JMB 2007; 26 (4) DOI: 10.2478/v10011-007-0038-0

UDK 577.1 : 61 **ISSN 1452-8258**

JMB 26: 300–308, 2007

Original paper
Originalni naučni rad

THE DIAGNOSTIC VALUE OF N-ACETYL-β-D-GLUCOSAMINIDASE AND MICROALBUMIN CONCENTRATIONS IN RHEUMATOID ARTHRITIS

DIJAGNOSTIČKA VREDNOST N-ACETIL-β-D-GLUKOZAMINIDAZE I MIKROALBUMINURIJE KOD REUMATOIDNOG ARTRITISA

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Summary: The purpose of this research was to compare the diagnostic values of laboratory variables, to present quantitative evaluations of the diagnostic sifted test with reference to sensitivity and specificity, the predictive value of the positive and negative test and precision of the test for N-acetyl-β-D-glucosaminidase (NAG), microalbumin, rheumatoid factor (RF), C-reactive protein (CRP), DAS 28 index, in early diagnosis of untreated rheumatoid arthritis (RA), and to define the effect of untreated rheumatoid arthritis on glomerular and tubular function. Using a colorimetric assay for the determination of N-acetyl-β-D-glucosaminidase and an immunoturbidimetric assay for the determination of urinary albumin, the samples of serum and urine have been examined in 70 participants (35 RA who were not treated, 35 healthy controls). RF was defined with the test for agglutination (Latex RF test) in the same participants. Out of 35 examined patients with RA, in 13 we found the presence of NAG enzymuria (sensitivity of the test 37.14%), while microalbuminuria appeared in 4 patients (sensitivity of the test 11.42%). RF appeared in 17 patients (sensitivity of the test 48.57%). Four patients were NAG and RF positive, while 3 patients were microalbuminuria and RF positive. Among 18 RF negative patients, 9 patients were NAG positive, and 1 patient presented with microalbuminuria. Among 17 RF positive RA, the presence of NAG was found in 4 patients, and the presence of microalbuminuria in 3 patients. Among 18 RF negative RA, NAG enzymuria appeared in 9 patients. Microalbuminuria was present in 1 patient. In the healthy control group, 8 patients were NAG positive, 2 patients were positive for microalbuminuria. RF appeared in 2 patients. NAG has higher sensitivity than microalbuminuria in the detection of asymptomatic renal lesions in untreated RA.

Keywords: N-acetyl-β-D-glucosaminidase, microalbumin, rheumatoid arthritis, rheumatoid factor

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Kratak sadržaj: Cilj ovog istraživanja bio je da se uporede dijagnostičke vrednosti laboratorijskih parametara, da se prikažu kvantitativne ocene dijagnostičkog testa za definisanje osetljivosti i specifičnosti, prediktivne vrednosti pozitivnog i negativnog testa, kao i preciznost testa kod N-acetil-β-D-glukozaminidaze (NAG), mikroalbumina, reumatoidnog faktora (RF), C-reaktivnog proteina (CRP), DAS 28 indeksa, u ranoj dijagnozi, kod netretiranog reumatoidnog artritisa (RA), kao i određivanje efekta netretiranog reumatoidnog artritisa na glomerularnu i tubularnu funkciju. Koristeći kolorimetrijsku metodu za određivanje N-acetil-β-D-glukozaminidaze (NAG), kao i imunoturbidimetrijske metode za detekciju mikroalbuminurije, ispitani su uzorci 70 učesnika (35 RA netretirani, 35 kontrolna zdrava grupa). RF je određen testom aglutinacije (Latex RF test) kod istih učesnika. Od ispitanih 35 pacijenata sa RA, kod 13 je otkriveno prisustvo NAG enzimurije (osetljivost testa 37,14%), a mikroalbuminurija je bila zastupljena kod 4 pacijenta (osetljivost testa 11,42%). RF je bio zastupljen kod 17 pacijenata (osetljivost testa 48,57%). Četiri pacijenta bila su NAG i RF pozitivna, dok je kod 3 pacijenta otkriveno prisustvo mikroalbuminurije i RF. Od 18 RF negativnih pacijenata, 9 su bili NAG pozitivni, 1 sa prisutnom mikroalbuminurijom. Kod 17 RF pozitivnih RA pacijenata, zastupljenost NAG nađena je kod 4 pacijenta, a 3 su imala mikroalbuminuriju. Od 18 RF negativnih RA, NAG enzimurija je nađena kod 9 pacijenata. Mikroalbuminurija je bila prisutna kod 1 pacijenta. U kontrolnoj zdravoj grupi, 8 pacijenata je imalo pozitivan NAG, 2 pacijenta su imala pozitivnu mikroalbuminuriju. RF je bio zastupljen kod 2 pacijenta. NAG ima veću osetljivost od mikroalbuminurije za detekciju asimptomatskih bubrežnih lezija kod netretiranog RA.

