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ORIGINAL ARTICLE

To what extent estimated or measured GFR could predict subclinical graft fibrosis: a comparative prospective study with protocol biopsies

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Summary

Monitoring of allograft function entails methods more accurate than serum creatinine and creatinine-based GFR equations (eGFR). This prospective trial aimed at investigating the diagnostic accuracy of creatinine- and cystatin C-based eGFR with measured GFR (mGFR) and compared them with graft fibrosis detected by protocol biopsies (PBx). Forty-four kidney transplant recipients were enrolled. PBx were obtained postengraftment and at 6th and 12th months. GFR was measured by Tc-99m DTPA at 3th, 6th, and 12th months after transplantation. Significant correlation existed between eGFR and mGFR at 3, 6, and 12 months ($P < 0.0001$). Cystatin C-based Hoek and Larsson equations had the lowest bias and highest accuracy. The sum of interstitial fibrosis and tubular atrophy score increased from implantation to 6th and 12th months (0.52 ± 0.79, 0.84 ± 0.88, 1.50 ± 1.35). This was accompanied by reduction of mGFR from 54.1 ± 15.2 to 49.9 ± 15.2 and 46.8 ± 16.5 ml/min/1.73 m^2, while serum creatinine, cystatin C, and eGFR remained stable. Neither creatinine- nor cystatin C-based GFR equations are reliable for detecting insidious graft fibrosis. In the first year after transplantation, mGFR, with its best proximity to histopathology, can be used to monitor allograft function and insidious graft fibrosis.

Introduction

Histological injury to renal allograft occurs early after transplantation and almost always precedes functional deterioration [1]. Serum creatinine (SCr)-based equations are insufficient to predict this insidious and progressive damage [1–3]. Histopathological evaluation of the graft by protocol biopsies is a sensitive and accurate method for detecting this progressive injury [4,5].

Direct measurement of glomerular filtration rate (mGFR) by renal clearance of inulin and by plasma disappearance curves of radioisotope-labeled substances is more sensitive to detect subtle early changes, but their application in routine practice is limited [6,7] and there is a need for a simple and accurate method capable to detect deterioration in renal function during the onset of pathological injury in the allograft [8,9].

It has been proposed that serum cystatin C (SCys-C)-based estimates of GFR are better than SCr-based estimates of GFR in the general population [10]. Similarly, in two studies comprising over a hundred consecutive kidney transplant recipients with stable function, SCys-C-based formulas (Hoek, Filler, and Le Bricon) were more accurate and correlated better with measured GFR than SCr- or

other SCys-C-based equations [11,12]. However, we know of no study that compared eGFR equations and mGFR with synchronous histopathological features in graft biopsies.

In this study, we prospectively investigated the correlation between SCr- and SCys-C-based eGFR equations with mGFR and histopathological alterations in biopsies performed synchronously at constant time intervals during the first post-transplant year.

Materials and methods

Adult patients (>18 years of age) with stable graft function (serum creatinine <2.0 mg/dl) and normal thyroid function tests throughout the first 3 months post-transplant were included. Exclusion criteria were a previous transplantation, a history of relapsing acute rejection or already rejecting patients during recruitment, CNI-drug withdrawal or conversion, urinary protein excretion >500 mg/day, and pretransplant malignancy. A baseline (postengraftment) biopsy was taken and written informed consent obtained.

Data were collected during routine visits at the 3rd, 4th, 5th, 6th, 8th, 10th, and 12th months post-transplant. Clinical events, vital signs, renal function parameters, immunosuppressive protocol, and histopathology reports were recorded at every visit.

All patients received induction immunosuppression with rabbit ATG or IL-2 receptor antibody. The usual dosage of ATG (Fresenius, Bad Homburg, Germany) was 2–4 mg/kg/day intravenously and was adjusted to keep CD3+ T-cell count below 50 cells/mm^3 until serum creatinine level has reduced to <3 mg/dl. Basiliximab (Simulect 20 mg vial; Novartis Pharmaceutical Co., East Hanover, NJ, USA) was administered on day 0 and 4.

