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Prognostic Value of Tubular Proteinuria and Enzymuria in Nonoliguric Acute Tubular Necrosis

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Background: **Acute tubular necrosis (ATN) has high mortality, especially in patients who require renal replacement therapy (RRT). We prospectively studied the diagnostic accuracy of the urinary excretion of low-molecular-weight proteins and enzymes as predictors of a need for RRT in ATN.**

Methods: **In 73 consecutive patients with initially nonoliguric ATN, we measured urinary excretion of α_1- and β_2-microglobulin, cystatin C, retinol-binding protein, α-glutathione *S*-transferase, γ-glutamyltransferase, lactate dehydrogenase, and *N*-acetyl-β-D-glucosaminidase early in the course of ATN.**

Results: **Twenty-six patients (36%) required RRT a median of 4 (interquartile range, 2–6) days after detection of proteinuria and enzymuria. Patients who required RRT had higher urinary cystatin C and α_1-microglobulin [median (interquartile range), 1.7 (1.2–4.1) and 34.5 (26.6–45.1) g/mol of creatinine] than patients who did not require RRT [0.1 (0.02–0.5) and 8.0 (5.0–17.5) g/mol of creatinine]. Urinary excretion of cystatin C and α_1-microglobulin had the highest diagnostic accuracies in identifying patients requiring RRT as indicated by the largest areas under the ROC curves: 0.92 (95% confidence interval, 0.86–0.96) and 0.86 (0.78–0.92), respectively. Sensitivity and specificity were 92% (95% confidence interval, 83–96%) and 83% (73–90%), respectively, for urinary cystatin C >1 g/mol of creatinine, and 88% (78–93%) and 81% (70–88%) for urinary α_1-microglobulin >20 g/mol of creatinine.**

Conclusion: **In nonoliguric ATN, increased urinary excretion of cystatin C and α_1-microglobulin may predict an unfavorable outcome, as reflected by the requirement for RRT.**

Acute renal failure (ARF)[4] is common in hospitalized patients, with a mortality rate of 30–90% *(1–5)*. The predominant cause of ARF is acute tubular necrosis (ATN) *(4, 6)*. Patients with ATN who require renal replacement therapy (RRT) have a markedly higher mortality rate than patients not requiring RRT *(5, 7)*. In the absence of effective therapies for ATN, early indices to predict the severity of ATN are important to prevent progression from nonoliguric to oliguric ATN and to improve its management and outcome *(6, 8, 9)*. Several severity indices have been developed for ATN, but their predominant purpose is to predict ATN-associated mortality *(4, 8, 10–15)*. However, to date markers to predict the requirement for RRT, a clinically relevant indicator of unfavorable outcome in ATN, are lacking *(16, 17)*. Initial proximal tubular injury is characteristic of ATN, in contrast to, e.g., prerenal or postrenal causes of ARF *(18)*. Increased urinary excretion of low-molecular-weight proteins (tubular proteins) and enzymes has been shown to indicate proximal tubular injury. Some of the best characterized tubular proteins and enzymes to detect proximal tubular injury are α_1- and β_2-microglobulin, cystatin C, retinol-binding protein *(19–23)*, α-glutathione *S*-transferase (GST), γ-glutamyltransferase (GGT), lactate dehydrogenase (LD), and *N*-acetyl-β-D-glucosaminidase (NAG) *(19, 24–28)*. The objective of this study was to prospectively test the diagnostic accuracy of the urinary excretion of these tubular proteins and enzymes to predict the requirement for RRT at an early time point in ATN.

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[4] Nonstandard abbreviations: ARF, acute renal failure; ATN, acute tubular necrosis; RRT, renal replacement therapy; GST, glutathione *S*-transferase; GGT, γ-glutamyltransferase; LD, lactate dehydrogenase; NAG, *N*-acetyl-β-D-glucosaminidase; AUC, area under the curve; and CI, confidence interval.

