

Renal function in rheumatoid arthritis patients treated with methotrexate and infliximab

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ABSTRACT

Objective. This study was aimed at monitoring the early and late effects of infliximab on renal proximal function in RA patients treated with methotrexate. N-acetyl-β-D-glucosaminidase (NAG) activity in urine served as an indicator of proximal tubular damage

Methods. NAG activity was estimated in 21 patients during the course of treatment with infliximab and methotrexate. In every patient NAG-enzymuria was estimated directly before and 60 min after infliximab infusions and 62 weeks after starting the therapy.

Results. The total of mean NAG activities observed before each infusion of infliximab was significantly lower ($p < 0.02$) than NAG-enzymuria before the start of infliximab treatment (7.4 UI/g vs 11.8 UI/g). The proportion of patients in whom NAG activity rose by more than 50% during treatment ranged from 5.3% to 25%. Administration of infliximab did not significantly change the mean serum creatinine levels or creatinine clearance. No significant differences were observed in the mean values of NAG values before and 60 min after infliximab infusion.

Patients who demonstrated elevated NAG activities during the course of the whole treatment demonstrated significantly more pronounced NAG enzymuria before treatment and one hour after the first infusion ($p < 0.0005$), as well as higher RA activity ($p < 0.05$). There was no observed influence of NSAIDs or prednisone on the frequency of elevated NAG activities. Raised creatinine concentrations (> 1.3 mg/dL) were noted before and during the course of infliximab treatment in 3 patients. In 16 patients abdominal fat aspiration biopsy was performed and in 3 the presence of amyloid deposits was demonstrated. In these patients NAG activity exceeded twice the upper normal limit.

Conclusion. The introduction of infliximab during methotrexate therapy demonstrated no early or delayed nephrotoxicity of the drug in patients with rheumatoid arthritis.

Introduction

In aggressive forms of the rheumatoid arthritis (RA) and when methotrexate

(MTX) remains only partially effective, combining this drug with tumour necrosis factor-α blocking agents (anti-TNF-α) such as infliximab or etanercept is recommended. The elimination of infliximab and its effect on renal function has not yet been fully recognized. In a female RA patient undergoing haemodialysis for coexisting terminal renal insufficiency, Singh *et al.* observed good effects of infliximab on RA activity (1). On the other hand, Yee *et al.* noted acute tubular necrosis in a female patient with sarcoidosis treated with infliximab and this complication required prolonged haemodialysis (2). Elevated levels of N-acetyl-β-D-glucosaminidase (NAG) in urine have been shown to be associated with reversible tubular damage (3,4). In the available literature, however, no data have been published on renal function in RA patients treated with infliximab and methotrexate. This study therefore aimed at investigating the early and late effects of infliximab on proximal renal functioning in methotrexate-treated patients with RA.

Material and methods

The study was performed on 31 RA patients whose mean age was 50.8 years (range 18-79). Patients were eligible for the repeated administration of infliximab if they had active rheumatoid arthritis (RA) despite treatment with MTX for at least 3 months. In these patients being treated with a stable oral dose of MTX of 10 to 17.5 mg once a week (mean dose 12.5 mg), infliximab was given in 2 h intravenous infusions at a dose of 3 mg/kg body weight according to the following schedule: week 0, weeks 2, 6, 14, 22, 30, 38, 46 and 54. In all 31 patients serum creatinine levels were measured and urinalysis carried out. NAG activity in the urine was monitored in 21/31 patients and this subgroup formed the subject of detailed analysis. Prednisone (10 mg daily, $n = 15$) and non-steroidal anti-inflammatory drugs (NSAIDs, $n = 15$) were administered in stable doses. The treatment procedure was approved earlier by the local Bioethical Committee.

NAG activity and the creatinine concentration were measured in 348 sam-

ples of urine in 21 patients during the course of treatment with infliximab and methotrexate. In every patient NAG-enzymuria was measured on a mean of 16 (range: 13 to 20) occasions: directly before and 60 min after the infliximab infusions and 62 weeks after starting the therapy.

