Delayed graft function in renal transplantation

Boom, H.

CHAPTER 3

DELAYED GRAFT FUNCTION IS CHARACTERIZED BY REDUCED FUNCTIONAL MASS MEASURED BY ^{99M}Tc-MAG3 RENOGRAPHY

Henk Boom, Tarek A.F. El-Maghraby, Jan A.J. Camps, Koos A.K. Blokland, Aeilko H. Zwinderman, Leendert C. Paul, Ernest K.J. Pauwels, Johan W. de Fijter.

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Background The mechanism that underlies delayed graft function (DGF) is still poorly defined. Previous studies using tubular function tests have shown that post-ischemic injury to the renal transplants results in profound impairment of para-immunohippurate extraction by the tubules.

Methods Using ^{99m}Technetium-mercaptoacetyltriglycine (^{99m}Tc-MAG3) renography and tubular function slope (TFS) we studied the tubular uptake of ^{99m}Tc-MAG3 in a prospective study of renal transplant recipients with immediate graft function (IGF) and those with DGF.

Results Thirty-seven consecutive recipients of a cadaveric graft and 5 kidneys from living donors were evaluated within 48 hours after transplantation and in week 2, month 3 and 6 and 3 years after transplantation. In addition to the protocol scans, recipients with DGF were examined every other day until function was resumed. Repeated measurement two-way ANOVA and a change point analysis were performed to determine the difference in the follow-up of TFS values between the two groups. Fourteen patients were classified as having DGF and 28 IGF. In the DGF group, the initial TFS value was significantly lower than in the IGF group [0.54 (+ 0.01) and 1.75 (+ 0.16) respectively; P = 0.002], a difference that persisted for up to 3 years. Change point analysis revealed that the post ischemic tubular excretion improved with time in both groups in the first 3 to 4 weeks, but both groups remained different up to 3 years after transplantation. Multi-variate analysis revealed that only the cold ischemic time (CIT) was an independent risk factor for a low TFS value. After the initial recovery from post-ischemic injury, the TFS may be used as a marker for functional renal mass.

Conclusion We propose that the tubular defect in DGF defined by ^{99m}Technetium-mercaptoacetyltriglycine (^{99m}*Tc*-MAG3) renography is irreversible and may be a marker of initial graft function.

INTRODUCTION

Despite increased donor awareness and actions, the actual number of organ donors has not increased to any meaningful extend. This disparity has led to an increased interest in the use marginal donors, including donors at the extremes of age, non-heart beating donors and donors with a history of hypertension or diabetes (1,2).
DGF is a common complication after renal transplantation, with a reported incidence of up to 50%, especially in recipients of kidneys from marginal donors (3-6). The underlying cause is usually ischemic damage, which may be further complicated by an increased likelihood of acute rejection episodes (3,7) or drug-related nephrotoxicity (8). There is still debate on the impact of DGF on late graft outcome. Some authors find an effect of DGF on renal allograft survival (9,10), but others only find an effect of DGF on graft survival in patients who experience acute rejection episodes (11).
To answer the question whether the occurrence of DGF is merely an expression of an insufficient amount of functional renal mass at the time of transplantation or an independent risk factor for subsequent graft loss, there is need for a marker of renal mass that is easy assessable and can be repeated frequently. Several studies have been undertaken to define the nature of graft dysfunction in DGF. Corrigan et al. found in human renal transplants with post-ischemic injury, a severely impaired para-aminohippurate (PAH) extraction, by the renal tubules that persisted for up to 7 days (12). The renal handling of ^{99m}Technetium-mercapto-acetyltriglycine (^{99m}Tc-MAG3) is similar to that of PAH (13). We prospectively evaluated serial ^{99m}Tc-MAG3 renographies in patients with immediate or delayed graft function, using a standardized method to estimate the active tubular extraction for up to 3 years.