Patients and Methods

All consecutive patients at the University Hospital Essen with potential ARF who reported to the Department of Nephrology from August 2000 to June 2001 were considered for this prospective study (n = 164; Fig. 1, step 1). We further evaluated 103 patients with confirmed ARF for enrollment (Fig. 1, step 2). These had nonoliguric ARF, defined as a doubling of baseline serum creatinine concentration from <106 μmol/L to at least 115 μmol/L, measured at least twice, and a urine output >500 mL/day in the 48 h between steps 1 and 2. We excluded 61 patients (Fig. 1, step 2) for the following reasons: (*a*) patients requiring RRT within the 48 h between steps 1 and 2 because of overt pulmonary edema, acute respiratory distress syndrome, or acute cardiac failure, because no clinically meaningful prediction of RRT requirement was possible in these patients; (*b*) patients with pre- and postrenal ARF because this is not caused primarily by proximal tubular injury; and (*c*) patients with preexisting renal parenchymal disease or chronic renal failure, renal surgery, renal transplantation, renal trauma, or renal surgery as determined from the history or clinical, laboratory, and radiologic criteria, because the latter conditions may be associated with increased urinary excretion of tubular proteins and enzymes unrelated to ATN. Prerenal ARF was defined by a fractionated sodium excretion <1% *(29–31)*, fractionated urea nitrogen excretion <35% *(31)*, blood urea nitrogen-to-serum creatinine ratio >15 *(6, 31)*, and urine osmolality >300 mosmol/kg of H_2O *(6)*. Postrenal ARF was diagnosed by typical morphologic changes visualized by ultrasound. The median time from initial report to inclusion and to the collection of urine samples was 2 days (interquartile range, 2–4 days).

On the day of inclusion, the 73 patients were enrolled who fulfilled the requirements for ATN, defined as at least five of the following nine criteria. We applied this series of criteria recently validated or established to indicate ATN because no single standard test exists to diagnose ATN (Fig. 1, step 3): (*a*) sepsis or systemic inflammatory response syndrome *(32)*; (*b*) arterial hypotension *(32)*; (*c*) intravenous administration of aminoglycosides, amphotericin B, or contrast media; (*d*) rhabdomyolysis, defined as serum myoglobin >10 mg/L; (*e*) fractionated sodium excretion >1% *(29–31)*; (*f*) fractionated urea nitrogen excretion >35% *(31)*; (*g*) blood urea nitrogen-to-serum creatinine ratio <15 *(6, 31)*; (*h*) urine osmolality <300 mosmol/kg of H_2O *(6)*; and (*i*) tubule cells or granular casts in the urine sediment *(33, 34)*.

On the day of inclusion, we collected untimed urine samples or samples from urine collection bags in the morning from each patient. All tubular proteins and enzymes were measured on the day of inclusion except for α-GST, which was measured later in samples stored at −20 °C after the addition of stabilization buffer. Serum and urine creatinine concentrations were determined by a modified Jaffe (alkaline picrate reaction) method with protein precipitation. Urinary concentrations of α_1- and β_2-microglobulin, cystatin C, and retinol-binding protein were measured by immunonephelometric methods (Dade Behring). As determined previously, the intra- and interassay imprecisions (CV) for the immunonephelometric measurement of urinary cystatin C were ≤4.8% and ≤5.2%, respectively, and the accuracy of the method was −0.8% ± 2.6% *(35)*. α-GST was measured by enzyme immunoassay (Biotrin), GGT was measured by a kinetic assay using L-γ-glutamyl-3-carboxyl-4-nitroanilide, and NAG by a kinetic assay using 3-cresolsulfonphthaleinyl-*N*-acetyl-β-D-glucosaminide as substrate (both from Roche). LD was measured by kinetic assay using pyruvate as substrate (Merck). Before measurement of GGT and LD, urine was subjected to gel filtration through columns prepacked with Sephadex G-25M (Pharmacia) to remove small molecular inhibitors *(36)*. We added 2.0 mL of urine to the column and eluted it with 3.5 mL of a solution containing 9 g/L sodium chloride. Results for GGT and

