## Early diagnostics of kidney damage in longstanding rheumatoid arthritis and amyloidosis

A. N. Tishko[1], S. V. Lapin[2], T. V. Vavilova[3], & A. A. Totolian[4]

[1]*The Federal State Institute of Public Health 'The Nikiforov Russian Center of Emergency and Radiation Medicine', The Ministry of Russian Federation for Civil Defence, Emergencies and Elimination of Consequences of Natural Disasters (NRCERM, EMERCOM of Russia), St. Petersburg Russia,* [2]*The Saint Petersburg Pavlov State Medical University, St. Petersburg, Russia,* [3]*The Saint Petersburg Mechnikov State Medical Academy, St. Petersburg Russia, and* [4]*Pasteur Institute of Epidemiology and Microbiology, St. Petersburg, Russia*



**Abstract**: We studied a group of patients with longstanding rheumatoid arthritis (27 patients), a group of patients with different degree of kidney insufficiency (8 patients), and 19 healthy people. We reviewed the main contemporary methods of kidney function examination and carried out comparison between biochemical characteristics (serum creatinine level, cystatin C), calculated data of glomerular filtration rate (modification of diet in renal disease (MDRD), cystatin C, and Cockroft–Gault formulas), results of screening for microalbuminuria, and presence or absence of amyloid deposits in subcutaneous fat tissue. As a result of our investigation, we showed that a complex of laboratory methods should be used for kidney damage diagnostics. The most informative of them are serum cystatin C and calculation of glomerular filtration rate (GFR) by MDRD and cystatin C formulas. Calculation of GFR by Cockroft–Gault formula showed to be less informative.

**Introduction**: The systemic amyloidoses are characterized by progressive extracellular deposition of amyloid fibrils. AA-amyloidosis, formerly known as secondary amyloidosis, is a common type caused by chronic inflammation [1]. However, early AA-amyloidosis is difficult to diagnose because of its rarity and long latency period. As a result, most patients present late in the course of the disease with clinical symptoms of overt organ damage. More than 80% of patients with AA amyloidosis have renal involvement at time of diagnosis [2]. The main cause of death (40–60%) in patients with AA-amyloidosis is complications of terminal phase of renal insufficiency [3]. Accurate and early diagnosis of systemic amyloidosis is considered to be essential in the clinical management of the disease. The aim of our study was to work out effective laboratory methods for early kidney damage diagnostics in asymptomatic amyloidosis in longstanding rheumatoid arthritis.

**Materials and methods**: We examined 27 patients with longstanding rheumatoid arthritis (RA $>$ 5 years, without any renal disease, diabetes mellitus, and pregnancy), 8 patients with different degree of kidney insufficiency and 19 healthy donors. Laboratory examination consisted of: screening for microalbuminuria, biochemical characteristics (serum creatinine and serum cystatin C level), subcutaneous fat tissue biopsy with Congo red staining. Serum cystatin C (Cys C) was measured by immunoturbidimetric method on biochemical analyzer 'KONELAB-20' ('Alfresa', Japan). Normal range for Cys C was considered 0.63–0.95 mg/l for men and 0.56–0.87 for women. The morning urine was tested with the HemoCue Albumin 201 system (HemoCue, Sweden). The negative result was considered to be for the concentration of albumin $<$20 mg/l, microalbuminuria was considered to be within 20–200 mg/l. Calculated data included glomerular filtration rate (GFR) according to modification of diet in renal disease (MDRD), Cys C and Cockroft–Gault (CG) formulas (normal range $>$ 90 ml/min/1.73 m$^2$).

For calculated data the following formulas were used:

$$\mathrm{GFR_{MDRD}} = 186 \times (\mathrm{Cr}/88.4)^{-1.154} + K \times a^{-0.203},$$

where $K = 1$ for men and $K = 0.72$ for women, *Cr* - serum creatinine ($\mu$mol/l), a – age;

$$\mathrm{GFR_{CG}} = (140 - a) \times m/K \times \mathrm{Cr},$$

where $K = 0.8$ for men and $K = 0.95$ for women, $a$ – age, Cr - serum creatinine ($\mu$mol/l), $m$ – weight (kg);

$$\mathrm{GFR_{CysC}} = 94,652 \times \mathrm{C}^{-1.2478},$$

where C – serum Cystatin C (mg/l) [4].

Fat tissue biopsy was taken with 16G needle from near umbilical area previously anesthetized with Lidocaine. Then it was fixed with acetone and stained with Congo red. The result was analyzed by apple-green birefringence observed with polarized light, using a microscope (Axiolab Pol, Carl Zeiss, Germany). Statistical analysis was performed using the statistical software package STATISTICA, version 8.0 (StatSoft Inc., USA). For all tests $p$ values less than 0.05 were considered significant.

**Results and discussion**: Analysis of the whole group of patients with longstanding RA showed the increased mean value of Cys C which was $1.47 \pm 0.91$ mg/l and was higher than 'cut-off' value in 75% of cases. Pathologic values of creatinine were found only in 33.3% of cases. In this group dependent correlation was shown between Cys C and serum creatinine values ($r = 0.88$; $p < 0.05$), it was present both in patients with increased and with normal concentration of serum creatinine. The calculation of GFR in patients with longstanding RA showed decreased filtration rate irrespective to the calculation method:

$$GFR_{MDRD} = 80.4 \pm 29.6 ml/min/1.73m^2;$$

$$GFR_{CG} = 65.7 \pm 21.1 ml/min/1.73m;$$

$$GFR_{CysC} = 70.8 \pm 43.4 ml/min/1.73m^2.$$

In the next step, we analyzed groups with presence and absence of microalbuminuria. The results are shown in Table I. Increased level of Cys C was found in 87.5% of patients with microalbuminuria ($r = 0.39$; $p = 0.05$), whereas increased level of serum creatinine was found only in 33.3% of these patients ($r = 0.36$; $p > 0.05$). At the same time Cys C was also slightly increased in patients without microalbuminuria. There was high correlation between Cys C level and $GFR_{MDRD}$ ($r = -0.71$; $p < 0.05$).

