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# Biomarkers in Follow-up of Post – transplanted Patients

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***Abstract:*** ***Background:*** *Acute graft rejection (AGR), acute tubular necrosis (ATN) and chronic allograft dysfunction (CAD) are the most common causes of renal transplant failure. The early detection of renal transplant failure using reliable and noninvasive biomarkers is crucial for the adequate treatment and outcome.* ***Material and Methods:*** *In order to find out noninvasive markers of AGR or other early post transplantation complications and potential indicators for the development of CAD, urinary excretions of enzymes NAG, AAP, γ-GT; LMW proteins: $\alpha_1$M, $\beta_2$M; serum/urine creatinine and serum Cystatin C were measured in 46 renal graft recipients within the first post transplantation month, and excretion of the same biomarkers in 29 graft patients six months after transplantation. Study group was divided in four groups: healthy individuals who served as control group (30), patients with stable graft function (21), acute renal injury(15) and acute tubular necrosis(10). The proximal tubular enzymes N-Acetyl-D-glucosaminidase (NAG), alanine aminopeptidase (AAP) and γ- glutamyl transferase (γGT) were measured with standardized kinetic method and glutathion transferase ( α/π form) was measured with the Biotrin test. LMW proteins - $\alpha_1$ microglobulin, $\beta_2$ microglobulin in urine and serum Cystatin C were determined with DAKO test. Serum and urine creatinine were analyzed with kinetic Jaffe method. Daily enzymuria, LMW proteins, serum Cystatin C and creatinine were measured in all consecutive renal allograft recipients for first 21 postoperative days. Graft dysfunction was defined as >20% increase in serum creatinine and >100% increase in enzymuria over the baseline.* ***Results:*** *During the first post transplantation month 15 patients had AGR, whereas 21 patients did not show any signs of rejection. In patients with AGR, urinary α1M/creatinine ratios increased a few days before rejection, whereas in patients with stable graft function urinary α1M/creatinine ratio decreased and during the follow-up period. Excretion of NAG was significantly higher in patients with AGR ($p<0.001$) and ATN ($p<0.01$) compared to patients with stable graft function (SGF). Highly increased π-GST excretion ($p< 0.001$) was noted in AGR patients compared to ATN and SGF. Acute tubular necrosis resulted in rapid elevation of urinary levels of both α –and π-transferase..* ***Concussion:*** *Enzymuria assay is a simple, reliable and noninvasive method for characterization of acute tubular necrosis and acute rejection in renal allograft recipients. Cystatin C has been described as an ideal GFR marker and has been reported to be at least as accurate as the commonly used serum creatinine to detect impaired renal function in various patients groups, including renal transplant patients.*

***Keywords:*** *Acute graft rejection, acute tubular necrosis, NAG, AAP,γ-GT, β2M, α1M/creatinin,α/πGST, renal transplantation*

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## I. Introduction

Renal function in the early post-transplantation period depends largely on factors affecting the kidney prior to implantation. Function of the graft may be also disturbed by the most common complications of the early post-operative period such as acute graft rejection (AGR), acute tubular necrosis (ATN) and may be modified by nephrotoxic action of cyclosporine A (CsA). The transplanted kidney is exposed to different forms of injury which result in tissue damage. Acute graft rejection (AGR) is defined as an immune response against donor antigens (1). It induces and mediates functional and structural deterioration of the graft and is an independent non-immunologic cause of renal dysfunction. The recent immunosuppressive regimens have progressively reduced the incidence of clinical acute rejections (First, 2003). It has been shown that the occurrence of AGR episodes associates with the development of chronic allograft dysfunction (CAD). All grafts that develop CAD have undergone previous tissue injury. Recent findings suggest that continuing or repetitive tissue injuries or impaired repair from the injury result in increased renal fibrosis and in the development of CAD (Sundberg et al., 1994). After renal transplantation, defects in the proximal renal tubules are the earliest

