

Investigation of the Diagnostic Performance of Dimethyl Arginine Derivate and Lisosomal Enzymes in Patients with Rheumatoid Arthritis

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Abstract

Introduction: Untreated Rheumatoid Arthritis (RA) has implications on renal tissue as one of the visceral manifestations of disease. Arthritis primarily damages the proximal renal tubules.

Aim: To compare the diagnostic values of laboratory variables; to find the predictive value of the positive and negative test and accuracy of the tests for Symmetric Dimethyl Arginine (SDMA), N-acetyl- β -D-glucosaminidase (NAG), microalbuminuria, rheumatoid factor (RF), C-reactive protein (CRP) and Disease Activity Score 28 index (DAS 28); and to detect the effect of untreated rheumatoid arthritis on glomerular and tubular function.

Patients and methods: Quantification of N-acetyl- β -D-glucosaminidase is used for colorimetric assay, ELISA method for detection of SDMA, immunoturbidimetry assay for microalbuminuria and agglutination test for RF. Serum and urine samples are examined in 70 participants (35 patients with untreated rheumatoid arthritis and 35 healthy individuals as control group).

Results: Of 35 examined patients with RA, we found presence of NAG enzymuria (sensitivity of the test; 37.14%) in 13, while microalbuminuria appeared in 4 patients (sensitivity of the test; 11.42%). SDMA was present in 26 patients (sensitivity of the test; 74.58%), while RF was detected in 17 patients (sensitivity of the test; 48.57%).

Conclusion: SDMA and NAG have higher sensitivity than microalbuminuria in the detection of asymptomatic renal impairment in untreated RA.

Keywords: Symmetric Dimethyl Arginine; N-acetyl- β -D-glucosaminidase; Microalbumin; Rheumatoid arthritis.

Introduction

Methylated derivative of the amino acid L-arginine is Symmetric dimethylarginine (SDMA). Body elimination of SDMA is exclusively performed by renal excretion. SDMA plasma concentration of SDMA is tightly related to renal function. SDMA concentration correlates with inulin clearance and is a reliable marker for renal function. In renal impairment elevated level of serum SDMA indicates future risk of cardiovascular attack and mortality correlates with the extent of renal impairment [1-3]. Most commonly used marker for renal excretory function-serum creatinine concentration, does not adequately reflect mild to moderate impairment of renal function.

The standard routine parameters used for assessment of glomerular filtration rate (GFR) have relatively low sensitivity, due to the large functional reserves of the kidney [4]. The kidney is an organ with great compensatory potential and up to 50% of its functional capacity can be lost before any increase in degradational products of nitrogen metabolism and appearance of proteinuria, which force the patient to ask medical help. The renal function can be determined by many methods, such as immune, radiologic and cytologic analysis. Biochemical analyses as non-invasive methods have significant importance in the early detection of some pathological conditions that appear during therapy. Determination of the activity of enzymes and their isoenzymes in urine is such an example, because their activity in serum has little diagnostic value. Pathogenic mechanisms that lead to destruction of epithelial cells of proximal tubules and are responsible for the appearance of enzymuria are: immune mechanism, complement, lysosomal enzymes, and tubular obstruction by cell debris, protein cylinders, medicaments or proteinuria. Each of them,

in a different degree contributes to the release of biochemical markers in urine, in a directly or indirectly.

Renal markers for assessment of renal dysfunction

Some of the classes of urine proteins are used for assessment of asymptomatic renal dysfunction, i.e.:

1. Enzymes with high molecular weight, usually not filtered via glomeruli, but produced in the proximal tubular cells (N-acetyl- β -D-glucosaminidase; NAG);

2. Medium molecular weight proteins normally filtered via glomeruli in a very small quantity, while the vast amount is resorbed in the tubules (microalbumin) [5-10].

The most studied protein of all urine enzymes is urinary NAG (U-NAG). The enzyme belongs to hydrolase class, usually present in the lysosomes of proximal tubular cells [11]. In the human tissue and in biological liquids exists two main forms of the enzyme: A (Acid) and B (Basic) [12]. The isoform A (U-NAG-A) is the dominant form in normal urine [13]. At the end of cell maturation process it is found in the resolved form of cytosol. Its excretion is related to turnover and is noted as functional enzymuria. Isoform B (U-NAG-B) depends on maturation and is more closely related to the basement membrane, where it appears. Its presence in urine is correlated to cell lysis and, because of that is noted as lesion enzymuria [14]. NAG can be detected in circulation. Due to its large molecular weight (1,40,000 daltons) NAG cannot pass through an intact glomerular membrane. In healthy people, urine NAG is a representative of the total quantity released from the walls of the renal tubular cells [15], and is very sensitive marker for renal tubular damage.