MATERIALS AND METHODS

Between August 1997 and January 1998, 37 consecutive recipients of a first or second cadaveric renal transplant and 5 recipients of a living related donor transplant gave informed consent and were entered into the study. DGF was defined based on previously described criteria (3) and never functioning grafts were excluded from the study. In short, cases were categorized as DGF when the serum creatinine level increased, remained unchanged, or decreased by less than 10 % per day immediately following surgery during three consecutive days within the first week. All other situations were designated as immediate graft function (IGF). An acute rejection episode as a cause of graft dysfunction was defined as a decline in renal function by more than 10 % and was confirmed by a percutaneous renal biopsy unless this could not be obtained. Cyclosporine (CsA) nephrotoxicity was diagnosed based on the criteria described in the Banff 1997 classification of renal allograft pathology (14) or when there was a normalization of renal function after reduction of the CsA dose. Renal function, expressed as the creatinine clearance was estimated using the formula developed by Nankivell, which showed a good correlation with ^{99m}Tc diethylenetriaminepentaacetate (^{99m}Tc DTPA) clearance (15):

GFR [ml/min] = 6.7 / creat. [mmol/l] + BW [kg] / 4 - urea [mmol / l] / 2 - 100 / (height [m])^2 + (35 [male] or 25 [female]).

All patients had an initial ^{99m}Tc-MAG3 study on the first post-transplant day. Protocol scans were performed at 2 weeks, 3 and 6 months and at three years after transplantation. In addition, patients experiencing DGF were followed with ^{99m} Tc-MAG3 studies every other day until the serum creatinine concentration decreased by more than 10 % per day on three consecutive days.

^{99m}Tc-MAG3 renogram

^{99m}Tc-MAG3 studies were done by positioning a large field of a view gamma camera (Toshiba GCA 501S) anteriorly over the patient in the supine position. The field of view included the transplanted kidney, the lower abdominal aorta and iliac arteries as well as the urinary bladder (fig. 1a). After a bolus injection of 100 MBq of ^{99m}Tc-MAG3 into a large caliber vein (usually the medial antecubital vein), frames were recorded initially at 1 sec intervals for 120 frames, followed by 90 frames at 20 sec intervals to complete the 32 minute study as shown in figure 1b and 2a. To calculate the dose activity administered, the syringe was counted before and after injection and when there was extravasation, the injection site was counted as well. A dedicated nuclear medicine computer (MAPS 10000 Web Link Medical, UK) was programmed for processing the data and calculating the Tubular Function Slope (TFS).

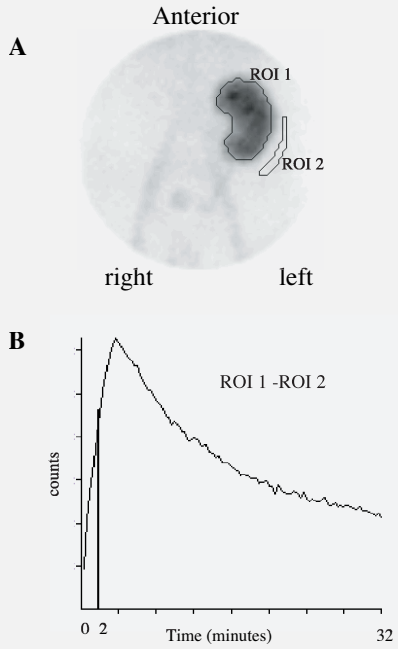
Tubular Function Slope

To study the radiopharmaceutical uptake by the renal tubular cells the tubular function slope (TFS) was designed. Two regions of interest were drawn semi-automatically; one around the graft (ROI - 1) and one representing the background (ROI - 2) (fig. 1a). The background region of interest was crescentic in shape and it was placed infero-lateral to the kidney, avoiding any vascular structures (fig. 1a). A background subtracted graft curve was generated during the first two minutes of the study. (fig. 2 a): it consists of a rapidly ascending initial phase until the first pass peak, which results from the initial perfusion, followed by a second phase which represents the tubular extraction phase (fig. 2 b). The graft curve was normalized to the injected dose and a measure of the slope was taken using a linear fit (least squared error estimate) of the curve between 50 seconds and 110 seconds (figure 2 b). This slope was defined as the TFS. As TFS is determined in the second phase of the time activity curves it is proposed to be independent of renal perfusion.