and a constant manifestation of acute graft rejection. The early detection of injuries and the follow-up of tissue response and recovery from injury is crucial for the adequate treatment and outcome. Many tubular enzymes have been studied as markers of the necrotic/apoptotic damage or dysfunction of proximal tubular cells (Donadio et al., 1996; Thompson et al., 1977). Three major origins have been identified: the lysosomes, the brush-border membrane, and the cytoplasm of the cells. Several studies have demonstrated that the cells of the proximal tubuli are extremely sensitive to the immunological and toxic processes, and that the release of enzymes or proteins such as alanin aminopeptidase, γ-glutamyl-transpeptidase,brush border glycoprotein SGP-240, N-acetyl-β-D-glucosaminidase (NAG), glutathione-S-transferase (α and π-GST), and various proximal and distal tubular derived antigens can be used as an early noninvasive indicator of proximal tubular cell damage due to graft rejection (Geber et al., 1998; Kotanko et al., 1986; Tolko-Rubin et al., 1996). The increased urinary excretion of these markers often precedes clinical signs of AGR by several days. Evaluation of excretion of enzymes and low molecular weight proteins (LMWP) has been proposed as a potential tool in making the differential diagnosis of the most common complications in the early postoperative period following acadaveric kidney transplantation such as AGR and ATN. Several studies have demonstrated that increased urinary amounts of enzymes are useful in detection of acute tubular damage at very early stage, but increased enzymuria may also be induced by a reversible mild dysfunction of the cells not necessarily associated with irreversible damage. Enzymuria may reflect mild injury or injury that is reversible, although Jung et al. (Jung et al., 1986) found that elevated initial levels of urinary alkaline phosphatase (AP) and *N*-acetyl-β-d-glucosaminidase (NAG) were associated with mortality in their population of patients with AGR. Previous studies have demonstrated that tubular enzymuria can detect tubular injury 12 h to 4 days earlier than standard parameters of renal function (Conti et al., 2005). Estimation of excretion of isoenzymes characteristic to different tubule segments: proximal (α-GST), and distal (π-GST) tubule, may allow the differentiation of the etiology of complications, which damage the tubule by divergent pathogenic mechanisms (Herger-Rosenthal et al., 2004). Higher excretion of π-GST, an isoenzyme localized and released from various nephron segments, in particular the distal tubule, can be a sign of widespread renal damage, which is distinctive for an AGR. Tissue localization of this isoenzyme may explain higher excretion in AGR than in ATN patients, where ischemic injury focuses mostly on the proximal tubule . Significantly raised π-GST excretion in AGR group compared to SGF and ATN recipients suggests the usefulness of its measurement for differentiation of AGR from other post-transplant renal disorders. However, enzymes are also released during chronic glomerular diseases, which might limit their use as markers of tubular injury only (Campbell et al., 1996; Crespo et al., 2001). Enzymuria seemed highly sensitive for renal tubular injury and directly correlates with elevated serum levels of cretonne, Cystatin C (Bornstein et al., 1996; Harrison et al., 1998).The low-molecular weight proteins that escape complete reabsorption when the proximal tubular cells are overloaded or damaged have been used as markers of damage or dysfunction of these cells. Some of the best-characterized tubular proteins for detection of proximal tubular injury are $\alpha_1$- and $\beta_2$-microglobulin, retinol-binding protein, and Cystatin C. Produced at different sites, they are freely filtered by the glomerulus and reabsorbed but not secreted by proximal tubular cells. At least one study showed that tubular proteinuria seems to be superior compared to enzymuria in predicting the need for renal replacement therapy in AGR. Urinary excretions of LMW-proteins usually increase long before the elevation of other markers of AGR, such as rise of serum creatinine or general proteinuria (Vincent et al., 1979; Bentham et al., 1993). As many small molecular weight proteins such as α1- and β2-microglobulins and cystatin C are excreted almost exclusively through glomerular filtration, their serum concentrations can be used as marker of GFR (Steinhoff @ Sack, 1993). By using SDS-polyacrylamide electrophoresis, Bosket et al. (1974) demonstrated LMW-proteinuria in 86% of patients with AGR, but in none of the patients with uncomplicated post transplantation course. Serum β2-microglobulin (β2M) has been extensively measured in renal transplanted patients (Wibell et al., 1997; Shea et al., 1981). High pretransplantation serum levels of β2M have been reported to decline toward normal at varying rates after successful renal transplantation (Bernier @ Post, 1973; Edwards et al., 1983). High concentrations of urinary β2M have been reported during or even several days before clinical signs of acute rejection (Hemminge et al., 1978; Pistil et al., 1994; Maury @ Tempo,1984), whereas a continuous decline of urinary β2M was found in the recipients with uncomplicated post transplant outcome (Maury @ Tempo,1984). Constantly high or increasing serum β2M have shown to predict clinical rejection by several days (Beckman et al., 1986). The increase of serum β2M is not, however, specific for acute rejection but may be associated with cyclosporine toxicity, bacterial, viral and fungal infections (Donaldson et al., 1996) and with the deterioration of GFR due to other causes (Uhland et al., 1996; Leach et al., 1992). Beckman et al. (1986) reported that the serum β2M is unable to distinguish between AGR and cyclosporine toxicity, but suggests that is helpful in the early detection of CMV infection. Measurement of urinary α1M has many advantages compared to that of β2M. α1M is stable in urine and unlike β2M, its urinary concentration is not affected by serum paraproteins or by CMV-infection (Cont et al., 1995; Uchida @ Goths, 2002). Cystatin C is a 13 KD molecular weight, non-glycosylated protein that is produced by all nucleated cells and eliminated from blood by glomerular filtration. Its production rate is constant and it is unaffected by inflammation, abnormal tissue growth, muscle mass, sex, age, diet or nutritional