Urine Albumin (molecular weight from 66 KDa) is quantitatively the most important protein in plasma and urine. It approximately composes up to 30% of urinary proteins, and appears to be a good indicator for assessment of glomerular permeability variations. These variations in glomerular permeability occur in diabetic and hypertensive nephropathy, nephritic syndrome, pre-eclampsia and glomerulonephritis. Urine albumin excretion has great individual variations and depends on physical activity or variations in food

intake. From the pathophysiological aspect, microalbuminuria is caused by increased glomerular permeability for albumin, increased glomerular pressure and/or reduced tubular albumin reabsorption. Renal endothelium is closely involved in the regulation of these processes [16,17].

Aim

The aim of this study was to define the effect of untreated rheumatoid arthritis on glomerular and tubular function. The urine microalbumin was used as marker for glomerular function, while NAG was an indicator of proximal tubular damage.

Implication for health policy/practice/research/medical education:

Dimethyl arginine derivate, symmetric isoform has higher sensitivity than N-acetyl- β -D glucosaminidase. It is a relevant marker in the assessment of asymptomatic renal damages in untreated RA. Both forms can be used in routine clinical practice.

Patients and Methods

Diagnosis of disease in the group of patients examined in this study was established on the basis of revised diagnostic criteria for the classification of rheumatoid arthritis, suggested in 1987 by the American Association for Rheumatism (ARA) [18-21]. Patients had to fulfill at least four out of seven criteria. Criteria from one to four had to be present for at least six weeks. In the study were included 35 patients (28 women, 7 men) suffering from RA and 35 healthy individuals (18 women, 17 men) in the control group. The mean age was 56.68 (\pm 6.79) years (40-65 years) in RA group, and 46.2(\pm 12.49) years (29-65 years) in control group. The mean disease duration was 43.97 (\pm 45.23) months (1-168 months). None of the patients had previous history of renal disease. Three patients had been previously treated with oral costeroids, while none of them used any non-steroidal anti-inflammatory drugs (NSAIDs). The rest of the patients refused to use any other medication before beginning of the study (Table 1).

	Ra group No of patients 35 value (m \pm sd)	Control healthy group No of subjects 35 value (m \pm sd)
Male / female ratio	7/28	17/18
Mean age (years)	56.68 (\pm 6.79)(40-65)	46.20 (\pm 12.49) (29-65)
Mean disease duration (month)	43.97 (\pm 45.23)(6-168)	0.00 (\pm 0.00) (0.00-0.00)

Table 1: Patients' data.

Inclusion criteria: The study involved newly diagnosed patients with untreated rheumatoid arthritis, aged 18-65 years.

Exclusion criteria: Patients with disease or condition that could directly or indirectly influence the results:

1. Patients with previous medical history for diseases of the spleen, thyroid gland, lung and hepatal damage, renal, cardiovascular, neurologic and hematologic diseases.

2. Patients with diabetes mellitus, acute infections, malignant neoplasms and febrile conditions.

3. Patients treated with antibiotics and salicylate six months prior to the beginning of the study.

4. Patients with hypertension, gouty arthritis, urinary infections, and various autoimmune diseases such as mixed connective tissue disorders and vaculitis.

5. Patients treated with antihypertensive, cardiac and antidiabetic therapy.

6. Patients who received blood transfusions and overweight patients.

7. Hypersensitivity to some medications or their components.

8. Excluded were patients whose results show that in 0 spot increased levels of degradation products: serum and urine creatinine, serum urea and disorders in hematologic or enzymatic status.

All patients provided informed consent.

Clinical evaluation of disease activity

A subspecialist in this field performed clinical evaluation. The disease activity was evaluated by Disease Activity Score 28 index (DAS 28) [22-25]. The index is a mathematical formula that allows us to get a uniquely composed quantitative score, which consists of palpation of painful sensitive joints (max number 28), swollen joints (max number 28), Westergren ESR, and the patient's global assessment of the activity of the disease (0–100 mm Visual Analogous Scale, VAS) and the morning stiffness (minutes). DAS 28 index is ranked from 0 to 10 and a score under 3.2 ranks the disease as low-activity. The assessment of glomerular filtration rate (GFR) was calculated by the Cockcroft-Gault formula [26].