The immunosuppressive protocol invariably consisted of a calcineurin inhibitor with mycophenolate mofetil (MMF, or equivalent doses of mycophenolate sodium) or azathioprine (AZA) and corticosteroid. Tacrolimus was administered 0.10–0.15 mg/kg/day as two divided doses to keep whole blood trough concentrations between 5–10 ng/ml for the first year. Cyclosporine A was given initially 8–10 mg/kg/day and continued with a maintenance dose of 5–8 mg/kg/day. The dosage was adjusted based on the trough levels 250–350 ng/ml in the first three months and 150–250 ng/ml, thereafter. Daily MMF dosage ranged between 1.5 and 2.0 gm. Corticosteroid dosage was 10 mg/day for the first 6 months followed by 7.5 mg/day for the following three and finally tapered to 5 mg/day during the last 3 months. All patients received TMP/SMZ prophylaxis during the first 6 months of the transplantation.

The primary biochemical tests were SCr and SCys-C for the estimation of GFR. SCr was determined by enzymatic creatinine assay using a Beckman LX20 (Beckman Coulter, Brea, CA, USA). SCys-C was determined by the particle-enhanced nephelometry (Dade Behring N Latex Cystatin C assay; Dade Behring, Deerfield, IL, USA). GFR was estimated from SCr using the Modification of Diet in Renal Disease (MDRD4) and Cockcroft–Gault equations [2,3].

The MDRD4 equation for creatinine in mg/dl is: GFR (ml/min/1.73 m^2) = 186 × $(S_{cr})^{-1.154}$ × $(Age)^{-0.203}$ × (0.742 if female) × (1.212 if African American).

The Cockcroft–Gault equation is: [(140-age). BW/Screa. 72].GF, where SCr is serum creatinine in mg/dl, BW is body weight in kg, GF is gender correction factor (0.85 if female, 1.0 if male).

SCys-C-based eGFR (ml*min^{-1} 1.73 m^{-2}) was estimated with the formulas of Hoek [13], Larsson [14], Le Bricon [15], and Filler [16] as follows:

Hoek – eGFR = −4.32 + 80.35 × 1/cys C,

Larsson – eGFR = 77.239 × CysC in mg/l$^{-1.2623}$

Le Bricon – eGFR = 78 × (1/cystatin C, in mg/l) + 4.

Filler – log(GFR) = 1.962 + [1.123xlog(1/Cystatin C)]

The plasma clearance of $Tc^{99\ m}$-diethylenetriaminepentaacetic acid (Tc-99m DTPA) was measured by single-injection and dual-plasma sampling as the reference standard of mGFR. Blood was sampled at 120 and 240 min. The average of the two measurements was calculated and corrected for body surface area. Tc-99m DTPA mGFR was performed at the 3rd, 6th, and 12th months after transplantation, and SCr and SCys-C were measured in a fasting blood sample on the same days.

Bias was defined as the mean difference between mGFR and eGFR and expressed as ml/min/1.73 m^2. Accuracy was defined as the percentage of eGFR values within 30% of the mGFR [17].

Renal biopsies were obtained using needle core biopsies of the renal cortex performed after revascularization (implantation) and at the 6th and 12th months. Serial 3- to 4-μm sections of paraffin-embedded tissue were stained with hematoxylin–eosin, periodic acid–Schiff, silver, and Masson trichrome. Initial histopathology was evaluated and reported by the local kidney transplant pathologist. At the end of the study, all biopsies were blindly reviewed by two pathologists (SŞ and FAT) and the final decision was given by consent. The number of glomeruli and percentage of sclerotic glomeruli were recorded. Histopathological grading for interstitial fibrosis (ci), tubular atrophy (ct), fibrous intimal thickening (cv), and arteriolar hyalinosis (ah) was performed based on the criteria suggested by the 1997 Banff scoring system [18].

The whole data were collected by a third party, and the participating investigators have not been informed about the results until after the completion of the study.

The study was approved by the Ege University School of Medicine Ethics Board.

Statistical analysis

Numerical variables are given as mean ± SD, and categorical variables are presented as percentages. Correlation analysis was performed using the Pearson or Spearman tests. Repeated analysis of variance was used for multiple comparisons of continuous variables. Comparison of the significance of mGFR values between the progressor and nonprogressor groups was performed with an independent *t*-test. *P* value <0.05 was considered as statistically significant. SPSS version 13.0 (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses.

Results

Between December 2008 and July 2009, 44 primary kidney transplant recipients of 21–56 years of age (mean: 37.3 ± 9.8) were enrolled in this dual-center study. Twenty-three patients received kidneys from living donors and 21 from deceased donors. Patient and donor characteristics are shown in Table 1.