Reproducibility and inter-observer reliability

To assess the intra-observer variability the TFS was calculated twice in two different sessions by one observer in a random set of 33 scintigrams of 10 patients. The average difference between the TFS values between the first and second processing was 0.062 (SD 0.016) with a correlation of 0.992. The intra-class correlation was 0.991. The inter-observer variability was assessed by processing 59 scans twice by two different operators. The aver-

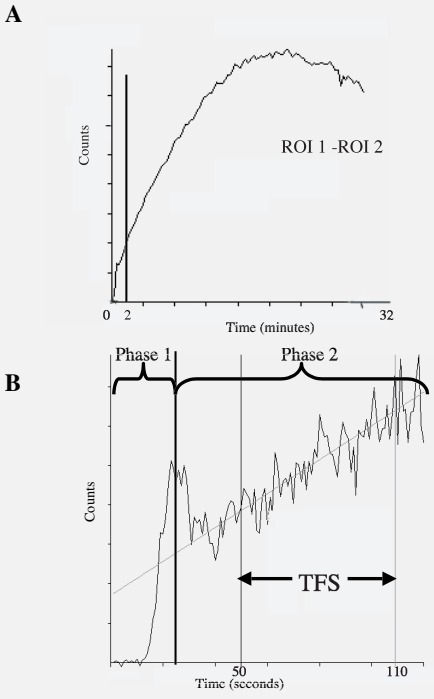
Figure 1



a: ^{99m}Tc-MAG3 renogram. The region of interest 1 (ROI -1) describes the whole kidney activity. The region of interest 2 (ROI - 2) describes the avascular reference region.

b: Backgound subtracted and dose adjusted ^{99m}Tc-MAG3 extraction and secretion curve of a kidney experiencing immediate graft function

Figure 2



a: Backgound subtracted and dose adjusted ^{99m}Tc-MAG3 extraction and secretion curve of kidney experiencing delayed graft function. (Note: time axis is until 32 minutes).

b: First 2 minutes of the ^{99m}Tc-MAG3 extraction and secretion curve in which Phase 1 (the rapidly ascending phase) represents the renal perfusion phase. Phase 2 represents the tubular extraction phase. Using a linear fit (least squared error estimate) the slope of this curve is determined and defined as tubular function slope (TFS).

age difference in TFS values between the two was 0.011 (SD 0.019) with a correlation of 0.997. The inter-class correlation was estimated to be 0.997. These excellent correlations were not unexpected as the calculations of the TFS, including drawing the regions of interest and the defined periods in the second phase of the early dynamic curve, are almost entirely automatic.

Immunosuppressive Regimen

The standard immunosuppressive regimen consisted of Prednisone, Cyclosporine (Neoral) and Mycophenolate Mofetil (MMF). Cyclosporine was administered intravenously in a dose of 3 mg/kg /day for the first 48 hours, starting at the onset of surgery. The initial oral dose of Neoral was 10 mg/kg/day from day 2 onward. Subsequent doses were adjusted according to cyclosporine 12-hour trough level monitoring. Mean cyclosporine dose and trough levels were not significantly different between the 2 groups. In the first three months, the target 12-hour trough level was 300 μg/l (range 250 μg/l –350 μg/l) and beyond 3 months it was 150 μg/l (range 100 μg/l - 200μg/l). All patients started on 20 mg/day of Prednisolone followed by a dose reduction of 2.5 mg every fortnight until the maintenance dose of 10 mg was reached. MMF, 1 gram b.i.d. was given from day 1 onward. Acute rejection episodes were treated with 1 gram of methylprednisolone intravenously for 3 consecutive days. Severe or steroid resistant acute rejection episodes or any second rejection episode were treated with rabbit anti-thymocyte globulin for 10 days, as previously described (16).