Ključne reči: N-acetil-β-D-glukozaminidaza, mikroalbuminurija, reumatoidni artritis, reumatoidni faktor

Introduction

The standard, routine parameters, which are used for assessment of glomerular filtration rate (GFR), have relatively low sensitivity because of the large functional reserve of the kidney (1). The kidney is a compensated organ and up to 50% of its functional capacity can be lost before the changes start with an increase of degradation products of nitrogen metabolism and appearance of proteinuria, which present (subjective) individual problems that will force the patient to ask for medical help. The renal function can be determined by many methods, such as immune, radiologic, cytologic analyses, but an important place is taken by biochemical analyses, as non-invasive methods which have big importance in the early detection of some pathological conditions appearing during therapy. Among them, very important is the regulation of activity of enzymes and their isoenzymes in urine because their activity in serum has small diagnostic value. Pathogenic mechanisms which lead to the destruction of epithelial cells of proximal tubules and are responsible for the appearance of enzymuria are: immune mechanism, complement, lysosomal enzymes, tubular obstruction by cell debris, protein cylinders, toxical noxes, medicaments or proteinuria. Each of them, to a different degree, in a direct or indirect way contributes to the release of biochemical markers in urine. The purpose of this study is to define the effect of untreated rheumatoid arthritis on glomerular and tubular function. The urine microalbumin is used as a marker for glomerular function, while NAG is an indicator of proximal tubular damage.

Renal markers for assessment of renal dysfunction

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

1. Enzymes with high molecular weight, which are not usually filtered in the glomerulus, with genesis in the proximal tubula (NAG).
2. Intermediate protein which is normally filtered in the glomerulus in a very small quantity, while the biggest part is resorbed in the tubules (microalbumin) (2–7).

Of all urine enzymes, the most studied protein is U-NAG. It is an enzyme of the hydrolase class usually present in the lysosomes of proximal tubular cells (8). In the human tissue and in biological liquids two main enzyme forms exist: A (Acid) and B (Basic) (9–11). The percentage of A isoform (U-NAG-A) is the biggest in normal urine (12, 13). At the end of cell maturation process it is placed in the resolved form of cytosol. Its excretion is connected with exfoliative turnover and is signed as functional enzymuria. B isoform, (U-NAG-B) is dependent on maturation and is more closely connected with the basal membrane in which it appears. Because of this localisation of B isoform, NAG massive is released in tubular lumen only in cases of cytolytic tubular lesion. Its presence in urine is in correlation with cell lysis and, because of that, is signed as lesion enzymuria (14, 15). NAG can be detected in circulation. But plasma NAG cannot pass through an intact glomerular membrane because of its large molecular weight (140 000 daltons). In healthy people, urine NAG is a representative of the total quantity released from the walls of renal tubular cells (16) and is a very sensitive marker for renal tubular damage (1, 17–19).

Albumin (molecular weight from 66 KDa) is quantitatively the most important protein in plasma and urine. Approximately up to 30% of proteins in the urine belong to it, and it appears to be a good indicator for assessment of the change of glomerular permeability. The change in glomerular permeability occurs in diabetic and hypertension nephropathy, nephrotic syndrome, pre-enclamsia and glomerulonephritic conditions. Urine albumin excretion has high individual variability and depends on physical activity or variations in food. From the pathophysiological aspect, microalbuminuria can be caused by increased glomerular permeability for albumin, increased glomerular pressure and/or reduced tubular albumin reabsorption. Renal endothelium is intimately involved in the regulation of these processes (20, 21).

