Urine neutrophil gelatinase-associated lipocalin is a marker of graft recovery after kidney transplantation

Maria E. Hollmen[1], Lauri E. Kyllönen[1], Kaija A. Inkinen[2], Martti L.T. Lalla[2] and Kaija T. Salmela[1]

[1]*Division of Transplantation, Department of Medicine, Helsinki University Hospital, Helsinki, Finland and* [2]*Department of Medicine, HUSLAB, Surgical Hospital, Helsinki University Hospital, Helsinki, Finland*

Delayed graft function (DGF), especially long-lasting DGF, complicates kidney transplant outcome. Neutrophil gelatinase-associated lipocalin (NGAL) is an acute kidney injury marker; therefore, we tested whether urine NGAL could predict DGF, prolonged DGF (lasting over 14 days), or the quality of kidney function in transplant recipients without DGF (non-DGF). We collected urine samples from 176 recipients transplanted with deceased donor kidneys before and various days after transplantation. A total of 70 transplantations had DGF, of which 26 were prolonged. Patients who developed DGF had a significantly slower decrease in urinary NGAL compared with those without DGF, such that day 1 NGAL predicted DGF (area under the curve (AUC) 0.75) and predicted DGF in 15 of 112 cases with day 1 urine output over 1 l (AUC 0.70) and in 19 of 86 cases with a day 1 decrease in creatinine over 50 μmol/l (AUC 0.74). The urinary NGAL level on day 1 predicted prolonged DGF (AUC 0.75), which had significantly worse 1-year graft survival (73%), compared with shorter DGF (100%). In non-DGF, high day 3 NGAL (greater than the mean) was associated with significantly worse kidney function at 3 weeks compared with low NGAL, but not at 3 months and 1 year. NGAL did not correlate with long-term function in DGF. Hence, day 1 urinary NGAL predicted DGF even when it was not clinically expected early on, and importantly, it predicted prolonged DGF that led to worse graft survival.

Kidney International (2011) **79,** 89–98; doi:10.1038/ki.2010.351; published online 22 September 2010

KEYWORDS: deceased donor; delayed graft function; kidney transplantation; NGAL

Correspondence: *Maria E. Hollmen, Division of Transplantation, Department of Medicine, Helsinki University Hospital, Kasarmikatu 11-13, Helsinki 00130, Finland. E-mail: maria.hollmen@helsinki.fi*

Received 29 March 2010; revised 8 July 2010; accepted 21 July 2010; published online 22 September 2010

Delayed graft function (DGF) is an increasing problem after deceased donor kidney transplantation, as more kidneys from expanded criteria donors are accepted for transplantation. DGF is associated with acute rejection, increases the need for dialysis and posttransplantation biopsies, extends the post-transplantation hospital stay, and causes considerable economic burden.[1–8] In addition, the length of DGF is associated with worse 1-year outcome.[4,9] Ischemia-reperfusion injury occurs in all deceased donor transplantations, and has a major role in the pathogenesis of DGF. DGF can thus be regarded as one type of acute kidney injury.[10] Despite extensive studying on the mechanisms of ischemia-reperfusion injury in the experimental models, very little has been achieved in the prevention and treatment of DGF from the clinical point of view. Therefore, finding new ways to diagnose DGF very soon after, or even before transplantation, would further the possibility of developing therapeutic methods to prevent DGF in a clinical setting.

Neutrophil gelatinase-associated lipocalin (NGAL) has emerged as a new, noninvasive diagnostic tool for acute kidney injury.[11–16] NGAL associates with DGF; the association has been shown in kidney transplant biopsies taken 1 h after reperfusion, and in urine and serum samples taken on the day of, and very soon after, transplantation.[17–21]

The aim of our study was to examine (1) how serial urine NGAL (uNGAL) concentrations change over time after kidney transplantation; (2) whether uNGAL predicts the onset of kidney graft function; (3) whether uNGAL predicts prolonged DGF; and (4) whether uNGAL correlates with the level of kidney function in transplantations with early graft function (EGF).

RESULTS

The study included 176 renal transplant recipients. Recipient pretransplantation, posttransplantation, and donor characteristics are presented in Tables 1, 2, and 3. All recipients were Caucasian, except for one. The DGF grafts started to function from mean 12.0 days (s.d. 7.0) after transplantation. The donors in the DGF group were older, expanded criteria donors[22] were more common, cold ischemia time was longer, pretransplantation hemodialysis was more common, and time on dialysis before transplantation was longer in the DGF group compared with the EGF group. Overall 1-year patient

Table 1 | Recipient pretransplantation characteristics

	EGF	DGF[a]	*P*-value
N	106/176 (60.2%)	70/176 (39.8%)	
Mean age, years (s.d.)	50.5 (12.8)	54.1 (13.3)	NS
Gender			
Female	45 (42.5%)	21 (29.6%)	NS
Male	61 (57.5%)	49 (69.4%)	
TX number			
First TX	99 (93.4%)	62 (88.6%)	NS
Re-TX	7 (6.6%)	8 (11.4%)	
Underlying kidney disease			
Polycystic disease	26 (24.5%)	16 (22.9%)	NS
Glomerulonephritis	21 (19.8%)	14 (20.0%)	
Diabetes mellitus	29 (27.4%)	19 (27.1%)	
Other	30 (28.3%)	21 (30.0%)	
Mode of dialysis			
Hemodialysis	62 (58.5%)	52 (74.3%)	0.032
Peritoneal dialysis	44 (41.5%)	18 (25.7%)	
Mean time on dialysis, days (s.d.)	770 (571.1)	975 (598.9)	0.007