The mGFR, eGFR, and SCr and SCys-C values during the study period are shown in Table 2. mGFR values progressively decreased from 3rd month to 12th month after transplantation. But SCr and SCys-C levels and eGFR values were remained relatively stable and did not change statistically whole period.

There was an excellent correlation between mGFR and both SCr and SCys-C values and SCr- and SCys-C-based GFR estimations during the 3rd, 6th, and 12th month measurements (Table 3).

The bias and accuracy of the different equations are given in Table 4. The Hoek equation had the best performance with the lowest bias and highest accuracy when compared with mGFR. Larsson eGFR values had better proximity to mGFR and Hoek equation results at every interval, whereas MDRD, CG, Le Bricon, and Filler equations overestimated mGFR in all stages of the study. Bland–Altman plot between mGFR and Hoek formula for 3rd, 6th, and 12th months is shown in Figs 1, 2, and 3.

Histopathological alterations in protocol biopsies are shown in Table 5. They showed mild but progressive increase in the scores of interstitial fibrosis (ci), tubular atrophy (ct), and sum of ci and ct. Glomerulosclerosis, arteriolar hyalinosis, and chronic intimal thickening did not change. ci and ct were present at implantation biopsies of eight patients (18%). New-onset or progressive ci + ct were detected in fourteen and 24 patients at the 6th and 12th month protocol biopsies, respectively.

Table 1. Patient and donor characteristics.

	n: 44
Recipient Age (years)	37.3 ± 9.8
Recipient Gender (male/female)	22/22
Donor age (years)	42 ± 14
Donor gender (male/female)	27/17
Living donor, *n*, (%)	23 (52)
Cold ischemia (hours)	16 ± 5
Number of mismatched antigen	3.0 ± 1.7
Immunosuppressive regimen	
CsA vs Tac	21/23
MMF vs AZA	43/1
ATG vs IL-2 receptor antibody	30/14

Table 3. Pearson correlation analysis between mGFR and SCr- and SCys-C-based GFR estimations (*r* values are shown, and all *P* values are <0.0001).

	mGFR at 3rd month	mGFR at 6th month	mGFR at 12th month
SCr	−0.667	−0.594	−0.688
SCys-C	−0.689	−0.760	−0.725
MDRD	0.678	0.741	0.833
Cockcroft–Gault	0.703	0.712	0.841
Le Bricon	0.715	0.767	0.758
Hoek	0.715	0.767	0.758
Larsson	0.711	0.743	0.723
Filler	0.712	0.766	0.756

Table 2. Alterations in renal function tests during the study period.

	3rd month	6th month	12th month	*P* (3rd vs 6th month)	*P* (6th vs 12th month)	*P* (3rd vs 12th month)
mGFR	54.1 ± 15.2	49.9 ± 15.2	46.8 ± 16.5	0.015	0.083	0.000
SCr (mg/dl)	1.07 ± 0.33	1.09 ± 0.36	1.13 ± 0.33	1.000	0.380	0.380
SCys-C (mg/dl)	1.27 ± 0.38	1.25 ± 0.38	1.33 ± 0.37	1.000	0.173	0.438
MDRD	78.9 ± 23.8	78.4 ± 2.3	73.6 ± 25.1	1.000	0.433	0.246
Cockcroft–Gault	85.1 ± 22.1	87.5 ± 26.2	83.6 ± 25.5	0.757	1.000	0.611
Le Bricon	70.5 ± 19.4	72.1 ± 20.2	67.9 ± 20.5	1.000	0.163	0.569
Hoek	64.2 ± 20.0	65.8 ± 20.8	61.5 ± 21.2	1.000	0.163	0.569
Larsson	66.9 ± 28.4	69.5 ± 31.4	64.1 ± 32.2	0.944	0.163	0.864
Filler	77.1 ± 25.3	79.1 ± 26.3	73.7 ± 26.9	1.000	0.170	0.601

Table 4. Bias and accuracy of the equations with respect to mGFR.