Patients and Methods

In the patients examined for this study, the diagnosis of the disease 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) (22). In order for a patient to be diagnosed with rheumatoid arthritis, he or she must fulfill at least four out of seven criteria. Criteria from one to four are present for at least six weeks. The study involved 35 patients (female 28, male 7) suffering from RA, and 35 healthy subjects (female 18, male 17) from the control group. Their average age was 56.68 years (± 6.79) (40–65 years) in the group with RA, and 46.2 years (±12.49) (29–65 years) in the control group. The average duration of the disease in months from the beginning was 43.97 (± 45.23), in the interval of 1–168 months. None of the patients who were involved in research had a medical record for past or present renal diseases. Three patients had been previously treated with oral corticosteroids, while none of them had used NSAID. The rest of the patients refused use of other medicines before taking the examinations.

Criteria for inclusion: the study involved patients who suffered from rheumatoid arthritis, aged 18–65 years, newly found and till now not treated.

Criteria for exception from the research: from the research were excepted all the patients with a disease

or condition which could directly or indirectly influence a change in results:

1. Patients with previous medical record for diseases of the spleen, thyroid gland, hepatal damage, renal, hematologic, cardiovascular, neurotic and lung damage, autoimmune disease, AIDS, aged <18 years.
2. Patients with diabetes mellitus, acute infections, malignant neoplasm, febrile conditions.
3. Patients treated with antibiotics and salycilate in the period of six months prior to the beginning of the study.
4. Patients with hypertension arterialis, uric arthritis, uric infections, SLE, Sy Sjögren, mixed conjunction texture disease, vasculitis.
5. Patients treated with antihypertension, antidiabetic and cardiac therapy.
6. Patients with anamnesis for transfusion of blood and overweight.
7. Hypersensitive to some of the medicines or their components.
8. Excepted patients who together with these medicines take medicines from basic line.
9. Excepted patients whose results show that in 0 spot there is a glycemia, or increased level of degraded products: creatinine in serum and urine, urea in serum and disorder of the hematologic and enzymatic status.

All patients took part in this study voluntarily, so the ethical criterion was not breached during our work.

Clinical evaluation of disease activity

A subspecialist in this field did the clinical evaluation. The activity of the disease was evaluated using DAS 28 Index (Disease Activity Score (DAS 28)) (23–26). The index is a mathematical formula that allows us to get a uniquely composed quantitative score, which constitutes from palpation painful sensitive joints (max number 28), swollen joints (max number 28), Westergren ESR, and the patient's global assessment of the activity of disease (0–100 mm Visual Analogous Scale, VAS) and the morning rigid (minutes). DAS 28 index is ranked from 0 to 10 and a score under 3.2 ranks the disease as low-active. The assessment of glomerular filtration rate (GFR) was calculated using the Cocroft-Gault formula (27).

Laboratory assessment

For a clinical assessment of the basic disease, the following laboratory variables needed to be measured: haemogram and differential haemoanalysis, reactors of acute phase, anti CCP 2, C-reactive protein (CRP), rheumatic factor (RF), alkaline phosphatase (AP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine kinase (CK), lactate dehydrogenase (LDH), urea and creatinine in serum. Examples of urine were taken not only for routine urine analysis, but for detecting NAG, creatinine in urine and microalbuminuria.

Immunoturbidimetric assay for the determination of urinary albumin: for random urinary albumin measurement, an early morning mid-stream specimen should be used. Centrifuge cloudy samples before use and analyse the clear supernatant. Reference values are 2.0–20.0 mg/L. An undiluted sample is added to a 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 sample urine. By constructing a standard curve from the absorbance of standards, the albumin concentration of the sample can be determined. The assay can be carried out manually (at room temperature) or with an automated analyser using DAKO tests (28, 29).