status (Le Brice et al., 2002; Rich et al., 2001). A single reference value can be used for children and adults of both sexes. A single measurement of serum cystatin C provides a precise and accurate estimation of GFR (Larsson et al.2004). During the first four post transplant days, cystatin C decreases more rapidly than serum creatinine. In the immediate post transplant period, cystatin C may thus detect graft dysfunction and its recovery more rapidly than serum creatinine (Nishi et al., 1992; Lijkenboom et al., 2001). Glucocorticoids increase the production of cystatin C and glucocorticoid medication should therefore be taken into account when interpreting serum cystatin levels in renal transplant patients (Bold et al., 2003; Cresses et al., 2002).

The aim of this study was to assay a broad number of markers of tubular injury in the early post-renal transplant period, and then to select a set which would enable to classify the etiology of this damage. The search for complication-specific excretion marker was justified by dissimilarity of path mechanisms of tubular lesions in each of the afore mentioned complications.

## II. Material And Methods

Study was performed on 46 cadaveric renal allograft recipients and 30 healthy individuals who served as controls. Post-transplant monitoring included determination of urinary enzymes, LMW proteins, serum creatinine and Cystatin C, urine analysis and urine volume. Patients were classified into one of three groups: stable graft function (SGF, n=21)—the patients with satisfactory and SGF during the period of initial 30 days after kidney transplantation, those with acute graft rejection (AGR, n=15) and those with acute tubular necrosis (ATN, n=10). Inclusion criteria into the AGR group involved the clinical symptoms of AGR confirmed by graft biopsy. Patients requiring at least one dialysis session in the first week after transplantation and without other reason for delayed graft function were included in the ATN group. Demographic and biochemical characteristics of patients enrolled in the study are presented in Table 1. Measurements of urine enzymes and LMWP were performed every day during the first 21 days after kidney transplantation. Measurements were done in the second morning urine samples of the day several times in each patient. At least two measurements were performed during initial twenty one days after renal transplantation-usually on day 1,2,3,7,11,14,17 and day 21. Elements of urinary sediment were removed by centrifugation at 3,000 rpm for 15 min. Serum Cystatin C and $\beta_2$Microglobulin ($\beta_2$M) and urinary $\alpha_1$Microglobulin ($\alpha_1$M) were determined with DAKO test. N-acetyl-β-D-glucosaminidase concentration in urine was measured by fluorimetric method. Concentration of NAG was measured with the 4-methyl-umbelliferyl-2-acetamido-2-deoxy-β-D-glucopyranoside substrate, and the 4-methylumbelliferone (4MU) product was determined in alkaline pH. Alanynaminopeptidase activity was determined calorimetrically with L-alanyl-β-naphtylamide, and released β-naphthylamide was bound to p-dimethylaminobenzaldehyde [12]. γ-Glutamyltransferase activity was determined calorimetrically with γ-glutamyl-p-nitroanilide using the Roche diagnostic test. The urinary enzyme activity was expressed in U/mmol creatinine. The concentration of α-GST and π-GST were quantified by the Biotin test using enzyme immunoassay technique. Serum and urine creatinine was measured by colorimetric techniques based on Jaffe reaction. Mean values and standard deviations were calculated and differences between groups were assessed by Student's t test or analysis of variance (ANOVA). Pearson's coefficients were used to calculate correlations between variables. Differences were classified as significant at $P<0.05$. All calculations were performed with Statistics 5.0 software package.