	3rd month		6th month		12th month	
	Bias	Accuracy 30%	Bias	Accuracy 30%	Bias	Accuracy 30%
MDRD	24.9 ± 17.5	31.8	28.4 ± 18.1	25.0	26.8 ± 14.5	22.7
Cockcroft–Gault	31.0 ± 15.7	20.4	37.6 ± 18.7	11.3	36.9 ± 14.6	2.2
Le Bricon	16.5 ± 13.6	56.8	22.1 ± 12.9	31.8	21.1 ± 13.4	36.3
Hoek	10.2 ± 14.0	70.4	15.8 ± 13.3	61.3	14.7 ± 13.8	50.0
Larsson	12.9 ± 20.6	65.9	19.6 ± 22.5	54.5	17.4 ± 23.2	47.7
Filler	23.0 ± 18.0	38.6	29.2 ± 17.6	20.4	26.9 ± 18.0	27.2

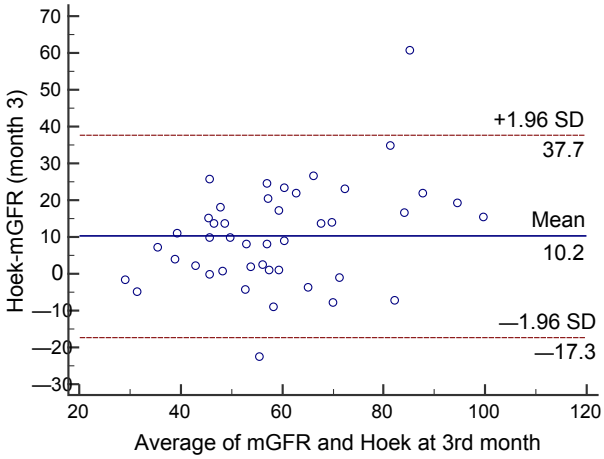

Figure 1 Bland–Altman plot for mGFR and Hoek at 3rd month.

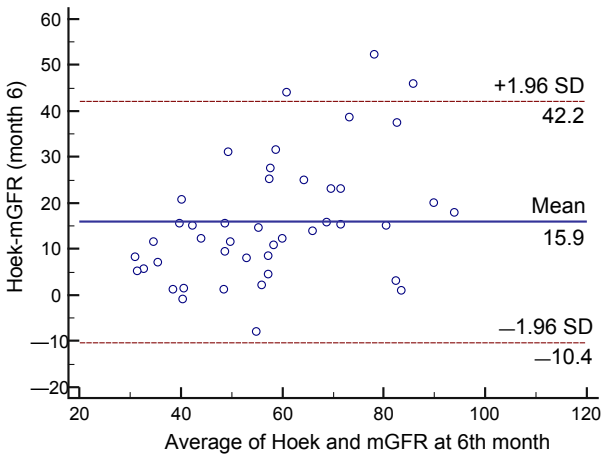

Figure 2 Bland–Altman plot for mGFR and Hoek at 6th month.

mGFR at 3rd month was significantly correlated with percentage of sclerotic glomeruli (*P*:0.005, *r*:−0.434), ci (*P*:0.048, *r*: −0.300), ct (*P*:0.034, *r*: −0.320), sum of ci + ct (*P*:0.040, *r*: −0.311) and ah (*P*: 0.030, *r*: −0.339) at zero hour biopsy and also correlated with ci (*P*:0.036, *r*: −0.317), and ah (*P*:0.021, *r*: −0.360) at 12th month. MDRD and CG at 3rd month were significantly correlated with ct (*P*:0.026, *r*: −0.334; and *P*:0.033, *r*: −0.321), sum of ci and ct (*P*: 0.041, *r*: −0.310; *P*:0.040, *r*: −0.311), and ah (*P*:0.012, *r*: −0.388; and *P*:0.010, *r*: −0.399) at 12th month. Le Bricon, Hoek, and Larsson at 3rd month were significantly correlated with ah (*P*:0.049, *r*: −0.309) at 12th month.

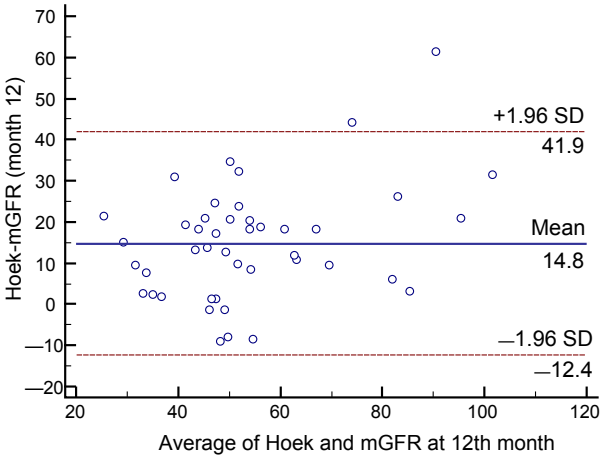

Figure 3 Bland–Altman plot for mGFR and Hoek at 12th month.