Laboratory assessment

For a clinical assessment of the basic disease, the following laboratory variables had to be measured: CBC, acute phase reactants, anti CCP2, C-reactive protein (CRP), Rheumatic Factor (RF), Alkaline Phosphatase (AP), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Creatinine Kinase (CK), Lactate Dehydrogenase (LDH), serum urea and creatinine. Urine samples were taken not only for routine urine analysis, but also for detection of SDMA, NAG, urine creatinine and microalbuminuria.

ELISA technology of DId-Diagnostika-Gmbh for Detection of Symmetric Dimethyl Arginine (SDMA)

Principle of the assay: The competitive SDMA-ELISA uses the microtiter plate format. SDMA is bound to the solid phase of the microtiter plate. SDMA in the samples is acylated and competes with solid phase bound SDMA for a fixed number of rabbit anti-SDMA antiserum binding sites. When the system is in equilibrium, free antigen and free antigen-antiserum complexes are removed by washing. The antibody bound to the solid phase SDMA is detected by anti-rabbit/oxidase. The substrate TMB/oxidase reaction is monitored at 450 nm. The amount of antibody bound to the solid phase SDMA is inversely proportional to the SDMA concentration in the sample.

Reference value: 0.3-0.7 $\mu\text{mol/L}$

Immunoturbidimetric assay for the determination of urinary albumin (Randox laboratories limited)

Principle of the assay: Immunoturbidimetric assay for determination of urine albumin: for random urine albumin measurement an early morning mid-stream specimen should be taken. Centrifuge cloudy samples before use and analyze the clear supernatant. Reference values are 2.0-20.0 mg/L. An undiluted sample

is added to the buffer containing the antibody specific for human serum albumin. The absorbance (340 nm) of the resulting turbid solution is proportional to the concentration of albumin in the urine sample. By constructing a standard curve from the absorbance of standards, the albumin concentration in the sample could be determined. The assay could be carried out manually (at room temperature) or with an automated analyzer using DAKO tests [27]. Sample collection and storage: for random urine albumin measurement use an early morning mid-stream sample. Centrifuge cloudy samples before use and analyze clear supernatant in the assay.

Reference value: 2.0-20.0 mg/L.

Colorimetric assay for the determination of N-acetyl- β -D-glucosaminidase (NAG) in urine (Roche)

Principle of the assay: Colorimetric assay for the determination of Acetyl- β -D-glucosaminidase in urine: 3-Cresolsulfonphthaleinyl- N-Acetyl- β -D-glucosaminidase, sodium salt, is hydrolyzed by N-Acetyl- β -D-glucosaminidase (NAG) with release of 3-cresol-sulfonphthalein, sodium salt (3-cresol purple), which is measured photometrically at 580 nm using a ROCHE test. Turbid urines should be centrifuged and the supernatant decant.

Reference values are: NAG urine 0.27–1.18 U/mmol creatinine.

Serum urea: Was detected by the method of Kassire. Reference value is 3–7.8 mmol/L.

Serum and urine creatinine: Were detected by the Jaffe method. Reference values for serum and urine creatinine are 45–109 $\mu\text{mol/L}$ and 7–17 $\mu\text{mol/L}$, respectively.

C-reactive protein (CRP): Was detected using the test of agglutination (Latex CRP test); (BioSystems S.A. Reagents & Instruments Costa Brava 30, Barcelona, Spain). Reference value is under 6 mg/L CRP in serum.

Rheumatoid factor (RF) was detected using the test of agglutination (Latex RF test) (BioSystems S.A. Reagents and Instruments Costa Brava 30, Barcelona, Spain). Reference value is under 8 mg/L RF in serum. For the specification of erythrocyte sedimentation rate (ESR) we used quantitative - Westergren method, and normal values are: 7–8 mm for men, 11–16 mm for women.

Ethical issues

The research followed the tenets of the Declaration of Helsinki. Informed consents were obtained. All patients took part in this study voluntarily. The research was approved by ethical committee of University Clinical Center of Skopje, Republic of Macedonia.