## III. Result

During the follow-up period (mean 3 post transplantation weeks), 16 patients ( 34.7 %) (9 men and 7 women) had AGR episode whereas 21( 45.6 %) patients (14 men and 7 women) did not show any signs of rejection. In ten patients we confirmed acute tubular necrosis initially requiring dialysis. Rejection occurred on days 3 to 21 (mean 12.0) after transplantation. The clinical characteristics of evaluated patients were shows in table 1.

**Table1. Clinical characteristics of the evaluated patients**

| Groups | | Controls (N=30) | SGF (N=21) | AGR (N=16) | ATN (N=10) |
|---|---|---|---|---|---|
| Sex | F<br>M | 12<br>18 | 7<br>14 | 4<br>12 | 1<br>9 |
| Age(years) | | 37.5+/-4.3 | 32.6+/-9.3 | 36.4+/-6.5 | 31.2+/-5.2 |
| Serum creatinine μmol/L | | 82+/-24 | 180+/-36* | 246+/-58* | 292+/-76* |
| Serum Cystatin C mg/L | | 0.95+/-0.45 | 1.85+/-0.75 | 3.26+/-1.15* | 2.2+/-0.95** |
| Proteinuria g/L | | 0.085+/-0.012 | 0.219+/-0.085 | 0.426+/-0.175* | 0.275+/-0.112** |

Data are expressed as the mean ± SD ; *****$p<0.001$;**$p<0.01$

The mean pre transplant urinary excretion levels of the enzymes NAG, AAP and γGT in the 46 patients with chronic renal failure during the present study are shown in table 2. The levels were found to be higher by 9.0 fold for NAG, 4.0 fold for AAP and 2.5 fold for γGT, as compared to the mean excretion levels of the corresponding enzymes in 30 healthy volunteers .

**Table2.** The mean excretion levels of urinary enzymes and LMW proteins in pre transplant patients with chronic renal failure compared with values of controls

| | Controls | Pretransplanted patients |
|---|---|---|
| NAG U/mmol creatinine | 0.7+/- 0.45 | 6.6+/-3.1* |
| AAP U/mmol creatinine | 0.5+/-0.25 | 2.3+/-1.6* |
| γGT U/mmol creatinine | 1.35+/-0.55 | 3.75+/-2.05* |
| α1M/creatinine ratio mg/mmol | 0.32 ±0.12 | 18.3 ± 5.6* |
| serum β2M mg/L | 1.7+/-0.98 | 16.8+/-12.3* |

Data are expressed as the mean ± SD *p <0.001

We selected a group of patients with acute graft rejection (n= 16) and compared their results with another group of patients (ATN) requiring dialysis directly after transplantation (n=10).The GS transferases were measured in urine collected during the 1st day after transplantation or as soon as urine was produced (Table 3, figure 1). In the group with AGR significantly higher levels of π-GST( p<0.001) and α-GST(p<0.05) were observed compared to the SGF group. When the patients with ATN were compared with those with AGR, the increase was found to be 11.8-fold for urinary π-GST and 3.3- fold for α-GST, respectively.