Statistical analysis

Risk factors for a low TFS were analyzed, using linear regression analysis. Differences in TFS and creatinine clearance were examined using the two way ANOVA for repeated measurements. Furthermore, the change of the ln(TFS)-parameter during follow-up was modeled using a change-point model with two linear regression lines, one involving a rapid increase after transplantation, and a second line, following a change-point, involving a slow change of the ln(TFS) parameter. The parameters of the regression lines, and the timing of the change-point was assumed to vary between patients. These parameters were handled as random, drawn from the multivariate normal distribution. The mean and (co)variance of this normal distribution were estimated separately for patients with immediate and delayed graft function. The change of the ln(TFS) of the "averaged" patient according to the model thus defined, was calculated and illustrated graphically. A p-value of 0.05 or less was considered significant. Statistical analysis was done using the SPSS 9.0 software package (Version 9.0; SPSS, Inc., Chicago, IL).

RESULTS

A total of 42 patients were prospectively followed with ^{99m}Tc-MAG3 renography; 28 experienced IGF and 14 had DGF. The characteristics of the study population, grouped according to the presence or absence of DGF are summarized in Table 1. In this cohort of patients none of the factors summarized in Table 1 were statistically different between

the two groups. Although donor age and the number of female donors were slightly higher in the DGF group, this did not reach significance. The type of rejection episodes was not different in the two groups. Three patients in the IGF group experienced recurrence of their renal disease (IgA nephropathy, membrano proliferative glomerulonephritis and focal segmental glomerulosclerosis) and one lost his graft after 432 days. Two patients in the DGF group lost their graft because of recurrence of their renal disease (micro angiopathic nephropathy and membrano proliferative glomerulonephritis) after 43 and 373 days respectively. A total of two hundred and seventy three ^{99m}Tc-MAG3 renographies were performed: 160 in the IGF group and 113 in the DGF group.

Table 1. Patient characteristics grouped according to the incidence of DGF

Characteristic	**IGF** (N = 28)	**DGF** (N = 14)	p-value
Pretransplantation factors			
HLA-AB mismatch	1.25 (1.07)	0.92 (0.86)	0.37
HLA-DR mismatch	0.46 (0.51)	0.38 (0.51)	0.67
Panel reactive antibodies (historic)	30 (33)	19 (27)	0.30
Donor age, years	46 (17)	48 (11)	0.81
Recipient age, years	45 (13)	44 (11)	0.80
Gender			
Recipient male (%)	64.3	78.6	0.34
Donor female (%)	50	35.7	0.38
Mismatch: female to male (%)	32.1	35.7	0.91
Cold ischemia time, (hours)	23 (8)	25 (5)	0.46
Warm ischemia time (minutes)	35 (12)	36 (11)	0.80
Pretransplantation MAP, (mmHg)	105 (16)	104 (12)	0.70
Cadaveric transplantation (%)	82	100	0.15
Cause of donor death			
Cardiovascular (%)	61	64	1.00
Retransplants (%)	21	14	0.39
Posttransplantation factors			
Immunosuppression			
Pred / neoral/ MMF (%)	100	100	1.00
Biopsy proven rejection episodes (%)	53	36	0.34

Data are expressed as $\pm$ SD, unless otherwise stated; MAP: mean arterial pressure; MMF:Cellcept; Pred.: prednisone

Follow-up of TFS

The TFS values after predefined time intervals for both the IGF and the DGF group are shown in figure 3a. In the DGF group the average TFS values (mean ± SEM) in the initial scans was 0.54 ± 0.01 and there was an increase to 1.8 ± 0.19 in the week 2 studies. In the IGF group the average TFS values (mean ± SEM) at the first examination and the week two studies were 1.75 ± 0.16 and 2.52 ± 0.22, respectively. At the month 3 and 6 and year 3 time points, the initial difference in TFS values between the two groups persisted; for the DGF group the TFS values were 1.83 ± 0.17, 1.84 ± 0.19 and 1.38 ± 0.24 respectively and for the IGF group they were 2.91 ± 0.22, 3.17 ± 0.23 and 2.76 ± 0.31. The differences between the two groups were highly significant ($P < 0.0001$). After correction for the initial TFS value, by subtracting the initial TFS values from the follow-up TFS values of each group, these differences were no longer present ($p = 0.85$) and the curves were superimposable. These data show that in both groups, despite of comparable recovery of tubular function as measured by TFS, there is an initial difference in the TFS values that persists for up to 3 years.