Colorimetric assay for the determination of N-acetyl-β-D-glucosaminidase in urine: 3-Cresolsulfonphthaleinyl-N-Acetyl-β-D-glucosaminide, sodium salt, is hydrolysed by N-Acetyl-β-D-glucosaminidase (NAG) with the 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.

Urea in serum was detected with the method of »Kassirer«. Reference values are 3–7.8 mmol/L.

Creatinine in serum and urine was detected with the »Jaffe« method. Reference values for creatinine in serum are 45–109 μmol/L and for creatinine in urine 7–17 μmol/dU.

C-reactive protein (CRP) was found using the test of agglutination (Latex CRP test), (BioSystems S.A. Reagents&Instruments Costa Brava 30, Barcelona, Spain) (30–34). Reference values are under 6 mg/L CRP in serum.

Rheumatic factor (RF) was determined using the test of agglutination (Latex RF test) (BioSystems S.A. Reagents&Instruments Costa Brava 30, Barcelona, Spain) (32, 33, 35–38). Reference values are under 8 mg/L RF in serum.

For the specification of red cell sedimentation (SER), we used the method after Westergren, and normal values were: for males 7–8 mm, for females 11–16 mm.

Statistical analysis

For testing the importance of the difference between two arithmetic means, with respect to proportion, a Student's t-test was used, which compares the mid-

dle values of certain numerical parameters between two groups (2), and a Wilcoxon-matched test for independent examples. Sensitivity and predictivity for positive and negative test of examined marks were defined with the test of sensitivity and specificity. P value between 0.05 and 0.1 was taken as statistically significant. Data processing was done with the statistics package Statistica 7.0.

Results

Of all the examined patients with RA, in 13 patients (37.14%) we found the presence of NAG enzymuria, while microalbuminuria was present 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%) we found the presence of microalbuminuria. In 13 patients (37.14%) NAG enzymuria was not detected and they were RF positive. In 14 patients (40%) microalbuminuria was not detected and they were RF positive. Among 18 RF negative patients, in 9 patients (50%) NAG enzymuria was detected, and microalbuminuria in 1 patient (5.55%). Among the 22 patients in whom NAG enzymuria was not detected, 13 patients (59.09%) were RF positive, but among the 31 patients without microalbuminuria, 14 patients (45.16%) were RF seropositive. Among the total of 35 examined patients with RA, sensitivity of NAG was 37.14%, sensitivity of microalbuminuria was 11.42%, while sensitivity of RF 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 positive for microalbuminuria, and RF was present in 2 patients (5.71%) (*Table I*).

Diagnostic value of N-acetyl-β-D--glucosaminidase and microalbumin in urine in patients with rheumatoid arthritis

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 II*.

NAG has better diagnostic performances than microalbuminuria in relation to sensitivity (sensitivity 37.14% vs. 11.42 %), but with lower specificity (specificity 77.14% vs. 94.28%) in the detection of renal tubular damage in untreated RA.

NAG, microalbumin 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 DAS 28 > 3.2 patients, 3 were NAG positive (20%) and their M ± SD (1.86 ± 1.06) was extended (1.2–3.1), while microalbuminuria was positive in 2 patients (13.13%) and their M ± SD (21.35 ± 0.21) was extended (21.2–21.5).

Among 18 seronegative RF patients, replacement of DAS 28 > 3.2 was found in 13 patients (72.22%). Of these 13 DAS 28 > 3.2 patients, 8 were NAG positive (61.53%) and their M ± 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 bigger titer of NAG than RF seropositive patients (1.12 (± 0.58) (0.32–2.1) vs. 1.07 (± 0.79) (0.25–3.1)), and bigger DAS 28 > 3.2 index (5.04 ± 1.33 (2.47–6.83) vs. 4.56 ± 1.76 (1.85–7.03)). Between these two groups of NAG there is no statistical relation (p=0.652918).

However, seropositive RF patients with DAS 28 > 3.2 have much bigger 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). Between these two groups of NAG there is no statistical connection (p=0.592980) (*Figure 1*).