Statistical analysis

For testing the importance of the difference between two arithmetic means, with respect to a proportion, we used the Student's t-test that compares the mean values of certain numerical parameters between two groups and a Wilcoxon-matched test for independent samples. Sensitivity and specificity for positive and negative test of examined variables were defined by the test of sensitivity and specificity. P value between 0.05 and 0.1 was taken as statistically significant. Data processing was done by statistical package - Statistica 7.0.

Results

We found NAG enzymuria in 13 patients (37.14%) patients with RA in our group, while microalbuminuria was detected in 4 patients (11.42%). RF was detected in 17 patients (48.57%). Four patients were NAG and RF positive (11.42%), while 3 patients were microalbuminuria and RF positive (8.57%). Among 18 RF negative patients, 9 patients (25.71%) were NAG positive, while in 1 patient (2.85%) microalbuminuria was detected. In 13 patients (37.14%) NAG enzymuria was not detected; they were RF positive. In 14 patients (40%), microalbuminuria was not detected; they were RF positive. Among 18 RF negative patients, NAG enzymuria was detected in 9 patients (50%), while microalbuminuria was detected in 1 patient (5.55%).

Among 22 patients without NAG enzymuria, 13 patients (59.09%) were RF positive. However, among the 31 patients without microalbuminuria, 14 patients (45.16%) were RF positive. Among 35 RA patients NAG sensitivity was 37.14%, microalbuminuria sensitivity was 11.42%, while RF sensitivity was 48.57%. Among 17 RF positive RA patients, presence of NAG was found in 4 patients and its sensitivity was 30.76%, while the presence of microalbuminuria was detected in 3 patients and its sensitivity was 17.34%. Among 18 RF negative patients, NAG enzymuria appeared in 9 patients and its

sensitivity was 50%. Microalbuminuria appeared in 1 patient and its sensitivity was 5.55%.

In the healthy control group, 8 patients (22.85%) were NAG positive, 2 patients (5.71%) were microalbuminuria positive and RF was present in 2 patients (5.71%). Table 2, summarizes the data. Diagnostic value of Symmetric Dimethyl Arginine (SDMA), N-Acetyl-β-D Glucosaminidase (NAG) and microalbuminuria in urine in patients with rheumatoid arthritis (RA) For NAG, microalbumin and for other laboratory variables in rheumatoid arthritis, sensitivity, specificity, predictive value of the positive or negative test and their precision are shown in Table 3. NAG has better diagnostic performances than microalbuminuria in term of sensitivity (sensitivity 37.14% vs. 11.42%), but lower specificity (specificity 77.14% vs. 94.28%) in the detection of renal tubular damage in untreated RA. SDMA, NAG, microalbuminuria and DAS 28 index of the intensity of disease Among 35 patients with RA, DAS 28>3.2 was replaced in 28 patients (80%). In 17 seropositive RF patients, replacement of DAS 28>3.2 was detected in 15 patients (88.23%). Of these 15 patients, 3 were NAG positive (20%) and their Mean±SD [1.86 (±1.06)] was extended (1.2–3.1), while microalbuminuria was positive in 2 patients (13.13%) and their Mean ± SD [21.35(± 0.21)] was extended (21.2–21.5).