The NAG activity in 21 patients was compared to that in 65 healthy individuals (35 men and 30 women); their mean age \pm SD was 36.2 ± 8.9 years (range 26–55). NAG activity was assessed by the method described by Chatterjee and Zwierz (5,6) in morning urine samples, and was compared to the creatinine concentration in the urine. Rheumatoid arthritis disease activity was assessed using the modified Disease Activity Score (DAS 28). Creatinine clearance (Cl_{cr}) was calculated according to the Cockcroft's and Gault's equation. The non-parametric Mann-Whitney's test and the χ^2 test with Yates correction were used to compare the patient groups with decreased or augmented NAG activity with respect to variables characterising disease activity. For the evaluation of NAG activity during the course of treatment in individual patients Wilcoxon's paired sequence test was used. Statistical significance was established at $p < 0.05$.

Results

In the control group of 65 healthy individuals, the mean NAG activity in urine was 3.52 ± 1.99 UI/g (1.0 – 10.4 UI/g). The enzyme activity higher than 7.50 UI/g (sum of the mean NAG activity and of two SD) was regarded to be abnormal.

Before the start of infliximab treatment abnormal NAG-enzymuria (> 7.5 UI/g) was observed in 13 out of 21 (62%) RA patients. In patients with increased NAG activity only a significantly higher level of blood platelets was noted (472 ± 189 vs 328 ± 99 $10^9/L$; $p < 0.05$), while older patients more often manifested higher values for urinary NAG activity (57 ± 12 vs 41 ± 17 years; $p < 0.04$). The methotrexate dose used, DAS 28 and its components, the duration of RA, administration of NSAIDs, serum creatinine, albumin, C-reactive protein (CRP) and Cl_{cr} were not corre-

lated with NAG activity in the urine.

Mean NAG-enzymuria measured directly before every consecutive infliximab infusion was lower than before the first infusion, but was not significantly different (Table I). The mean NAG activity observed before the 2, 3, 4, 5, 6, 7, 8 and 9 infusion of infliximab was significantly lower ($p < 0.02$) than the activity of the enzyme before the start of infliximab treatment (7.4 UI/g vs 11.8 UI/g).

The frequency of elevated NAG activity (by more than 50%) as compared to NAG-enzymuria before the first infusion ranged from 5.3% to 25% (median 15%). Abnormal NAG activity in the urine was present before every infusion in 35% to 62.5% (median 55.2%). Administration of infliximab did not significantly change serum creatinine levels at every infusion (median 0.785; 0.72 – 0.87) or creatinine clearance (median 93 ml/min; 85–94).

NAG activity was evaluated before each of the consecutive infliximab infusions and 60 min after its administration. No significant differences were observed in the mean values for these parameters during the course of the anti-TNF treatment (Table I).

Patients treated with infliximab and methotrexate were divided into two groups: 12 patients in whom the mean NAG activity assessed before every infusion (except first one) exceeded 7.5 IU/g and 9 patients with a normal mean value for NAG activity (< 7.5 UI/g) during the entire course of therapy. Patients who demonstrated elevated NAG activity during the entire course of treatment demonstrated a significantly more pronounced NAG enzymuria before treatment and one hour after the first infusion ($p < 0.0005$), as well as higher RA activity ($p < 0.05$), as indicated by higher number of swollen joints, a higher Patient Global Assessment of Disease Activity (PGADA) and a higher DAS28 index (Table II).

Concomitant treatment with NSAIDs did not influence the frequency of abnormal NAG values (Table II). Serum creatinine above 1.3 mg/dL was noted before and during the course of infliximab treatment in 3 of 31 examined patients. Persistence of serum creatinine

Table I. Comparison of NAG activities before and 1 h after infliximab infusion in rheumatoid arthritis patients.

Time of testing (week of treatment)	Median NAG activity (UI/g)	
	Before infusion	1 h after infusion
1 st infusion (0)	11.8	11.0
2 nd infusion (2)	7.1	7.2
3 rd infusion (6)	9.4	10.1
4 th infusion (14)	7.6	11.4
5 th infusion (22)	8.8	8.4
6 th infusion (30)	6.9	8.4
7 th infusion (38)	8.3	9.2
8 th infusion (46)	9.5	9.0
9 th infusion (54)	8.3	10.2

two-fold higher than normal was demonstrated in only one patient. In the remaining 2 patients the increase in serum creatinine was detected only before the start of therapy and/or during the first 6 weeks of infliximab treatment.