Neglectable was the difference in the value of microalbuminuria in seronegative RF patients when compared to seropositive (15.03 (± 6.24) (8.30–35.2) vs. 15.30 (± 4.90) (5.50–25.7)); between two groups of microalbuminuria no statistical connection was found (p=0.717381) (*Figure 2*).

A statistical connection between DAS 28 index in RF seropositive and negative patients (p=0.379375) and between the two groups DAS 28 > 3.2, NAG positive, does not exist nor does it exist between RF seropositive and negative patients (p=0.285050).

No statistical connection was found using a Wilcoxon-matched test between: NAG in RA and healthy control group for p<0.05 (p= 0.555426); microalbuminuria in RA and healthy control group (p= 0.287038). There exists a statistical relation between NAG and microlbuminuria in frames of RA group for p<0.05 (p= 0.000000).

A statistical relation was found using a Wilcoxon-matched test between: NAG in RA and age, duration of disease in months, DAS 28 index, RF, CRP, SER, morning rigid, creatinine in serum and urine and serum urea in the same group for p<0.05 : NAG

Table I NAG, microalbumin and other laboratory variables in RA and healthy control group.

Parameter	RA untreated group (n=35) value (M ± SD)	RA $^{sero-}$ (n=18) value (M ± SD)	RA $^{sero+}$ (n=17) value (M ± SD)	Healthy control group (n=35) value (M ± SD)
	Positive / Negative	Positive / Negative	Positive / Negative	Positive / Negative
NAG + >1.18 (U/mmol/crea)	13/22 1.096 (± 0.68) (0.25–3.1)	9/9 1.12 (± 0.58) (0.32–2.1)	4/13 1.07 (± 0.79) (0.25–3.1)	8/27 1.00 (± 0.50) (0.26–1.91)
Microalbuminuria + >20 (mg/L)	4/31 15.16 (± 5.55) (5.50–35.2)	1/17 15.03 (± 6.24) (8.30–35.2)	3/14 15.30 (± 4.90) (5.50–25.7)	2/33 15.56 (± 12.46) (1.75–56.4)
Serum creatinine < 49–109 > μmol/L	3/32 67.55 (± 14.76) (41–108)	1/17 68.24 (± 14.16) (44–108)	2/15 66.82 (± 15.77) (41–99)	2/33 74.95 (± 19.72) (44–135)
Serum creatinine < 7–17> μmol/dU	9/26 10.41 (± 4.71) (3.1–25.4)	6/12 9.26 (± 4.54) (3.1–18)	3/14 11.62 (± 4.72) (5.8–25.4)	5/30 9.15 (± 4.22) (1.8–20.4)
Serum urea + > 7.8 mmol/L	4/31 5.66 (± 1.46) (3.00–8.60)	0/18 5.52 (± 1.33) (3.00–7.5)	4/13 5.82 (± 1.62) (3.80–8.6)	1/34 4.94 (± 1.28) (2.50–7.2)
GFR + >90 mL/min	14/21 99.19 (± 24.46) (56.08–157.30)	7/11 99.19 (± 24.46) (64.67–142,59)	7/10 99.19 (± 25.22) (56.08–157.30)	4/31 113.80 (± 30.86) (69.98–177.74)
DAS 28 + > 3.2	28/7 4.79 (± 1.56) (1.85–7.03)	13/5 4.56 (± 1.76) (1.85–7.03)	15/2 5.04 (± 1.33) (2.47–6.83)	0/35 0.00 (± 0.00) (0.00–0.00)
Morning rigid + > 0 min	26/9 43.20 (± 65.13) (0–300)	14/4 57.50 (± 81.40) (0–300)	12/5 28.05 (± 38.72) (0–120)	0/35 0.00 (± 0.00) (0.00-0.00)
RF +30 > IU/mL	17/18 346.15 (± 625.22) (0.00–1920)	0/18 0.00 (± 0.00) (0.00–0.00)	17/0 712.67 (± 743.72) (30–1920)	2/33 13.71 (± 38.73) (0.00–120)
CRP +12 > mg/L	14/21 46.86 (± 79.19) (0.00–384)	3/15 8.66 (± 24.62) (0.00–96)	13/4 87.31 (± 96.44) (0.00–384)	4/31 5.48 (± 12.80) (0.00–48)
Sedimentation +> 16	27/8 48.62 (± 39.81) (2.0–120)	13/5 43.94 (± 39.82) (2.0–120)	14/3 53.58 (± 40.39) (5.0–120)	4/31 9.42 (± 8.21) (2.0–44)
Anti CCP 2 > 1.26	23/12 1.71 (± 0.69) (0.92–3.0)	11/7 1.56 (± 0.59) (0.93–2.6)	12/5 1.87 (± 0.77) (0.92–3.0)	1/34 0.95 (± 0.10) (0.90–1.38)