mGFR at 6th month was significantly correlated with percentage of sclerotic glomeruli (*P*:0.007, *r*: −0.416) at zero hour, ci (*P*:0.041, *r*: −0.309), ct (*P*:0.023, *r*: −0342), sum of ci + ct (*P*:0.011, *r*: −0.381) at 6th month, and ci (*P*:0.045, *r*: −0.304) at 12th month. MDRD (*P*:0.016, *r*: −0.375) and CG (*P*:0.005, *r*: −0.429) at 6th month were significantly correlated with ah at 12th month. Le Bricon, Hoek, and Larsson were significantly correlated with ct (*P*:0.038, *r*: −0.313) and sum of ci + ct (*P*:0.023, *r*: −0.343) at 6th month.

mGFR at 12th month was significantly correlated with percentage of sclerotic glomeruli (*P*:0.031, *r*: −0.338) at zero hour, ci (*P*:0.015, *r*: −0.365), and sum of ci + ct (*P*:0.046, *r*: −0.303) at 12th month. CG was significantly correlated with ci (*P*:0.028, *r*: −0.331), ct (*P*:0.048, *r*: −0.299), and sum of ci + ct (*P*:0.031, *r*: −0.326) at 12th

Table 5. Histopathological features at protocol biopsies.

	Zero Hour	6th month	12th month	*P* (zero hour vs 6th month)	*P* (6th vs 12th month)	*P* (zero hour vs 12th month)
Glomerulosclerosis (%)	4.8 ± 8.8	5.9 ± 7.5	6.2 ± 7.5	1.000	1.000	1.000
ci	0.33 ± 0.47	0.43 ± 0.50	0.83 ± 0.73	0.970	0.015	0.000
ct	0.21 ± 0.41	0.40 ± 0.49	0.69 ± 0.71	0.175	0.151	0.001
ci + ct	0.52 ± 0.79	0.84 ± 0.88	1.50 ± 1.35	0.182	0.034	0.000
ah	0.28 ± 0.70	0.22 ± 0.54	0.42 ± 0.80	1.000	0.152	1.000
cv	0.28 ± 0.56	0.33 ± 0.53	0.39 ± 0.59	1.000	1.000	1.000

month. Le Bricon, Hoek, Larsson, and Filler were significantly correlated with percentage of sclerotic glomeruli (p:0.045, r: −0.315) at zero hour.

True histopathological progression was defined as ci + ct difference (Δci + ct) ≥ 2 at protocol biopsies. Progression was analyzed for all PBx intervals (from zero hour to 6th month, from 6th month to 12th month, and from zero hour to 12th month) (Table 6). Decrease in mGFR and SCys-c-based formulas in progressor group from 6th month to 12th month was more noticeable than nonprogressor group. There was no significant change in mGFR or eGFR for other intervals.

Discussion

The aim of our study was to investigate which of the many proposed eGFR formula showed the best correlation with mGFR and to elucidate the association of mGFR and eGFR values with subclinical histopathological alterations in protocol biopsies. We hoped to find a substance-based eGFR that correlated with small decrements in graft function undetectable with other routine measures. Unfortunately, both creatinine- and cystatin C-based equations failed to demonstrate similar GFR values with mGFR and did not correlate with biopsy findings. These results thus substantiated our perception that true kidney function is best estimated by mGFR.

Depending on the demographic characteristics, even the allografts from live donors may have significant baseline histological damage [19]. Indeed, almost 18% of the patients in our study have demonstrated ci + ct at implantation biopsies. These emphasize the necessity of implantation biopsies for the assessment of pre-existing histological injury. Monitoring renal function of kidney transplant recipients requires reliable methods that can detect early pathological changes in the graft. This may lead to interventions that prevent progressive renal damage [20,21].

Table 6. GFR decline between 6th and 12th month PBx.