In the third step, we compared patients with presence and absence of amyloidosis (Table II). Amyloidosis was found in six patients with longstanding RA and microalbuminuria. We compared biochemical characteristics of patients of these groups and the largest difference was shown in serum creatinine ($p = 0.05$) and Cys C ($p = 0.07$) values. The calculated values of GFR occurred to be lower in patients with amyloidosis which also showed renal damage in these patients, but the difference between these characteristics was not significant.

Difference between cystatin C concentration in RA patients without amyloidosis and healthy donors was not significant ($1.27 \pm 0.49$ mg/l and $0.82 \pm 0.18$ mg/l, respectively, $p > 0.05$) whereas level of Cys C in RA patients with amyloidosis was significantly higher than in healthy donors ($2.89 \pm 1.42$ mg/l and $0.82 \pm 0.18$, respectively, $p < 0.05$), but did not reach the level of it in patients with different degree of kidney insufficiency.

According to the results of fat tissue biopsy the sensitivity of urine examination for microalbuminuria was 85%, specificity – 80%, positive prognostic value – 75% and negative prognostic value – 88%. Cys C examination in patients with longstanding RA and amyloidosis had sensitivity 83%, specificity – 50%, positive prognostic value – 27% and negative prognostic value – 90%.

The calculated values of GFR occurred to be lower in patients with amyloidosis but showed no significant difference and calculation of GFR by Cockroft–Gault formula occured to be less informative.

Cys C was recently shown to be more informative marker of kidney damage than serum creatinine [5], but taking into account the increased level of Cys C in patients with longstanding RA, we can conclude that only a complex of laboratory methods should be used for kidney damage diagnostics in this group of patients. It should be started with microalbuminuria and serum Cys C at the first step, then measuring serum creatinine concentration and calculating $GFR_{CysC}$ and $GFR_{MDRD}$. In any case of pathological results of kidney function characteristics in patients of this group histological examination of fat tissue biopsy must be performed to exclude amyloidosis.

Table I. Characteristics of kidney function in patients with longstanding RA depending on presence or absence of microalbuminuria.

| Characteristics | Microalbuminuria is present, $n = 11$ | Microalbuminuria is absent, $n = 15$ | $p$ |
|---|---|---|---|
| Creatinine, μmol/l | 105.6 ± 37.0 | 85.5 ± 14.0 | 0.47 |
| Cystatin C, mg/l | 2.65 ± 1.25 | 0.85 ± 0.19 | 0.0001 |
| GFR (ml/min/1.73 m$^2$) | | | |
| MDRD | 64.9 ± 25.6 | 67.2 ± 11.8 | 0.89 |
| CG | 77.7 ± 33.0 | 85.7 ± 49.8 | 0.74 |
| CysC | 48.2 ± 32.5 | 116.2 ± 37.5 | 0.036 |

Table II. Characteristics of kidney function in patients with longstanding RA depending on presence or absence of amyloidosis.

| Characteristics | Amyloidosis is present ($n = 6$) | Amyloidosis is absent ($n = 6$) | $p$ |
|---|---|---|---|
| Creatinine, μmol/l | 121.6 ± 33.5 | 76.3 ± 11.4 | 0.05 |
| Cystatin C, mg/l | 2.89 ± 1.42 | 1.27 ± 0.49 | 0.05 |
| GFR (ml/min/1.73 m$^2$) | | | |
| MDRD | 57.1 ± 22.1 | 74.3 ± 11.6 | 0.29 |
| CG | 76.3 ± 36.6 | 84.4 ± 21.6 | 0.71 |
| CysC | 45.9 ± 34.4 | 95.8 ± 45.0 | 0.12 |

**Acknowledgements**: Authors thank Tatyana Shemerovskaya, Aleksey Maslyansky, and Elena Ilivanova for providing the opportunity to include patients with longstanding RA in our investigation.

***Declaration of interest:*** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

## References

1. Hazenberg BP, van Rijswijk MH. Clinical and therapeutic aspects of AA amyloidosis. Baillieres Clin Rheumatol 1994;8:661–690.
2. Janowski R, Kozak M, Abrahamson M, Grubb A, Jaskolski M. 3D domain-swapped human cystatin C with amyloidlike intermolecular β-sheets. Proteins 2005;61:570–578.
3. Maniatis A. Pathophysiology of paraprotein production. Ren Fail 1998;20:821–828.
4. Larsson A, Malm J, Grubb A, Hansson LO. Calculation of glomerular filtration rate expressed in mL/min from plasma cystatin C values in mg/L. Scand J Clin Lab Invest 2004;64:25–30.
5. Kyhse-Andersen J, Schmidt C, Nordin G, Andersson B, Nilsson-Ehle P, Lindström V, Grubb A. Serum cystatin C, determined by a rapid, automated particle-enhanced turbidimetric method, is a better marker than serum creatinine for glomerular filtration rate. Clin Chem 1994;40:1921–1926.

The online version first published on 14 July 2011 showed the incorrect page numbers of this article. They have been now been corrected on this version.

DOI: 10.3109/13506129.2011.574354081