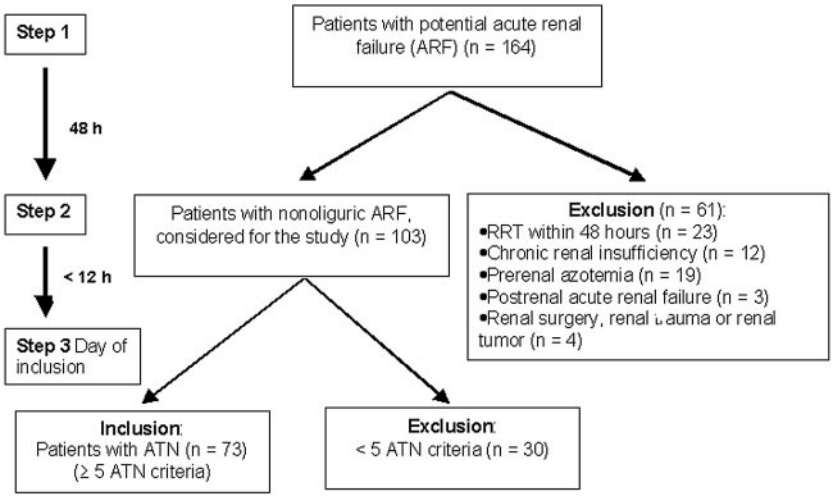

Fig. 1. Flow chart showing the study design with inclusion and exclusion of patients.

Numbers in *parentheses* represent the respective numbers of patients.

LD were multiplied by a dilution factor of 1.75. Urinary excretion rates for enzymes and proteins were normalized for millimoles of urinary creatinine to compensate for differences in urine flow rate, according to recent recommendations *(37)*. All measurements were performed by two experienced laboratory technicians, without knowledge of patient status regarding RRT. Clinical and laboratory data were extracted from records of the entire hospital stay. For comparison, the "Liano severity of ATN score" (Liano score) was recorded from clinical and laboratory data obtained on the day of inclusion by the following equation *(12)*: 0.032 (age in decades) − 0.086 (male) − 0.109 (nephrotoxic origin of ATN) + 0.109 (oliguria) + 0.116 (hypotension) + 0.122 (jaundice) + 0.15 (coma) − 0.154 (consciousness) + 0.182 (assisted ventilation) + 0.21.

RRT in the further course of ATN after the day of inclusion was always initiated on need, independently of the investigators, by a team of three nephrologists in our department for the following indications: pulmonary edema, oliguria or anuria for more than 48 h, metabolic acidosis or hyperkalemia not responding to conventional treatment, overt symptoms of uremia, and urea nitrogen >36 mmol/L. RRT was available 24 h a day, and no patient requiring RRT was denied dialysis for futility. In the further follow-up of the study, no nephrologist, other physician in charge, or laboratory technician had access to the collected data, including low-molecular-weight proteinuria, enzymuria, or the Liano score. No adverse events occurred as a result of testing for the purpose of classifying patients as having ATN or as a result of measurements for proteinuria or enzymuria. The study protocol was approved by the local Institutional Review Board and was in accordance with the Helsinki Declaration of 1975 as revised in 1996. Informed consent was obtained from all patients before enrollment.

STATISTICAL ANALYSIS

The primary endpoint was the requirement for RRT in the course of ATN from the day of inclusion to hospital discharge or death. Data are presented as median values with interquartile ranges in parentheses because data were not normally distributed. Continuous data were compared by the Mann–Whitney rank-sum test and categorical data by a two-tailed Fisher exact test. $P < 0.05$ was considered statistically significant. Data for proteinuria and enzymuria were available and completely analyzed in all patients studied. ROC curves were generated for sensitivity and specificity with the respective areas under the curves for α_1- and β_2-microglobulin, cystatin C, retinol-binding protein, α-GST, GGT, LD, NAG, and the Liano score. Cutoff values were derived from ROC curves by the Youden index, defined as sensitivity + specificity − 1, giving equal weight to sensitivity and specificity *(38)*. Analyses were performed with SAS, Ver. 8.0 (SAS Institute).

