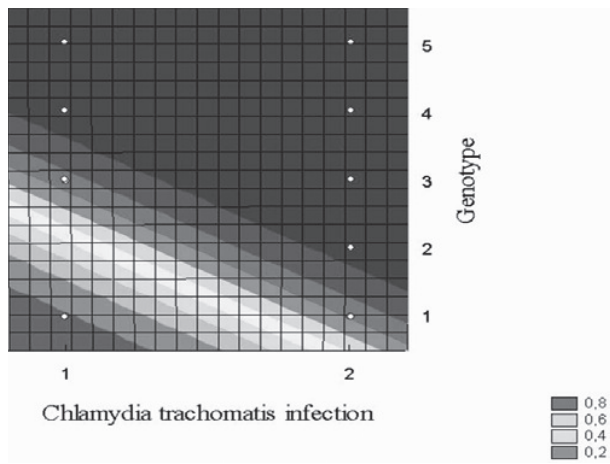

Fig. 8
Model of logit regression for prognosis of secondary amyloidosis risk according C. trachomatis infection and genotype variant.

Discussion

Secondary amyloidosis is rare, but rather serious complication of chronic inflammatory processes and chronic infections. Therefore, the detection of risk factors takes great interest. Nowadays, SAA1 gene polymorphism influence is discussed all over the world. In a number of Japanese studies, γ/γ genotype was considered as "risk genotype." The influence of the γ/γ genotype as well as γ haplotype itself on the development of secondary amyloidosis in patients with RA in Asian population is described in number of studies [31]. SAA 1.3 gene did not influence ESR rate, while the correlation between serum SAA and CRP elevated levels, as well as SAA/CRP, was described.

There are few European studies describing SAA gene polymorphism in Caucasians [32, 33, 34].

Therefore, different isoforms of SAA1 gene, α and γ, are genetic risk factors for developing secondary amyloidosis in different ethnic groups: SAA γ in the Japanese and SAA α in the European population. Thus, gene SAA1 allele frequency considerably varies in different ethnic groups. According to the obtained data, SAA1 α/α (allele variants 2995T and 3010C) are the genetic risk factors for the development of secondary amyloidosis in Belarusian patients with rheumatoid arthritis (OR=45.26). Moreover, α/α genotype met 2.9 times more often in comparison with RA patients without amyloidosis. Besides, SAA1 α/α genotype dominated in both groups and distributed as 95.6% and 32.2% in 1st and 2nd groups, respectively. Genotypes α/γ, β/β, and β/γ were revealed only in the 2nd group, and distributed as 16.9%, 15.3%, and 8.5%, respectively. However, one can consider γ allele as "protective," OR=0.

The −13 T allele frequency of the SAA1 gene (polymorphism −13 T/C) was 10.2% in the 2nd group; among AA-positive RA patients (1st group), this allele was absent. In both groups, there were no −13T/T homozygotes. Consequently, the−13 T allele has no influence on the

manifestation of AA-amyloidosis in Belarusian patients with RA.

Proinflammatory cytokines and persistent infections induce the activity of serum amyloid A (SAA) gene and, consequently, SAA synthesis by liver. In clinical practice, we commonly observed the combination of rheumatoid arthritis, mostly seronegative, with persistent *Chlamydia trachomatis* infection [29]. Therefore, one can suppose that the presence of persistent *C. trachomatis* infection in patient with rheumatoid arthritis may act as supplementary stimuli for the development of secondary amyloidosis. Besides, it influences clinical manifestations in RA, keeps high disease activity and interferes treating efficacy.

According to the obtained data, *C. trachomatis* infection is a risk factor for developing secondary amyloidosis in RA patients. However, *C. trachomatis* infection (by PCR or cultural method) was detected in 38 (84.4%) out of 45 patients with RA complicated by renal amyloidosis. In the 2nd group, the association of *C. trachomatis* and RA was found only in 10 (16.9%) out of 59 patients. There were no patients with diagnostic G and M antibodies titer against *C. trachomatis*. These antibodies were not found (16 cases) or present in non-diagnostic titer (39 cases). It is known that low expression of major outer membrane proteins (MOMP) – a major antigen for immune response stimulation – is characteristic feature of persistent *C. trachomatis* infection. Great part in microorganism survival takes inhibition of II histocompatibility complex proteins expression, and therefore, cell was not detected as infected and was not attacked by B- and CD4+ (Th1)-aggression factors. It leads to insufficiency of production of specific antibodies, which makes difficulties in the elimination of microorganism and in the detection of serum *C. trachomatis* by immunoenzyme assay.

C. trachomatis infection in RA patients significantly correlates with the high probability of secondary amyloidosis (R=0.93) (P<0.0001). Besides, OR for *C. trachomatis* infection was 26.6; 95%CI 9.26–76.37.

Therefore, *C. trachomatis* can induce rapid progression of amyloid deposits, and activate progression of "silent" amyloidosis. Clinical signs of *C. trachomatis* infection in RA patients (asymmetric arthritis, involvement of "exceptions RA" joints, longstanding hyperthermia) demand PCR verification. Probably, the course of antibiotics would allow to reduce the risk of secondary amyloidosis and to improve life span in patients with RA. To estimate this hypothesis, further clinical studies should be conducted.

The obtained results allowed us to create the prognostic statistical model for the risk assessment of secondary amyloidosis. Further, the possibility of risk estimation gives us an opportunity to choose optimal therapeutic strategy and monitoring scheme in RA patients with high risk of secondary amyloidosis.

Conclusions

1. In contrast to the Japanese data, our results revealed that in Belarusian citizens (Caucasians), SAA1 α/α was the most amyloidogenic isotype. Relative risk of developing secondary amyloidosis in RA patients significantly increases in α/α genotype. The presence of the 3T allele in SAA1 gene allele had no influence on the risk of developing AA-amyloidosis in the investigated Belarusian patients with RA. We revealed a strong correlation between genotype variant and presence of secondary amyloidosis: R=0.93, P<0.0001. OR for α/α genotype was 45.26; 95%CI (9.9–206.8).
2. The presence of persistent *C. trachomatis* infection in patients with rheumatoid arthritis is a risk factor for the development of secondary renal amyloidosis. *C. trachomatis* infection in RA patients significantly correlates with the high probability of secondary amyloidosis (R=0.93) (P<0.0001). Besides, OR for *C. trachomatis* infection was 26.6; 95%CI 9.26–76.37.
3. The logit regression model of risk prognosis is described by the equation:

$$Y = \frac{exp(-7.3612 + (3.26752) * x + (1.74919) * z)}{(1 + exp(-7.3612 + (3.26752) * x + (1.74919) * z))}$$

$$\chi^2 = 78.999, P < 0.001,$$

where x – presence/absence of *C. trachomatis* infection (1 – presence or 2 – absence), z – SAA1 genotype variant
and allows correct positive prognosis in 80%.

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