vs. age (p=0.000000); NAG vs. duration of disease in months (p=0.000000); NAG vs. DAS 28 p=(0.000000); NAG vs. RF (p=0.018345); NAG vs. CRP (p=0.040620); NAG vs. SER (p=0.000000); NAG vs. creatinine in serum (p=0.000000); NAG vs. creatinine in urine (p=0.000000); NAG vs. serum urea (p=0.000000).

A statistical relation was found using a Wilcoxon--matched test between: microalbumin in RA and age, duration of disease in months, DAS 28 index, RF, SER, creatinine in serum and urine and serum urea in the same group for $p<0.05$: microalbumin vs. age (p=0.000000); microalbumin vs. duration of disease in months (p=0.004845); microalbumin vs. DAS 28 (p=0.000000); microalbumin vs. RF (p=0.049358); microalbumin vs. SER (p=0.000044), microalbumin vs. serum creatinine (p=0.000000); microalbumin vs. creatinine in urine (p=0.000324); microalbumin vs. serum urea (p=0.000000).

There was found no statistical relation using a Wilcoxon-matched test between: microalbumin in RA with CRP and morning rigid in frames of the same group: microalbumin vs. CRP (p=0.094787); microalbumin vs. morning rigid (p=0.113973).

Table II Diagnostic performance of NAG, microalbumin and other laboratory variables in rheumatoid arthritis.

	NAG (RA=35)	NAG (RA^-=18)	NAG (RA^+=17)	Micro-albumin (RA=35)	Micro-albumin (RA^-=18)	Micro-albumin (RA^+=17)	Serum creatinine (RA=35)	Serum creatinine (RA^-=18)	Serum creatinine (RA^+=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 of the positive test %	61.90	52.94	33.33	66.66	33.33	60	60	33.33	50
Predictive values of the negative test %	44.89	25	32.5	48.43	34	29.78	49.23	34	31.25
Precision %	57.14	67.92	59.61	52.85	64.15	69.29	51.42	64.15	67.30
	Urine creatinine (RA=35)	Urine creatinine (RA^-=18)	Urine creatinine (RA^+=17)	Serum urea (RA=35)	Serum urea (RA^-=18)	Serum urea (RA^+=17)	GFR (RA=35)	GFR (RA^-=18)	GFR (RA^+=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 of the positive test %	64.28	54.54	37.5	80	0	80	77.77	63.63	63.63
Predictive values of the negative test %	46.42	28.57	31.88	47.69	34.61	27.65	40.38	26.19	24.39
Precision %	55.71	67.92	63.46	54.28	64.15	73.07	64.28	71.69	73.03
	RF (RA=35)	RF (RA^-=18)	RF (RA^+=17)	CRP (RA=35)	CRP (RA^-=18)	CRP (RA^+=17)	SER (RA=35)	SER (RA^-=18)	SER (RA^+=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
Predictive values of the positive test %	89.47	0	89.47	77.77	42.85	76.47	87.09	76.47	77.77
Predictive values of the negative test %	35.29	35.29	0	40.38	36.60	11.42	20.51	13.88	8.82
Precision %	71.42	62.26	96.15	64.28	64.15	84.61	82.85	83.01	86.53