Urine pathology defined as the presence of more than 5 erythrocytes per microscopic field or the presence of protein in individual urine samples was detected in 7 patients (trace of protein in 2 patients and erythrocyturia in 5 patients detected on three occasions). Only in one patient was more frequent erythrocyturia present due to coexisting nephrolithiasis, but her NAG-enzymuria was within normal limits.

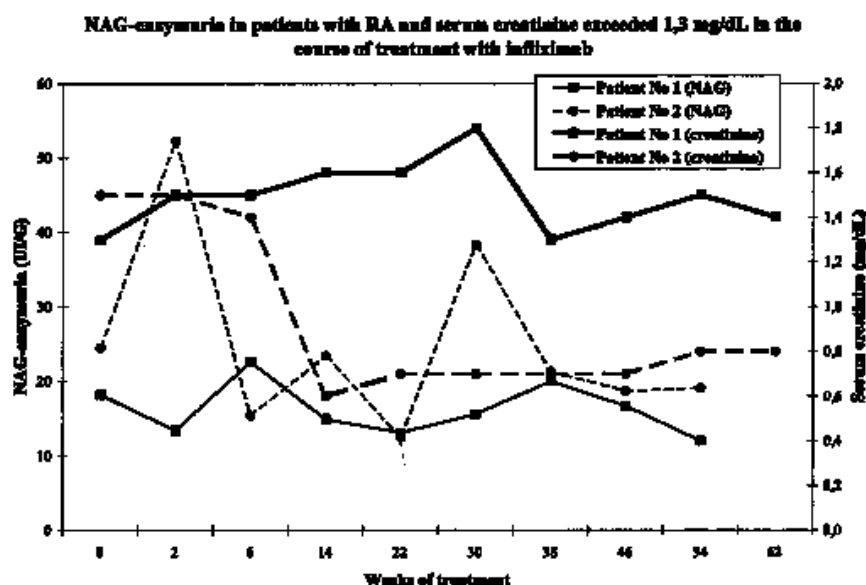
Among 3 RA patients with the most marked increase in NAG enzymuria during the course of treatment and before the administration of anti-TNF therapy, in 2 serum creatinine levels higher than 1.3 mg/dL were also found (Fig. 1).

In 16 patients an abdominal fat aspiration biopsy was performed and in 3 patients the presence of amyloid deposits was demonstrated. In these patients NAG activity exceeded twice the upper normal limit, ranging between 16.3 and 21.5 UI/g, but only in one patient did serum creatinine range between 1.3 and 1.8 mg/dL during therapy; periodically traces of protein were noted in the urine and creatinine clearance was decreased (Fig. 1). In patients with amyloid deposits mean NAG activity during the course of infliximab administration was lower than before start of the treat-

Table II. Comparative characteristics of rheumatoid arthritis patients as related to NAG activity in the course of treatment with methotrexate and infliximab.

Parameter	Value of tested parameter in patients with mean NAG activity during entire treatment		p
	7.5 UI/g	< 7.5 UI/g	
Number of patients	12	9	
Age (years)	57 ± 13	43 ± 17	NS
Duration of disease (years)	12.2 ± 9.9	10.9 ± 7.3	NS
Age at the start of disease	44 ± 14	32 ± 16	NS
Dose of MTX/week (mg)	13.5 ± 2.0	13.3 ± 2.8	NS
Number of patients taking NSAIDs	9/12 (75%)	6/9 (67%)	NS
NAG activity before treatment	14.7 ± 5.4	7.1 ± 3.5	0.0022
NAG activity after 1st infusion	16.3 ± 6.5	7.7 ± 3.2	0.0018
Serum creatinine (mg/dL)	0.82 ± 0.31	0.69 ± 0.21	NS
Creatinine clearance (ml/min)	80 ± 29	110 ± 43	NS
Blood platelets (10 ⁹ /L)	469 ± 201	347 ± 97	NS
Haemoglobin concentration (g/dL)	11.1 ± 1.2	11.6 ± 1.4	NS
ESR (mm/h)	60 ± 29	41 ± 15	0.1
Concentration of CRP(mg/dL)	6.1 ± 5.1	3.3 ± 2.1	NS
Concentration of albumin (g/dL)	3.31 ± 0.50	3.62 ± 0.55	0.063
Number of tender joints	18.7 ± 6.3	14.7 ± 4.5	NS
Number of swollen joints	17.7 ± 4.0	13.6 ± 3.7	0.046
PGADA* - VAS (mm)	74 ± 21	52 ± 19	0.025
DAS 28	7.37 ± 0.83	6.42 ± 0.65	0.033

*PGADA: Patient Global Assessment of Disease Activity (PGADA).