Results

Twenty-six of the patients with nonoliguric ATN on the day of inclusion required RRT, whereas 47 did not require RRT in the further course of ATN. Patients requiring RRT developed oliguria more often than patients not requiring RRT [23 (88%) vs 1 (2%); $P < 0.001$]. Patient characteristics and renal function at the time of inclusion as determined by serum creatinine and urinary flow rate did not differ significantly between the groups (Table 1). On the day of inclusion, median (interquartile range) fractional sodium excretion, fractional urea nitrogen excretion, blood urea nitrogen-to-creatinine ratio, and urine osmolality were 4.2 (1.9–8.5)%, 42.2 (36–51.7)%, 10 (7–14), and 229 (117–277) mosmol/kg of H_2O, respectively, for all ATN patients studied. ATN was caused in the majority of patients who required RRT by a combination of etiologies, whereas 86% of the patients who do not require RRT had a single etiology. In patients requiring RRT, the median time from inclusion to initiation of RRT was 4 (2–6) days. At the time of RRT initiation, median serum creatinine was 345 (301–433) μmol/L and urinary flow rate was 10 (4–17) mL/h. In patients not requiring RRT, serum creatinine was 221 (176–290) μmol/L and urinary flow rate 48 (34–75) mL/h 4 days after inclusion. Twenty-one patients requiring RRT received continuous veno-venous hemodialysis, and 5 received intermittent hemodialysis. Before ATN, 24 patients requiring RRT (92%) and 37 patients not requiring RRT (79%) were treated in intensive care units. During the course of ATN, all patients requiring RRT and 41 patients not requiring RRT (87%) were treated in intensive care units. Significantly more ATN patients requiring RRT died in the hospital than patients not requiring RRT [22 (85%) vs 6 (13%); $P < 0.001$]. The 22 ATN patients requiring RRT died a median of 12 (9–17) days after inclusion and 7 (3–12) days after RRT was initiated. The six ATN patients not requiring RRT died 15 (10–22) days after the day of inclusion. One of the four surviving patients who

Table 1. Characteristics of patients in this study.[a]

	RRT+ (n = 26)	RRT− (n = 47)
Age,[b] years	67 (50–73)	70 (57–74)
F/M, n	9/17	17/30
Etiology of ATN, n (%)		
Ischemia	3 (11%)	15 (32%)
Nephrotoxicity	2 (8%)	13 (28%)
Sepsis/SIRS[c]	7 (27%)	12 (26%)
Combination	14 (54%)	7 (14%)
S-Creatinine at inclusion,[b] μmol/L	194 (177–221)	159 (142–213)
Urine flow rate at inclusion,[b] mL/h	67 (54–100)	75 (58–113)
Time from inclusion to RRT,[b] days	4 (2–6)	NA

[a] RRT+, patients with ATN who required RRT; RRT−, patients with ATN who did not require RRT.
[b] Median (interquartile range).
[c] SIRS, systemic inflammatory response syndrome, NA, not applicable.

required RRT underwent hemodialysis after hospital discharge.

On the day of inclusion in the study, urinary excretion of the tubular proteins α_1-microglobulin, cystatin C, and retinol-binding protein and the enzymes α-GST, GGT, and NAG was significantly higher in the patients who required RRT in the further course of ATN than in patients who did not require RRT (Table 2). The differences between these two groups were not significant for β_2-microglobulin and LD. The ability of the ROC curves for tubular proteins to differentiate between ATN patients requiring and those not requiring RRT suggested good diagnostic performance for cystatin C and α_1-microglobulin, with areas under the ROC curves (AUC) of 0.92 [95% confidence interval (CI), 0.86–0.96] and 0.86 (0.78–0.92), respectively (Fig. 2). Retinol-binding protein showed intermediate (AUC, 0.80; 95% CI, 0.72–0.87) and β_2-microglobulin the lowest (0.51; 95% CI, 0.42–0.60) diagnostic value, as shown in Fig. 2. Of the enzymes studied, NAG had the best diagnostic performance for predicting the need for RRT in ATN, as demonstrated by the ROC curve (AUC, 0.81; 95% CI, 0.73–0.88). The AUCs for α-GST, GGT, and LD were lower and similar to each other: 0.64 (95% CI, 0.55–0.72), 0.64 (0.55–0.73), and 0.59 (0.50–0.69), respectively (Fig. 3). The Liano score demonstrated intermediate diagnostic value (AUC, 0.83; 95% CI, 0.75–0.90; Figs. 2 and 3).