	ra untreated group No of patients 35 value (m ± sd)	ra group sero- No of patients 18 value (m ± sd)	ra group sero+ No of patients 17 value (m ± sd)	control healthy group No of subjects 35 value (m ± sd)
	Positive / Negative	Positive / Negative	Positive / Negative	Positive / Negative
nag + >1.18 (u/mmol/crea)	13/221.096 (± 0.68) (0.25-3.1)	9/91.12 (± 0.58) (0.32-2.1)	4/131.07 (± 0.79) (0.25-3.1)	8/271.00 (± 0.50) (0.26-1.91)
microalbuminuria + >20(mg/dl)	4/3115.16 (± 5.55) (5.50-35.2)	1/1715.03 (± 6.24) (8.30-35.2)	3/1415.30 (± 4.90) (5.50-25.7)	2/3315.56 (± 12.46) (1.75-56.4)
serum creatinine < 49-109 > mol/L	3/3267.55 (±14.76) (41-108)	1/1768.24 (± 14.16) (44-108)	2/1566.82 (± 15.77) (41-99)	2/3374.95 (± 19.72) (44-135)
urine creatinine < 7-17> mol/L	9/2610.41 (± 4.71) (3.1-25.4)	6/129.26 (± 4.54) (3.1-18)	3/1411.62 (± 4.72) (5.8-25.4)	5/309.15 (± 4.22) (1.8-20.4)
serum urea + > 7,8 mmol/L	4/315.66 (± 1,46) (3.00-8.60)	0/185.52 (± 1.33) (3.00-7.5)	4/135.82 (± 1.62) (3.80-8.6)	1/344.94 (± 1.28) (2.50-7.2)
Gfr + >90 ml/min	14/2199.19 (± 24.46) (56.08-157.30)	7/1199.19 (± 24.46) (64.67-142.59)	7/1099.19 (± 25.22) (56.08-157.30)	4/31113.80 (± 30.86) (69.98-177.74)
das 28 + > 3,2	28/74.79 (± 1.56) (1.85-7.03)	13/54.56 (± 1.76) (1.85-7.03)	15/25.04 (± 1.33) (2.47-6.83)	0/350.00 (± 0.00) (0.00-0.00)
Sdma +>0,7 μmol/L	26/943.20 (± 65.13) (0-300)	14/457.50 (±81.40) (0-300)	12/528.05 (±38.72) (0-120)	0/350.00 (± 0.00) (0.00-0.00)
Rf +30 > iu/ml	17/18346.15 (± 625.22) (0.00-1920)	0/180.00 (± 0.00)(0.00-0.00)	17/0712.67 (± 743.72) (30-1920)	2/3313.71 (± 38.73) (0.00-120)
Crp +12 > mg/l	14/2146.86 (± 79.19) (0.00-384)	3/158.66 (± 24.62)(0.00-96)	13/487.31 (± 96.44) (0.00-384)	4/315.48 (±12.80) (0.00-48)

sedimentation > 16 mm/h	27/848.62 (± 39.81) (2.0-120)	13/543.94 (± 39.82) (2.0-120)	14/353.58 (± 40.39) (5.0-120)	4/319.42 (± 8.21) (2.0-44)
acpa antibody > 1,26 u/ml	23/121.71 (± 0.69) (0.92-3.0)	11/71.56 (± 0.59) (0.93-2.6)	12/51.87 (± 0.77) (0.92-3.0)	1/340.95 (± 0.10) (0.90-1.38)

Table 2: SDMA (µmol/l), NAG (u/mmol/crea), microalbuminuria (mg/dl) and other laboratory variables in RA and healthy control group.

Among 18 seronegative RF patients, replacement of DAS 28>3.2 was found in 13 patients (72.22%).

	Nag ra untreated group No of patients 35	Nag ra group sero- of patients 18	Nag ra group sero+ of patients 17	Micro albuminuria untreated group No of patients 35	Micro albuminuria group sero- No of patients 18	Micro albuminuria group sero+ No of patients 17	serum creatinine ra untreated group No of patients 35	serum creatinine ra group sero- No of patients 18	serum creatinine ra group sero+ No of patients 17
Sensitivity %	37.14	50	30.76	11.42	5.55	17.64	8.57	5.55	11.76
Specificity %	77.14	77.14	77.14	94.28	94.28	94.28	94.28	94.28	94.28
predictive values for positive test %	61.9	52.94	33.33	66.66	33.33	60	60	33.33	50
predictive values for negative test %	44.89	25	32.5	48.43	34	29.78	49.23	34	31.25
Accuracy %	57.14	67.92	59.61	52.85	64.15	69.29	51.42	64.15	67.3
	urine creatinine ra untreated group No of patients 35	urine creatinine ra group sero- of patients 18	urine creatinine ra group sero+ of patients 17	serum urea ra untreated group No of patients 35	serum urea ra group sero- No of patients 18	serum urea ra group sero+ No of patients 17	gfr ra untreated group No of patients 35	gfr ra group sero- No of patients 18	gfr ra group sero+ No of patients 17
Sensitivity %	25.71	33.33	17.64	11.42	0	23.52	40	38.88	41.17
Specificity %	85.71	85.71	85.71	97.14	97.14	97.14	88.57	88.57	88.57
predictive values for positive test %	64.28	54.54	37.5	80	0	80	77.77	63.63	63.63
predictive values for negative test %	46.42	28.57	31.88	47.69	34.61	27.65	40.38	26.19	24.39
Accuracy %	55.71	67.92	63.46	54.28	64.15	73.07	64.28	71.69	73.03
	RF Ra untreated group No of patients 35	RF ra group sero- No of patients 18	RF ra group sero+ No of patients 17	CRP ra untreated group No of patients 35	CRP ra group sero- No of patients 18	CRP ra group sero+ No of patients 17	SER ra untreated group No of patients 35	SER ra group sero- No of patients 18	SER ra group sero+ No of patients 17
Sensitivity %	48.57	0	100	66.66	16.66	76.47	77.14	72.22	82.35
Specificity %	94.28	94.28	94.28	88.57	88.57	88.57	88.57	88.57	88.57