	Morning rigid (RA=35)	Morning rigid (RA^-=18)	Morning rigid (RA^+=17)	DAS 28 (RA=35)	DAS 28 (RA^-=18)	DAS 28 (RA^+=17)
Sensitivity %	74.28	77.77	70.58	80	72.22	88.23
Specificity %	100	100	100	100	100	100
Predictive values of the positive test %	100	100	100	100	100	100
Predictive values of the negative test %	20.45	10.25	12.5	16.16	12.5	5.40
Precision %	87.14	92.48	90.38	90	90.56	96.15

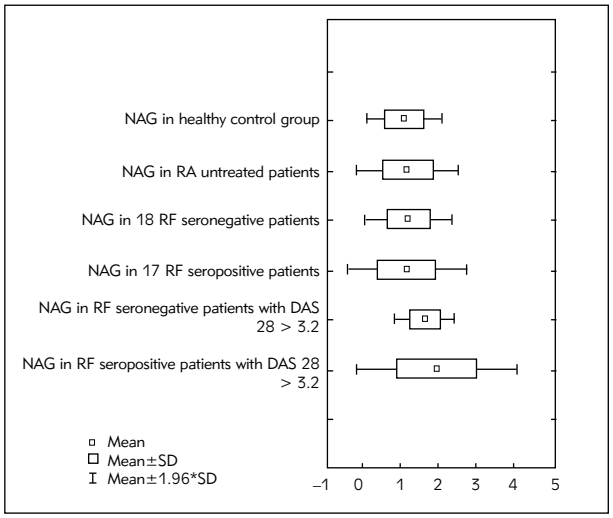

Figure 1 Distribution of N-acetyl-β-D-glucosaminidase (NAG) (U/mmol creatinine).

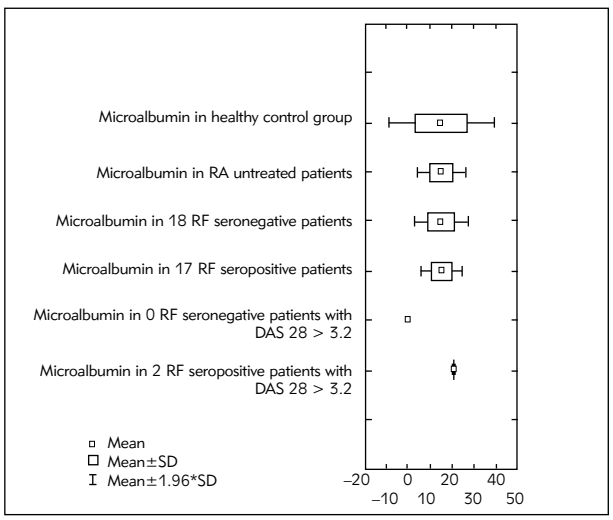

Figure 2 Distribution of microalbuminuria (mg/L).

Discussion

Statements for working on RA using renal tissue analysis (39–47) expose NAG as the most relevant marker for the assessment of asymptomatic renal dysfunction. Sensitivity of NAG in comparison with the sensitivity of microalbumin is higher (37.14% vs. 11.42%). This sensitivity is close to the sensitivity of GFR calculated with calculated creatinine clearance after Cocroft-Gault (40%), as the mathematical score is composed of serum creatinine, age and body weight. NAG is an isolated laboratory variable dominant in its performance in the diagnosis of asymptomatic renal tubular dysfunction. The rest of the standard working analyses used for assessment of renal function have shown low sensitivity: serum creatinine, urine creatinine, serum urea (8.57% vs. 25.71% vs. 11.42%).

Seropositivity influences the appearance of NAG induction (43), which has shown in our example that seropositive RF patients with DAS 28 > 3.2 have much higher NAG induction after circumference than seronegative RF with DAS 28 >3.2. Statistical connection between the duration of the disease in months and NAG enzymuria (p=0.000000) shows that untreated RA works on the renal texture as one of visceral appearances of the disease. Untreated RA primary damages the tubular, but in a very small circumference (percentage) also the glomerular apparatus.

In conclusion, NAG has higher sensitivity than microalbuminuria. It is a relevant marker in the assessment of asymptomatic renal damages in untreated RA. NAG can be used in everyday clinical practice.

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Received: September 21, 2007

Accepted: November 15, 2007