From ROC curves, we derived the optimum cutoff values giving equal weight to sensitivity and specificity for the four markers with the highest AUC: cystatin C (1 g/mol of urinary creatinine), α_1-microglobulin (20 g/mol of urinary creatinine), NAG (4.5 U/mmol of urinary creatinine), and the Liano score (0.60). At these cutoff values, patients requiring or not requiring RRT were best differentiated by cystatin C (Fig. 4). Hence, cystatin C demonstrated the highest sensitivity (92%; 95% CI, 83–96%) and specificity (83%; 95% CI, 73–90%), and was followed by α_1-microglobulin [sensitivity, 88% (95% CI, 78–93%); specificity, 81% (70–88%)]. The sensitivities of NAG and the Liano score were lower: 85% (95% CI, 75–91%) and 77% (66–85%), respectively. Furthermore,

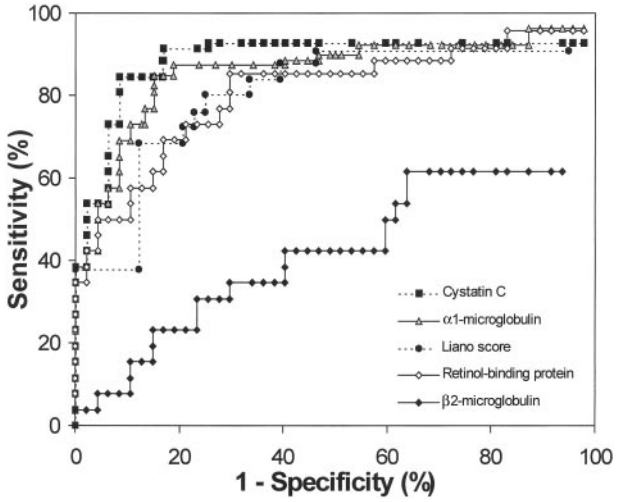

Fig. 2. ROC plot demonstrating the diagnostic performance of tubular proteinuria and the Liano score to predict the requirement for RRT in ATN.

both markers demonstrated lower specificities than cystatin C: 62% (95% CI, 50–72%) and 74% (63–83%), respectively. Combining the most sensitive and specific markers, cystatin C and α_1-microglobulin, did not increase the diagnostic value beyond that of each marker considered separately (data not shown).

Discussion

In initially nonoliguric ATN, increased urinary excretion of cystatin C and α_1-microglobulin may predict an unfavorable outcome, as reflected by the requirement for RRT. To our knowledge, urinary cystatin C and α_1-microglobulin are the first laboratory markers described to predict severe ATN in humans. This is important because these proteins predicted RRT when the patients were still nonoliguric. Detection in this early stage of ATN may allow adequate time to prevent progression to oliguric ATN, through intensified supportive care, and to initiate potential future therapies *(6, 8, 9)*. This may improve the outcome in ATN because oliguric, or RRT-requiring, ATN

Table 2. Urinary excretion of tubular proteins and enzymes and Liano score on the day of inclusion.[a]

			Median (interquartile range)		
	M_r, × 10^3	URL[b]	RRT+	RRT−	*P*
β_2-Microglobulin, g/mol Cr	12	0.05	0.5 (0.1–2.1)	0.7 (0.08–1.5)	0.17
Cystatin C, g/mol Cr	13	0.03	1.7 (1.2–4.1)	0.1 (0.02–0.5)	<0.001
Retinol-binding protein, g/mol Cr	22	0.03	10.5 (5.5–13.9)	1.5 (0.3–5.0)	<0.001
α_1-Microglobulin, g/mol Cr	26	1.6	34.5 (26.6–45.1)	8.0 (5.0–17.5)	<0.001
α-GST, pg/mol Cr	51	0.09	3.2 (0.9–11.4)	1.0 (0.3–4.1)	0.006
GGT, U/mmol Cr	90	2.8	5.2 (2.7–7.4)	3.7 (2.4–5.9)	0.039
LD, U/mmol Cr	120	0.6	4.2 (1.2–6.2)	2.9 (2.1–4.0)	0.17
NAG, U/mmol Cr	150	0.7	8.9 (5.4–14.7)	3.5 (2.3–6.8)	<0.001
Liano score	NA	NA	0.79 (0.64–0.91)	0.34 (0.19–0.57)	<0.001

[a] RRT+, patients with ATN who required RRT; RRT−, patients with ATN who did not require RRT.
[b] URL, upper reference limit; Cr, urinary creatinine; NA, not applicable.