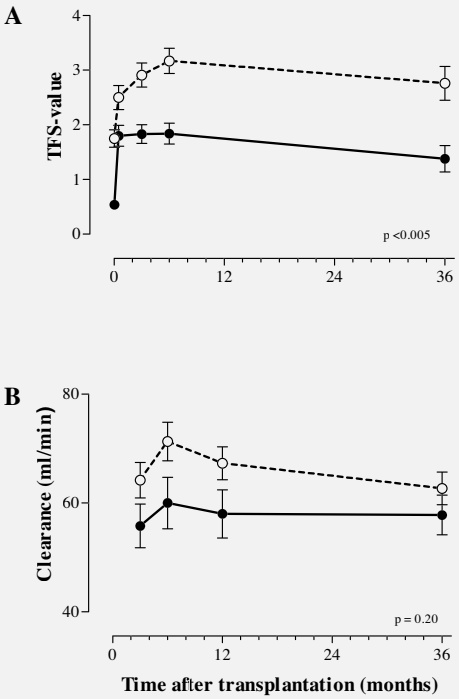
Change point model

To further evaluate whether there was a difference in time to reach the maximum TFS values between the two groups, all 273 available scans were studied in a change–point analysis model. The results of this analysis are plotted in figure 4. The slopes of the sharp increasing regression lines prior to the change-point were not different for recipients with DGF and patients with IGF (0.06 ln(TFS-units)/day and 0.06 ln(TFS-units)/day respectively; $p = 0.87$), but the absolute level achieved was different between the two groups ($P = 0.005$). Interestingly the timing of the change-point was also the same in DGF-and IGF-patients (29/21 days, $p=0.85$) as was the slow ln(TFS)-increase thereafter [-0.0003 ln(TFS) units and -0.00002 ln(TFS) units ($p=0.24$)]. This means that TFS remains at a constant level from about 3 to 4 weeks onwards both in the presence or the absence of DGF.

Factors influencing the TFS value and renal function

To evaluate the risk factors that correlated with an initial low TFS value and the creatinine clearance and TFS values at the predefined time-points, several factors concerning the donor and the transplantation procedure were retrospectively evaluated. In the multivariate analysis, the only significant risk factor for a low post-transplant TFS value was the cold ischemia time (correlation coefficient (CC): - 0.27 per hour; -0.54 – 0.00). In the multivariate analysis, only the TFS value at 6 months was correlated with the TFS value found after 3 years (CC: 0.75; 0.58 – 1.16) and the estimated creatinine clearance at 1 year was the only variable that was correlated with the graft function at 3 years (CC: 0.875; 0.59 - 0.89). The creatinine clearances at 3 and 6 months and 1 and 3 years are plotted in figure 3b and were 56 ml/min, 60 ml/min, 58 ml/min and 58 ml/min for the DGF group and 64 ml/min, 71 ml/min, 67 ml/min and 63 ml/min for the IGF group. The estimated clearances tended to be lower in the DGF group, but this did not reach a statistical significance ($p = 0.20$), because of the limited sample size.

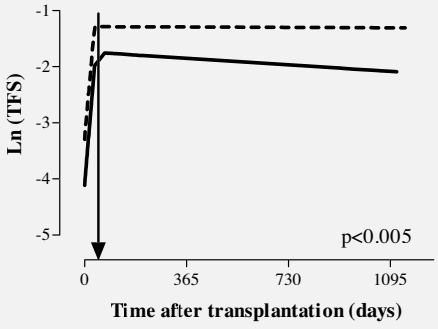
Figure 3



a: TFS values in the two groups, which are different (p = 0.005). After correction for the initial TFS value, by subtracting the inital TFS values from the TFS values in the follow-up of each group, these differences were no longer present (p = 0.85) and the curves were superimposable, indicating that the differences between the two groups were determined by the differences already present in the early post transplant ^{99m}Tc-MAG3 renographies
(IGF: dashed rule ; DGF: solid rule)

b: Creatinine clearances at the different time points. The values tended to be lower in patients that experienced DGF but did not reach statistical difference (p = 0.2).
(IGF: dashed rule ; DGF: solid rule).