**Fig. 1.** NAG-enzymuria in patients with RA and serum creatinine exceeded 1.3 mg/dL in the course of treatment with infliximab.

ment (13.5 UI/g vs 18.2 UI/g), which did not differ from patients without amyloid deposits (7.4 UI/g vs 11.8 UI/g).

Discussion

In the controlled studies, infliximab administration has not been associated with undesirable renal signs (7). In recent

years, initial reports appeared on attempts to treat with blockers of TNF-glomerulonephritis (8), nephrotic syndrome (9), as well as of Wegener's granulomatosis with accompanying renal lesions (10). Infliximab contains a glycosylated immunoglobulin G1 structure, which undergoes degradation to

aminoacids. The study on the protein inhibiting effects of TNF- showed that after its intravenous administration, no alterations have been noted in the early indices of tubular injury, including NAG activity (11). The present study has shown also that directly after the infusion of infliximab no changes in urine NAG activity were observed.

Under physiological conditions, TNF- interacts with mesangial cells, affects their phagocytic capacity, and controls by these cells the degree of glomerular filtration (12). Suranyi *et al.* have found that TNF- may play a significant role in the development of proteinuria in nephrotic syndromes (13). Clinical progression and activity of the inflammatory process observed by histological examination remain more closely linked to intra-renal concentrations of TNF- than with its blood levels. In patients with proliferative glomerulonephritis a relationship has been demonstrated between urinary excretion of TNF- and proteinuria and the clinical activity of the disease (14). Administration of infliximab may immediately inhibit local activity of TNF- in the kidneys and induce the rapid cessation of proteinuria (15).

In our previous study of 43 patients with RA who started with low dose MTX, we showed that the use of this drug with or without NSAIDs did not influence renal tubular function. Before the administration of MTX NAG-enzymuria was increased in 72% of patients and the continuation of therapy resulted in a decrease in NAG-enzymuria and serum CRP(16).

In this study we observed that in patients treated with methotrexate and infliximab, NAG activity in the urine decreased during 54 weeks of therapy. The phenomenon may be linked to an alleviation of inflammation in RA patients caused by infliximab infusions and, in particular, by neutralization of TNF in the organism, including the kidneys. The disturbed functioning of the proximal tubules may be linked to glomerular filtration of pro-inflammatory cytokines, which has been found in patients after surgery (17). The blocking of these cytokines may decrease NAG activity, as was confirmed by our study. In this

study a significant relationship was noted between the increased NAG activity in the urine during the course of treatment and indices of active RA, such as the PGADA, while the correlation with ESR values approached the level of significance. No significant effect of NSAIDs was observed, which confirms the results of our earlier observations (16,18). The results presented here indicate that in a significant number of cases impaired functioning of the proximal tubule may be related to the inflammatory process itself during the course of RA.

In patients with secondary amyloidosis in the course of RA high levels of TNF have been noted, which correlated with the presence of nephrotic syndrome (19). Infliximab may decrease the levels of amyloid A (SAA) in the serum and inhibit the continued deposition of amyloid. It may lead to decrease amyloidosis-induced proteinuria (15, 20). In the group of patients with confirmed amyloid deposits we did not find a significant decrease in creatinine concentrations during the course of infliximab treatment. On the other hand, renal function in this case did not markedly deteriorate.

Summing up the results of our observations, the introduction of infliximab during methotrexate therapy demonstrated no early or delayed nephrotoxicity in patients with rheumatoid arthritis.