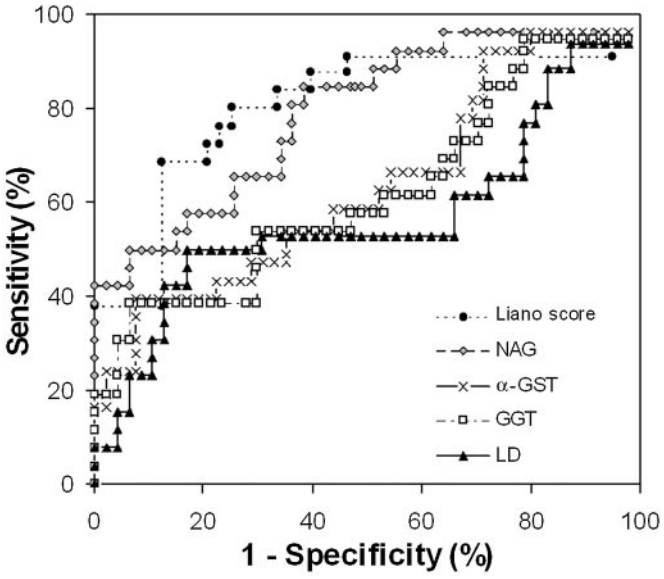

Fig. 3. ROC plot demonstrating the diagnostic performance of enzymuria and the Liano score to predict the requirement for RRT in ATN.

is associated with dramatically increased mortality *(5, 7)*. Of further note, our results indicate that cystatin C and α_1-microglobulin were superior to other proteins, enzymes, and the Liano score in differentiating ATN patients who subsequently required RRT from those who did not. However, the Liano score was not initially developed to predict the requirement for RRT, but to predict mortality in ATN *(4, 12)*. Our findings are consistent with recent studies indicating that urinary concentrations of lipocalin-type prostaglandin D synthase and neutrophil gelatinase-associated lipocalin, which are, like α_1-microglobulin, low-molecular-weight proteins of the lipocalin superfamily, may reflect the severity of tubular injury in human chronic renal disease and of ATN in animal models *(39, 40)*. Additionally, methods for measuring of urinary cystatin C and α_1-microglobulin are precise, simple, and readily available in clinical chemistry laboratories *(20, 35)*.

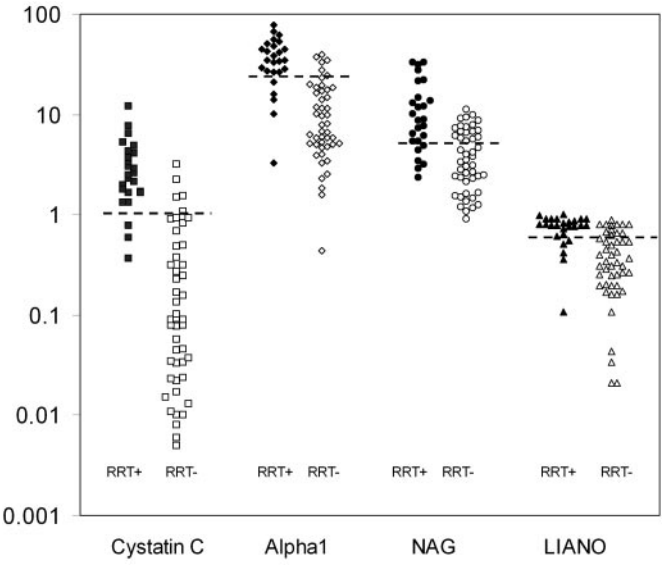

Fig. 4. Differentiation of ATN patients requiring RRT (*RRT+*; *closed symbols*) and not requiring RRT (*RRT−*; *open symbols).*

Patients were differentiated on the basis of cystatin C (g/mol of urinary creatinine) or α_1-microglobulin (*Alpha1*; g/mol of urinary creatinine) proteinuria; NAG (U/mmol of urinary creatinine) enzymuria; and the Liano score (*LIANO*). The *dashed lines* indicate the cutoff values for cystatin C (1 g/mol of urinary creatinine), α_1-microglobulin (20 g/mol of urinary creatinine), NAG (4.5 U/mmol of urinary creatinine), and the Liano score (0.60).