89,47	0	89.47	77.77	42.85	76.47	87.09	76.47	77.77	
predictive values for negative test %	35.29	35.29	0	40.38	36.6	11.42	20.51	13.88	8.82
Accuracy %	71.42	62.26	96.15	64.28	64.15	84.61	82.85	83.01	86.53

Table 3: Diagnostic performance of SDMA, NAG, microalbuminuria and other laboratory variables in rheumatoid arthritis

Of these 13 patients, 8 were NAG positive (61.53%) and their $M \pm SD$ [1.54 (± 0.40)] was extended (1.19–2.1), while microalbuminuria was not present in any of these patients. Seronegative RF patients have greater titer of NAG than RF positive patients [1.12 (± 0.58)] (0.32–2.1) vs. [1.07 (± 0.79)] (0.25–3.1), and greater DAS 28>3.2 index [5.04 (± 1.33)] (2.47–6.83) vs. [4.56 (± 1.76)] (1.85–7.03). There is no statistical relation between these two groups of NAG ($p=0.65$).

However, positive RF patients with DAS 28>3.2 have greater NAG induction of circumference than seronegative RF with DAS 28>3.2 [1.86 (± 1.06)] (1.2–3.1) vs. [1.54 (± 0.40)] (1.19– 2.1). There is no statistical correlation between these two groups of NAG ($p=0.59$) (Figure 1).

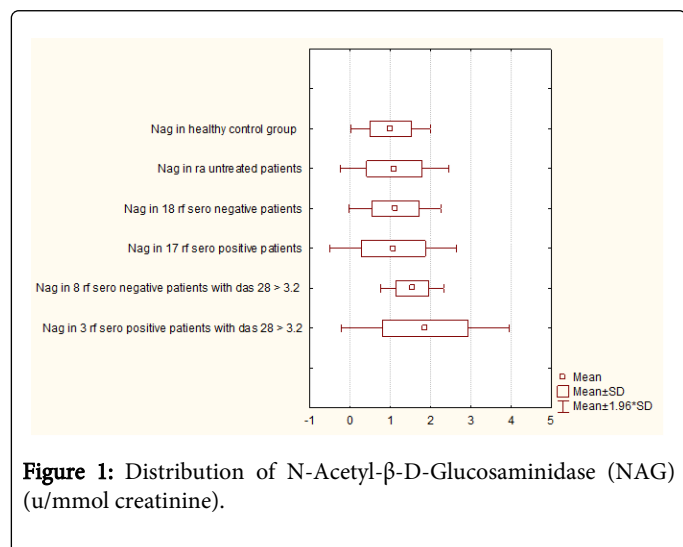


Figure 1: Distribution of N-Acetyl-β-D-Glucosaminidase (NAG) (u/mmol creatinine).

The difference in microalbuminuria in seronegative RF patients was neglectable when compared to seropositive RF patients [15.03 (\pm