References

1. SINGH R, CUCHACOVICH R, HUANG W, ESPINOZA LR: Infliximab treatment in a patient with rheumatoid arthritis on hemodialysis. *J Rheumatol* 2002; 29: 636-7.
2. YEE AM, POCHAPIN MB: Treatment of complicated sarcoidosis with infliximab anti-tumor necrosis factor-alpha therapy. *Ann Intern Med* 2001; 135: 27-31.
3. DIENER U, KNOLL E, LANGER B, RAUTENSTRAUCH H, RATGE D, WISSER H: Urinary excretion of NAG and alaninoaminopeptidase in patients receiving amikacin or cisplatin. *Clin Chim Acta* 1981; 112: 149-57.
4. WIELA-HOJEŃSKA A, ORZECZOWSKA-JUZWENKO K: N-acetyl- -D-glucosaminidase and its clinical significance (in Polish). *Pol Arch Med Wewn* 1999; 101: 359-66.
5. CHATTERJEE S, VELICER LL, SWEETLEYCC: Glycosphingolipid glycosyl hydrolases and glycosidases of synchronized human KB cells. *J Biol Chem* 1975; 250: 4972-9.
6. ZWIERZ K, GINDZIENSKI A, GLOWACKA D, POROWSKI T: The degradation of glycoconjugates in the human gastric mucous membrane. *Acta Med Acad Sci Hung* 1981; 38: 145-52.
7. LIPSKY PE, DESIREE MFM, VAN DER HEIJDE DM *et al.*: Infliximab and methotrexate in the treatment of rheumatoid arthritis. *N Engl J Med* 2000; 343: 1594-602.
8. DUCLOUX D, BRESSON-VAUTRIN C, CHALOPIN J: Use of pentoxifylline in membranous nephropathy. *Lancet* 2001; 357: 1672-3.
9. DREWE E, MCDERMOTT EM, POWELL RJ: Treatment of the nephrotic syndrome with etanercept in a patient with the tumour necrosis factor receptor-associated periodic syndrome (letter). *N Engl J Med* 2000; 343: 1044-5.
10. BOOTH AD, JEFFERSON HJ, AYLIFFE W, ANDREWS PA, JAYNE DR: Safety and efficacy of TNF blockade in relapsing vasculitis. *Ann Rheum Dis* 2002; 61: 559 (letter).
11. BENDELE A, SEELY J, RICHEY C, SENNELLO G, SHOPP G: Short communication: renal tubular vacuolation in animals treated with polyethylene-glycol-conjugated proteins. *Toxicol Sci* 1998; 42: 152-7.
12. NIEMIR ZI: What is the trigger of renal cytokine production in glomerular diseases? *Adv Clin Exp Med* 1998; 7: 55-6.
13. SURANYI MG, GUASCH A, HALL BM, MYERS BD: Elevated levels of tumor necrosis factor-alpha in the nephrotic syndrome in humans. *Am J Kidney Dis* 1993; 21: 251-9.
14. KACPRZYK F, CHRZANOWSKI W: Tumour necrosis factor (TNF) and interleukin-6 (IL-6) in glomerulonephritis (in Polish). *Pol Arch Med Wewn* 1996; 96: 224-33.
15. VERSCHUEREN P, LENSEN F, LERUT E, CLAES K, DE VOS R, VAN DAMME B, WESTHOVEN R: Benefit of anti-TNF- treatment for nephrotic syndrome in a patient with juvenile inflammatory bowel disease associated spondyloarthropathy complicated with amyloidosis and glomerulonephritis. *Ann Rheum Dis* 2003; 62: 368-9.
16. WILAND P, WIELA-HOJEŃSKA A, ŚWIERKOT J, HURKACZ M, ORZECZOWSKA-JUZWENKO K, SZECHINSKI J: Renal tubular dysfunction in patients with rheumatoid arthritis starting with low dose methotrexate (in Polish). *Pol Arch Med Wewn* 2003; 110: 855-62.
17. GORMLEY SM, MCBRIDE WT, ARMSTRONG MA *et al.*: Plasma and urinary cytokine homeostasis and renal dysfunction during cardiac surgery. *Anesthesiology* 2000; 93: 1210-6.
18. WILAND P, ŚWIERKOT J, SZECHINSKI J: N-acetyl- -D-glucosaminidase urinary excretion as an early indicator of kidney dysfunction in rheumatoid arthritis patients on low-dose methotrexate treatment. *Br J Rheumatol* 1997; 36: 59-63.
19. TSYBUL'KOV SV, BARANOV AA, BORODIN AG, KORSHUNOV NI, KIRDIANOV SI: Tumour necrosis factor-alpha and kidney damage in rheumatoid arthritis. *Ter Arkh* 2001; 73: 8-11.
20. ELKAYAM O, HAWKINS PN, LACHMANN H, YARON M, CAPSI D: Rapid and complete resolution of proteinuria due to renal amyloidosis in a patient with rheumatoid arthritis treated with infliximab. *Arthritis Rheum* 2002; 46: 2571-3.