**Table3. Levels of α-GST and π-GST in the urine**

| Groups / Biomarkers | Controls (N=30) | SGF (N=21) | AGR (N=16) | ATN, (N=10) |
|---|---|---|---|---|
| α-GST ng/ml | 2.5+/-1.3 | 4.6+/-0.8 | 6.1+/- 2.9 | 17.2 +/- 13.5* |
| π-GST ng/ml | 13.4+/-5.3 | 15.1+/-1.3 | 24.3 +/- 6.8* | 18.4 +/- 8.9* |

Data are expressed as the mean ± SD; *p< 0.001.

Excretion of most of the markers of tubular function was significantly higher in patients than in controls (Table 4).The levels of NAG was significantly higher in patients with AGR (p<0.001) and ATN (p <0.01) than in SGF recipients. Significant differences (p<0.05) were found in excretion of AAP, γ-GT and β2M between SGF and those with AGR and ATN. Averagely one week after transplantation, the mean α1M/creatinine ratio of transplanted patients was over 57 times higher than that of the controls (18.3 ± 5.6 vs. 0.32 ± 0.12 mg/mmol), and the urinary excretion of α1M was associated with cold ischemia time (r=0.347,p< 0.001). At that time, no significant difference in the urinary excretion of α1M/creatinine was noticed between patients who developed AGR and those with SGF (19.6+/-7.8 mg/mmol vs. 15.3+/-6.1 mg/mmol, NS).

**Table 4.Urinary excretion of biomarkers in the evaluated groups in the first 21 days**

| Groups / Biomarkers | Controls (N=30) | SGF (N=21) | AGR (N=16) | ATN (N=10) |
|---|---|---|---|---|
| NAG U/mmol creatinine | 0.7+/- 0.45 | 4.9+/-2.3 | 14.4+/-3.9* | 12.3+/-4.2* |
| AAP U/mmol creatinine | 0.5+/-0.25 | 1.8+/-0.7 | 2.8+/-1.05** | 2.1+/-1.3 |
| γGT U/mmol creatinine | 1.35+/-0.55 | 1.45+/-0.68 | 2.95+/-0.92** | 2.7+/-1.1 |
| α1M/creatinine ratio mg/mmol | 0.32 ±0.12 | 15.3+/-6.1* | 21.6+/-7.8* | 15.8+/-8.6* |
| Serum β2M mg/L | 1.4+/-0.9 | 13.0+/-11.6* | 15.6+/-12.9* | 30.8+/-14.2* |

Data are expressed as the mean ± SD;*p<0.001;**p <0.05

**Table5.** Urinary α1M/creatinine ratios in patients with and without AGR at various days after renal transplantation.

| | Days after transplantation | alfa1M/creatinine ratio (mg/mmol) mean(range) |
|---|---|---|
| Acute graft rejection n= 16 | 7 ( 3 – 11) | **17 (2.5 – 31)** |
| | 14 (12 – 20) | **20 (6.0 – 35)** |
| | 21(17 – 31) | **28 (9.0 – 47)** |
| Stable Graft function n=21 | 7 ( 5 – 10) | 16 (3.0 – 27) |
| | 14 (11 – 21) | 15 (2.0 – 18) |
| | 21 (15 – 23) | 11 (2.0 – 16) |

During the second postransplantation week, α1M/creatinine ratio decreased in 14 of 21 patients (66.6 %) with stable graft function, but only in 3 of 15 (20 %) of those with acute rejection ($p<0.01$). During the follow-up period (mean 3 post transplantation weeks), 16 patients (32.6%) had AGR episode whereas 21 patients did not show any signs of rejection. Rejection occurred on days 5 to 25 (mean 15.0) after transplantation.

On the other hand, in patients with AGR the α1M/creatinine ratio between first and second urine samples increased in 20 of 27 patients (74.1%). Averagely two weeks after transplantation, the mean α1M/creatinine ratio was significantly higher in patients who developed AGR than in those who did not (Table 3). After the first post transplantation week, the decreasing α1M/creatinine ratio had 95% positive predictive value in detecting non rejecting patients, and the increasing ratio had 71% predictive value in detecting rejecting patients. At 6 months from transplantation, the mean α1M/creatinine ratio was more than two times higher in the AGR than in the SGF (mean ± SD, 4.2 ± 2.2 vs.1.3 ± 1.1, $p< 0.01$).