Abbreviations: DGF, delayed graft function; EGF, early graft function; NS, not significant; s.d., standard deviation; TX, transplantation.
[a]DGF defined according to the Halloran criteria.[23]

Table 2 | Recipient posttransplantation characteristics

	EGF (*n*=106 (60.2%))	DGF (*n*=70 (39.8%))[a]	*P*-value
Initial CNI			
Tacrolimus	24 (22.6%)	17 (24.3%)	NS
Cyclosporine A	82 (77.4%)	53 (75.7%)	NS
Induction therapy with IL-2 receptor antagonist	15 (14.2%)	13 (18.6%)	NS
Mean change in plasma creatinine from pre-TX to day 1 (μmol/l (s.d.))	−117 (145.5)	+17 (129.1)	<0.0001
Mean plasma creatinine (μmol/l (s.d.))			
Day 1	445 (198.8)	664 (191.8)	<0.0001
Day 3	250 (148.3)	644 (191.9)	<0.0001
Day 7	141 (57.2)	458 (193.9)	<0.0001
3 Weeks	120 (38.6)	206 (112.5)	<0.0001
3 Months	110 (27.4)	148 (48.8)	<0.0001
1 Year	109 (38.8)	128 (41.9)	0.002
Mean eGFR, ml/min (s.d.)			
3 Weeks	64.2 (19.9)	46.4 (21.2)	<0.001
3 Months	69.7 (23.2)	58.9 (21.1)	0.003
1 Year	74.8 (24.4)	67.7 (23.5)	0.050
Mean urine output (ml per 24 h (s.d.))			
Day 1	2544 (1526.5)	2406 (809.2)	<0.0001
Day 3	574 (613.8)	713 (713.7)	<0.0001
Day 7	2412 (779.8)	1274 (1007.5)	<0.0001
Day 14	2661 (690.6)	1888 (1060.3)	<0.0001
Number of rejections	4 (3.8%)	6 (8.6%)	NS
Mean time to rejection (days (s.d.))	8.7 (2.1)	20.8 (13.8)	NS
1-Year patient survival	99.4%	98.6%	NS
1-Year graft survival	99.1%	90.0%	0.005

Abbreviations: CNI, calcineurin inhibitor; DGF, delayed graft function; EGF, early graft function; eGFR, estimated glomerular filtration rate (Cockcroft–Gault); IL-2 receptor antagonist, interleukin-2 receptor antagonist (either basiliximab (*n*=19) or daclizumab (*n*=9)); NS, not significant; TX, transplantation; s.d., standard deviation.
[a]DGF defined according to the Halloran criteria.[23]

survival was 98.9%, and graft survival was 95.5%. The 1-year graft survival and long-term (3 month and 1 year) graft function were inferior in the DGF group, compared with the EGF group.

A pretransplantation urine sample was obtained from 70 patients, as 106 patients were anuric or oliguric before transplantation. The pretransplantation uNGAL did not correlate with residual diuresis from the native kidneys of the recipients ($R = 0.089$, $p = $ NS). The mean uNGAL levels decreased after transplantation in both DGF and EGF groups (Figure 1). The mean uNGAL concentrations were significantly lower in the EGF group compared with the DGF group at all measured time points after transplantation. Recipient's posttransplantation uNGAL was not affected by donor age, gender, or by induction immunosuppression given to the recipient (data not shown).

We included in the multivariate analysis (multilogistic regression method, forward, conditional) factors significantly differing between the DGF and EGF groups in the univariate analyses and also the clinically relevant factors in this respect, such as recipient age, donor plasma creatinine, and donor-estimated glomerular filtration rate (eGFR) (Table 4). Day 1 urine output, day 1 uNGAL, and the mode of dialysis emerged as significant, independent predictors of DGF.

Receiver operating characteristic (ROC) analyses were performed to assess the potential of uNGAL in predicting DGF. The area under the curve (AUC) for day 1 uNGAL was 0.750 (confidence interval (CI) 0.663–0.837; $P<0.0001$). At the optimal cutoff level of 560 ng/ml, the sensitivity was 68% and the specificity was 73%. The odds ratio for this cutoff level was 5.4 (CI 2.4–12.3). For comparison, day 1 urine output predicted DGF with an AUC of 0.931 (CI 0.894–0.967; $P<0.0001$). At the optimal cutoff level of 1035 ml, the sensitivity was 91% and the specificity was 80%.

The correlations between uNGAL and kidney function (plasma creatinine, eGFR) at 3 weeks, 3 months, and 1 year after transplantation were studied (Table 5). uNGAL correlated with kidney graft function up to 3 months. The mean length of stay in the hospital after kidney transplantation was 21 days (ranging from 15 to 43 days). Day 1 uNGAL did not correlate with length of stay in the hospital ($R = 0.047$, $P = $ NS).

We wanted to study whether uNGAL predicts DGF in cases in which EGF was expected on the basis of early urine output and decreasing plasma creatinine. First, we focused on the 112 transplantations with day 1 urine output >1 l. Despite good diuresis, 15 of these transplantations developed DGF. Their mean day 1 uNGAL concentration was significantly higher, 1217 ng/ml (s.d. 1228.9), compared with 460 ng/ml (s.d. 481.3, $P<0.0001$) of the 97 transplantations with EGF. Day 1 uNGAL predicted DGF in this subgroup