Figure 4

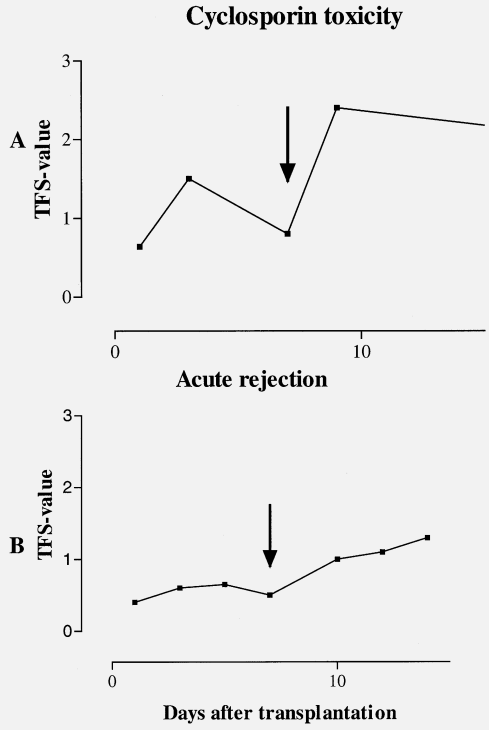


The change point model, using all 273 available renographies. The improving of the tubular extraction impairment occurred within the first post-transplant month and to the same extend in both groups.
(IGF: dashed rule ; DGF: solid rule)

Use of TFS in detection of acute rejection episodes or cyclosporine toxicity

In the total study population 24 episodes were identified with a decline of TFS of more than 20 % in 2 consecutive ^{99m}Tc-MAG3 renographies performed within the first three weeks. In 17 (71 %) cases an acute rejection episode or cyclosporine toxicity was identified and in 7 (29 %) cases no obvious explanation could be found. In the DGF group there were 14 cases with a decline of TFS of more than 20 % in 2 consecutive scans. In 10 (71%) cases an acute rejection episode or acute cyclosporine toxicity was identified, in 4 (29 %) cases no obvious explanation was found. Figure 5 shows two representative examples of the relation between the decline of TFS and an acute rejection episode (fig 5a) and he TFS and cyclosporine toxicity (5b) in patients who experienced DGF. Both curves show complete reversibility of the TFS value after treatment of the acute rejection episode or lowering of the CsA dose respectively.

Figure 5



a: A representative case of an acute rejection episode (Grade II a) in a patient experiencing DGF. TFS values are reversible after treatment.

b: A representative case of an episode of cyclosporine toxicity (CyA 12 hours trough level: 600 μmol/l) in a patient with DGF. TFS values are reversible after correction of the neoral dose.

DISCUSSION

In the present study we demonstrated that transplantation of a renal allograft is associated with a low level of active tubular extraction of the radiopharmaceutical ^{99m}Tc-MAG3 as assessed by renography. The tubular extraction is expressed as the tubular function slope (TFS). The TFS values were particularly decreased in the immediate post-operative days in recipients who experienced delayed graft function. Independent of whether the graft showed immediate good function or delayed function, this tubular extraction impairment of ^{99m}Tc-MAG3 showed improvement within the first post-transplantation weeks in both groups, but remained always lower in the DGF group.

After the first post-transplant month, the difference in the absolute TFS values between recipients with immediate function and those with delayed function persisted in subsequent ^{99m}Tc-MAG3 renographies for up to three years. The results where not different when the data of the living related transplants or the data of the grafts that showed recurrence of their renal disease were left out of the analysis.