## IV. Discussion

Acute graft rejection is defined as an immune response against donor antigens. It induces and mediates functional and structural deterioration of the graft and is independent of non-immunologic causes of renal dysfunction. It usually occurs within weeks to months of transplantation. The recent immunosuppressive regimens have progressively reduced the incidence of clinical acute rejections (First, 2003). Still, at least one episode of clinical and/or histological acute rejection occurs in 40-50% of cadaver kidney allograft recipients (Crespo, 2003; Gaber, 1998). More than half of the rejection episodes occur within the first 30 days, and almost 90% occur in the first 3-6 months post transplant, but they may occur much later (Gaber, 1998). Acute rejection episodes have been documented as late as in the third decade after transplantation. The interpretation of the results of the function of a transplanted cadaveric kidney requires consideration of its initial state, which is affected by case-specific factors in the donor terminal period, the ischemic reperfusion damage, factors related to the surgery, and post-operative period, mainly immunosuppressant. Concurrent influence of these factors results in elevation of all biomarkers of proximal tubule injury in the early post-transplant period, reflecting its extreme sensitivity to ischemia and various nephrotoxic factors (Price, 1982). Acute graft rejection, ATN, and nephrotoxicity to CsA are the most common complications resulting from these factors. Making a differentiation between these three etiologies is crucial for the achievement of the long-term graft success. As AGR, ATN and CsA have dissimilar pathogeneses; one could expect that profiles of enzymes excreted in these entities will be dissimilar. Among the multitude of enzymes excreted in urine, lysosomal NAG enzyme and enzymes of brush border of epithelial cells of proximal tubule-AAP and GGP have proved to be the most useful. The mean levels of pre-transplant enzymuria from the 47 patients with chronic renal failure was significantly higher ($p<0.001$) compared to 30 healthy volunteers. Our data is in agreement with previous reports of increased urinary excretion of the brush border enzymes in patients with interstitial nephritis, glomerulonephritis and diabetic nephropathy (Tempo, 2005; Burke et al., 1995). The levels were found to be higher by 9.0 fold for NAG, 4.0 fold for AAP and 2.5 fold for γGT. Conflicting reports are present regarding the relative occurrence of enzymuria during the episodes of graft dysfunction in renal transplant recipients). Bornstein et al. (1996) reported that enzymuria increases concurrently or after the serum creatinine is increased in response to graft dysfunction, while some others reported that it precede an increase(ment) of serum creatinine (Polak et al., 1999; Beckman, 1998). The important findings of our investigation are that the increase in excretion of NAG preceded the serum creatinine in all 21 episodes of graft dysfunction (AGR, ATN), whereas the γGT and AAP preceded it in 15 and 11episodes**.** In our study the NAG excretion was significantly higher in AGR and ATN groups compared to SGF patients; the highest NAG excretion was observed in the AGR group. The difference between AGR and ATN group was not statistically significant. These results are also supported by earlier reports, which suggest that excretion of NAG fails to identify a specific cause of increased enzymuria in patients after renal transplantation (Bourbouze et al., 1985; Sandburg et al., 1994). Independently of the elevated NAG excretion, increased excretion of brush border enzymes-AAP and GGT were observed in AGR and ATN groups in the early post-transplant period. Lack of significant difference in excretion of these enzymes did not allow