	Delta ci + ct between 6th and 12th month		
	<2	≥2	*P*
mGFR	−1.0 ± 7.4	−7.0 ± 10.8	0.038
Le Bricon	−0.6 ± 12.1	−10.4 ± 15.2	0.024
Hoek	−0.6 ± 12.5	−10.7 ± 15.6	0.024
Larsson	−0.7 ± 15.1	−13.5 ± 20.0	0.021
Filler	−0.7 ± 15.7	−13.5 ± 19.8	0.023

SCr is the most commonly used to evaluate post-transplant kidney function. However, its reliability is troubled by poor correlation with mGFR. SCr-based equations were promising in this context, but these are thoroughly affected by age and gender (i.e., MDRD) [22]. More than 500 studies focused on comparing the MDRD equation with the Cockcroft–Gault (CG) formula in patients with chronic kidney disease and kidney transplantation. Overall MDRD exhibited higher precision than CG, particularly in patients with low range of GFR, and had the best approximation to mGFR [2,3,7,23]. The drawbacks of SCr-based GFR estimations have led to alternatives: Grubb and colleagues were the first to recommend SCys-C levels as an index of GFR and have suggested to replace the MDRD prediction by a SCys-C-based equation corrected with a prepubertal coefficient [24]. SCys-C is produced by all nucleated cells with a remarkably constant rate, eliminated by glomerular filtration, and is not affected by muscle mass and gender. It has been reported to be a better index and superior to SCr for GFR estimation either in chronic kidney disease or in kidney transplant recipients [10,20,22,25,26].

However, both hyper- and hypothyroidism and large doses of glucocorticoids alter SCys-C concentrations, while low or medium doses do not seem to exert any effect [10,27,28]. In our study, we prevented nonrenal influences on SCys-C by administering small and constant doses of corticosteroids and including only euthyroid patients. In addition, we observed a strong correlation between mGFR and SCr- or SCys-C-based eGFR. However, the problem existed with respect to bias and accuracy of the eGFR equations. From this perspective, Hoek and Larsson formulas gave the best result. All patients received TMP/SMZ for the first 6 months after transplantation. After cessation of therapy, no improvement was observed in SCr-based equations. This may be due to the use of low dose (80 mg/day) TMP or complex nature of transplanted kidney.

The clinical use of inulin clearance has been accepted as a gold standard for the GFR measurement. However, this measurement is cumbersome, time-consuming, and noneconomic for routine use clinical practice [29–31]. Radioisotopic filtration markers such as Cr-51 EDTA, Tc-99m DTPA, and I-125 iothalamate are used because of easy applicability as well as the simplicity, accuracy and precision of their measurement. Tc-99m DTPA is readily available radiopharmaceutical of choice for serial GFR determination in clinical practice. However, it is well known that the quality of the commercial preparations varies. Unlike inulin and depending on difference among commercial kits, 10–13% of Tc-99m DTPA binds to plasma proteins. [32]. Therefore, the renal clearance of Tc-99m DTPA underestimates GFR by about 10% when compared with inulin [33]. Despite these limitations, GFR estimation by Tc-99m DTPA may offer a simple and accurate method for the follow-up of GFR over time. The same DTPA kit was used for all tests to prevent the effect of different protein binding rates.

One compared with SCys-c-based formulas, decrements in mGFR were more stable in three periods of study, while eGFR values remained relatively stable over the first year. mGFR values had the better correlation with histopathological scores at protocol biopsies when compared to eGFR formulas and thus are more suitable for the surveillance of kidney function of allografts with stable and well graft function in the first year post-transplant. Creatinine- and SCys-c-based eGFR equations did not have a longitudinal and constant relationship with histopathological changes and have yielded to variable results in different phases of the study. Therefore, they are not reliable for monitoring subclinical graft fibrosis in routine clinical practice.

In the first year after kidney transplantation, mGFR might contribute substantially to assessing graft injury over time when compared with protocol biopsies. Therefore, mGFR might be implemented to evaluate allograft function and considered as the predictor of a protocol biopsy to evaluate emerging injury.

Authorship

AU: participated in study design, data collection, interpretation of the results, writing of the manuscript and surgical procedures. EH: participated in patient management and data collection. ÇŞ and AA: performed radionuclide GFR measurements. SŞ and FAT: performed pathological analysis of allograft biopsies. AN: performed surgical procedures and participated in patient management. MY: participated in patient management, data collection and writing of the manuscript. HT: participated in study design, data collection, interpretation of the results, writing of the manuscript and patient management.

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