Previously, a firm correlation between histological tubulo-interstitial changes and renal outcome has been established (17). Since the TFS is a marker of the proximal tubular function and the tubular compartment represents approximately 80 to 85% of the renal mass, we propose that it may serve as a marker of renal mass after the initial recovery from post-ischemic injury.

It has been difficult to quantitate the amount of renal mass transplanted. The degree of glomerulosclerosis and glomerular size have been proposed as a measure of functional renal mass but these parameters have a poor correlation with outcome (18,19). However as stated before, there is a good correlation between tubulo-interstitial changes and outcome (17).

Radionuclide renography, using ^{99m}Tc-MAG3 is a non-invasive method that can be repeated frequently. To improve its diagnostic accuracy, various quantitative parameters have been proposed (20,21). In ^{99m}Tc-MAG3 renography the second part of the early phase of the curve represents the active extraction of the radiopharmaceutical by epithelial cells of the proximal renal tubules (22-26). No objective correlations with graft dysfunction have been established yet (23,26,27). Therefore, we defined the tubular function slope (TFS) in ^{99m}Tc-MAG3 renography, as a putative objective marker of tubular extraction capacity. The hypothesis that the TFS value of the renogram measures active tubular excretion is supported by studies using para-aminohippurate (PAH). Since the renal handling of ^{99m}Tc-MAG3 shares the same characteristics with the handling of PAH (13), it is likely that both substances are handled similarly. In a study of transplanted human kidneys with ischemic injury, no correlation between the PAH clearance and renal plasma flow, measured by phase contrast–cine-magnetic resonance imaging (MRI), was found (12). The authors concluded that the severely depressed clearance was explained by the reduced tubular extraction of the PAH. Because the tubular extraction of ^{99m}Tc-MAG3 is an energy dependent transport mechanism and occurs along the entire length of the proximal tubules, it will be affected by ischemic injury or inflammation. These conditions are characterized by the loss of tubular cell polarity, which impairs the transport of ^{99m}Tc-MAG3 by the dislocation of the Na+-K+-ATP-ase from the basolateral cell membrane into the cytoplasm (28,29).

A decrease in TFS value of more than 20% in consecutive ^{99m}Tc-MAG3 renographies in the early post-transplant period can be helpful in detecting additional complicating factors, such as an acute rejection episode or cyclosporine toxicity. As these events also influence long term graft outcome(3,7,8), we looked at the reversibility of the TFS values after treatment of an acute rejection episode or after adjusting the neoral dose. We found that TFS values were completely reversible in both conditions and we therefore assume that these events have a no major influence on the TFS values, during follow up.

We found that cold ischemic time (CIT) is an independent risk factor for a low initial TFS value in both groups. CIT has been reported as an independent risk factor for the occurrence of DGF(3,30-32). We hypothesize that in the presence of a long CIT, the quality and the amount of the functional renal mass transplanted, explains why some grafts experience DGF and others do not. It remains to be seen whether other risk factors for the occurrence of DGF, like donor age, pretransplant mean arterial pressure (MAP) and gender mismatch (3) are correlated with a low initial TFS-value when the numbers are increased.

We conclude that the TFS as defined in ^{99m}Tc-MAG3 renography may serve as a marker for the functional renal mass. Most renal transplants will experience a recovery phase from post-ischemic injury, but grafts from marginal donors are more likely to experience delayed graft function. After the initial recovery, grafts experiencing DGF have lower TFS values, than grafts without DGF, which remained evident up to 3 years of follow-up. Therefore, the TFS after for example 1 month provides information about renal mass that is not obtained by measures of glomerular filtration rate and may serve as a measure for initial renal mass post-transplantation. Furthermore a decrease of TFS in subsequent ^{99m}Tc-MAG3 renographies helps in the management of grafts experiencing DGF by planning additional diagnostic procedures like doing a renal biopsy or adapting calcineurine dose. Previous findings showed that DGF was associated with an increased risk of acute rejection episodes and a suboptimal function at 1 year but DGF alone was not independently associated with graft loss (3). The current study confirms that delayed graft function is associated with an initially decreased renal tubular function that appears irreversible.

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