Recently, the high stability of cystatin C in urine at routine storage conditions, such as those used in the present study, and the independence of urinary cystatin C from the mode of urine collection were demonstrated, as have also been described for α_1-microglobulin *(35, 41, 42)*. These features are in contrast to the complexity of ATN severity scores, including the Liano score as the best evaluated one, which has limited their widespread clinical use *(8, 10–15)*.

Our study focused on ATN because it is the most frequent cause of ARF and is associated with the highest mortality *(4, 6)*. In contrast to other causes of ARF, proximal tubules are characteristically injured early in ATN *(6, 18)*. We therefore selected tubular proteinuria and enzymuria as logical markers in ATN. To select a uniform cohort, patients with chronic renal failure, renal transplantation, renal tumors, or trauma were excluded because they may demonstrate increased tubular proteinuria and enzymuria unrelated to ATN. Cystatin C and α_1-microglobulin may have performed better than the other low-molecular-weight proteins because nonrenal factors may not influence their production and urinary excretion and because they are degraded more slowly in the urine *(35, 41–43)*. We evaluated enzymuria because it was previously shown to indicate proximal tubule dysfunction *(19, 24–28)*. However, tubular proteinuria was superior to enzymuria in predicting the need for RRT in ATN, which is consistent with the higher sensitivity of tubular proteins in detecting proximal tubular injury compared with enzymes *(28, 40, 44)*. Possibly, early peak urinary excretion of enzymes was missed because ATN patients were not included in the study at the time of the insult that caused the proximal tubular injury, but with some time delay, as is common in clinical practice. This could be important because enzymuria was demonstrated to occur more rapidly after renal injury than tubular proteinuria *(21, 23, 25, 27)*. Additionally, urinary excretion of enzymes is markedly diuresis dependent *(45)*. Although we normalized all measurements for urinary creatinine, this might not have entirely compensated for minor differences in urinary flow rates in this study. The more selective location of NAG in the proximal tubule compared with GGT and LD may be the cause for its higher accuracy *(46)*. Other investigators have proposed enzymuria as a marker of ATN severity *(26, 27, 47)*. Their data implied lower sensitivity and higher specificity of enzymuria compared with our data differentiating be-

tween ATN with severe or good prognosis. In contrast to our study, those study sizes were small, and mortality and increases in serum creatinine were chosen as endpoints, both of which have limitations *(16)*.

There are limitations to the present study. We did not aim at predicting mortality as an even more relevant clinical outcome because it would require large cohorts to detect an independent effect of ATN on mortality *(3, 6, 7)*. Instead, we studied the requirement for RRT as an outcome measure because it is clinically meaningful and strongly associated with mortality *(1, 7, 16, 17)*. Although RRT was initiated according to uniform center standards, the clinical judgment of individual nephrologists cannot be excluded when deciding to initiate RRT. In the present study, it seems unlikely that death preceding the need for RRT is a substantial bias: Only 13% of patients not requiring RRT died, and in these patients, death occurred more than 1 week after RRT was initiated in the patients requiring RRT. Furthermore, because we performed a single-center study, our results require independent validation. Because we focused on patients with ATN, who comprise most, but not all, patients hospitalized with ARF, the results may not be valid for patients with ARF of non-ATN etiologies, renal transplant ARF, or ARF in the presence of chronic renal failure *(4, 6)*. In addition, differentiation between prerenal ARF and ATN may be difficult. Although the ATN criteria applied were validated recently or are commonly acknowledged, they may not completely differentiate both conditions *(6, 30, 31, 33)*.

In conclusion, our data suggest that increased urinary cystatin C and α_1-microglobulin may be early predictors of an unfavorable clinical outcome in ATN, reflected by the requirement for RRT. The availability of cystatin C and α_1-microglobulin as simple markers may contribute to wider application of severity prediction in ATN. Severity prediction with these markers could assist in improving the outcome of ATN.

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