characterization of patients with ATN, AGR from those with SGF. This may be a result of several injurious processes that affect tubules at that time. Estimation of excretion of isoenzymes characteristic to different tubule segments: proximal (α-GST), and distal (π-GST) tubule, may allow the differentiation of the etiology of complications, which damage the tubule by divergent pathogenic mechanisms (Beckman et al., 1995; Donadio et al., 1989). Higher excretion of π-GST, an isoenzyme localized and released from various nephron segments, in particular the distal tubule, can be a sign of widespread renal damage, which is distinctive for an AGR. Tissue localization of this isoenzyme may explain higher excretion in AGR than in ATN patients, where ischemic injury focuses mostly on proximal tubule (51). Significantly raised π-GST excretion in AGR group compared to SGF and ATN recipients suggests the usefulness of its measurement for differentiation of AGR from other post-transplant renal disorders (Polak et al., 1995).The highest levels of alfa GST were found in patients with a well-presented renal function (SGF), whereas the highest levels of π GST were found in patients with acute renal failure. As compared with αGST, the excretion of πGST correlated with the excretion of NAG ($r=0.62$, $p<0.001$) and beta 2 microglobulin ( $r=0.48$, $p< 0.001$). A significant negative correlation was found between the urinary excretion of π GST and creatinine clearance ($p=-0.24$, $p<0.001$). Our observation may also explain high π-GST excretion has been described in the first post transplant day in patients with ATN. Impaired reabsorbtive and catabolic functions of proximal tubule in the early post transplant period is a consequence of various injuries of this nephron segment that is vulnerable to ischemia and toxicity. The damage is evidenced by the greater excretion of LMWP–alfa1M and b2M in comparison to controls. This observation is supported by reports of elevated LMWP excretion even in patients with normal renal graft function (Marchewka et al., 1999). Our presented study shows that averagely one week after transplantation the mean α1M/creatinine ratio in transplant recipients was about 57-fold higher than that of the healthy subjects. At that time the urinary excretion of α1M correlated with the duration of cold ischemia and was suggested to reflect ischemia induced injury. The mean α1M/creatinine ratios were about the same in patients with stable graft function and in those who later developed rejection. In patients with uncomplicated post transplantation course the ratio decreases. The decrease of α1M/creatinine ratio in consecutively collected urine samples thus indicates recovering tubular reabsorption capacity and rules out complications. The results shows that the α1M/creatinine ratio during the first post transplantation week serves as the measure of ischemia- induced injury, there after the decrease of α1M/creatinine ratio in consecutively collected urine samples indicates recovering tubular function and rules out rejection, whereas the increase of α1M/creatinine ratio signals acute rejection or other complications. However, at 6 months from transplantation, the mean urinary α1M/creatinine ratio of all transplanted patients was still 8 times higher than that of the healthy subjects. Same results were found by Tempo (2005) where alfa 1M/creatinin ratio was 20 time higher compared to healthy patients. These findings suggest the occurrence of persistent or prolonged injury in the majority of patients. Beta2M excretion is particularly difficult to interpret without concomitant estimation of its blood levels, due to its increased excretion in transplant patients (55). High pre transplantation serum levels of β2M have been reported to decline toward normal at varying rates after successful renal transplantation. Constantly high or increasing serum β2M levels have shown to predict clinical rejection by several days. The increase of serum β2M is not, however, specific for acute rejection but may be associated with cyclosporine toxicity (Pistil, 1989), bacterial, viral and fungal infections (Edwards et al., 1983; Pistil, 1989) and with the deterioration of GFR due to other causes (Maury @ Tempo,1984).The normal concentration of cystitis C is low and independent of age and body mass. In renal tubular disorders, it was demonstrated that urinary cystitis C concentrations increased approximately 200-fold (Leach et al., 2002). In the absence of circadian variations, cystitis C can be quantified in a single urine sample, and results may be confirmed within 1 h (Le Brice et al., 1999).Our results showed than during the first four post transplant days, cystitis C decreases more rapidly than serum creatinine. In the immediate post transplant period, cystitis C may thus detect graft dysfunction and its recovery more rapidly than serum creatinine.

## V. Conclusion

In conclusion, non-invasive measurements of urinary tubular biomarkers can provide information of the microenvironment of the allograft in transplant recipients. Enzymuria assay is simple, reliable, noninvasive method which can be used to distinguish acute tubular necrosis and acute rejection in renal allograft recipients. High NAG values are present in both ATN and AGR patients. Evaluating urinary excretion of π-GST may be helpful in differentiating AGR from ATN. The decrease of α1M/creatinine ratio in consecutively collected urine samples indicates recovering tubular function and rules out rejection, whereas the increase of this ratio indicates acute rejection or other complications. Increased urinary excretion of α1M associates with progressive deterioration of graft function and serves as an early and sensitive indicator of poor graft outcome.

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