6.24)] (8.30–35.2) vs. [15.30 (± 4.90)] (5.50–25.7). Between these two groups with microalbuminuria no statistical correlation was found ($p=0.71$) (Figure 2). Statistical correlation was found between DAS 28 index in RF positive and negative patients ($p=0.37$), while statistical correlation was not found between two groups DAS 28>3.2, NAG positive. There was not also between RF positive and negative patients ($p=0.28$). Statistical correlation was not found using Wilcoxon-matched test between NAG in RA and healthy control group for $p<0.05$ ($p=0.55$), microalbuminuria in RA and healthy control group ($p=0.28$). There was statistical correlation between NAG and microalbuminuria in the RA group for $p<0.05$. A statistical correlation was found using a Wilcoxon-matched test between: NAG in RA and age, duration of disease in months, DAS 28 index, RF, CRP, ESR, SDMA, serum and urine creatinine and serum urea in the same group for $p<0.05$: NAG vs. age ($p<0.001$); NAG vs. duration of disease in months ($p<0.001$); NAG vs. DAS 28 $p=(p<0.001)$; NAG vs. RF ($p=0.01$); NAG vs. CRP ($p=0.04$); NAG vs. ESR ($p<0.001$); NAG vs. serum creatinine ($p<0.001$); NAG vs. urine creatinine ($p<0.001$); NAG vs. serum urea ($p<0.001$). Statistical correlation was found using Wilcoxon-matched test between microalbuminuria in RA and age, duration of disease in months, DAS 28 index, RF, ESR, serum and urine creatinine and serum urea in the same group for $p<0.05$: microalbuminuria vs. age ($p<0.001$); microalbuminuria vs. duration of disease in months ($p<0.001$); microalbuminuria vs. DAS 28 ($p<0.001$); microalbuminuria vs. RF ($p=0.04$); microalbuminuria vs. ESR ($p<0.001$); microalbuminuria vs. serum creatinine ($p<0.001$); microalbuminuria vs. urine creatinine ($p<0.001$); microalbumin vs. serum urea ($p<0.001$). There was no statistical correlation using Wilcoxon-matched test between microalbuminuria in RA with CRP and SDMA in the same group: microalbuminuria vs. CRP ($p=0.09$); microalbuminuria vs. SDMA ($p=0.11$).

	SDMA ra untreated group No of patients 35	SDMA ra group sero- No of patients 18	SDMA ra group sero+ No of patients 17	DAS 28 ra untreated group No of patients 35	DAS 28 ra group sero- No of patients 18	DAS 28 ra group sero+ No of patients 17
Sensitivity %	74,28	77,77	70,58	80	72,22	88,23
Specificity %	100	100	100	100	100	100
predictive values for positive test %	100	100	100	100	100	100
predictive values for negative test %	20,45	10,25	12,5	16,16	12,5	5,40

accuracy%	87,14	92,48	90,38	90	90,56	96,15
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Table 4: NAG has better diagnostic performances than microalbuminuria in term of sensitivity.

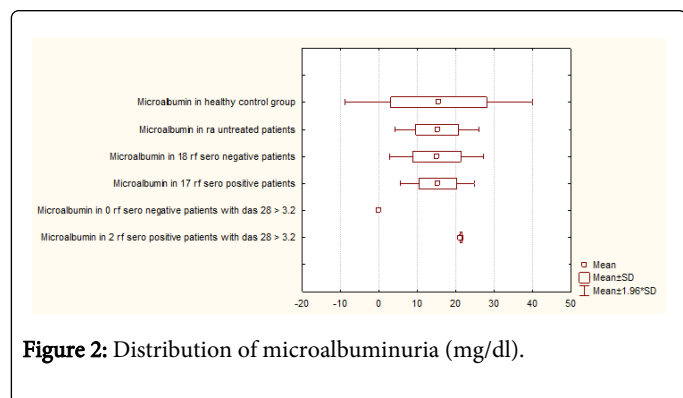


Figure 2: Distribution of microalbuminuria (mg/dl).

Discussion

Our study shows that NAG is the most relevant marker for assessment of asymptomatic renal dysfunction. NAG sensitivity is higher in comparison with the microalbuminuria sensitivity (37.14% vs. 11.42%). It is close to the GFR sensitivity calculated with creatinine clearance by Cocroft-Gault (40%), as the mathematical score is composed of serum creatinine, age and body weight. NAG is an isolated laboratory variable dominant in the diagnosis of asymptomatic renal tubular dysfunction [19-27]. The other standard analyses used for assessment of renal function have shown low sensitivity: serum and urine creatinine, serum urea (8.57% vs. 25.71% vs. 11.42%). Seropositivity influences the appearance of NAG induction, as was shown in our example - seropositive RF patients with DAS 28>3.2 have much higher NAG induction than seronegative RF with DAS 28>3.2. Statistical correlation between disease duration in months and NAG enzymuria ($p < 0.001$) shows that untreated RA have implications on renal issue as one of the visceral manifestations of disease. Untreated RA primarily damages the tubules, but in a very small amount also the glomerular apparatus. SDMA, in our study shows very high diagnostic value.

Conclusion

In conclusion, SDMA has higher sensitivity than NAG and microalbuminuria. It is a relevant marker in the assessment of asymptomatic renal impairment in untreated RA. These renal markers could be used in routine clinical practice.

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