Decline in $^{51}\text{Cr}$-labelled EDTA measured glomerular filtration rate following lung transplantation

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Abstract

Background. The nephrotoxicity of calcineurin inhibitors in lung-transplanted patients is well described, but previous studies have estimated rather than directly measured glomerular filtration rate (GFR). This study describes the decline of measured GFR in a large cohort of lung-transplanted patients from a national centre, and the correlation between measured and calculated GFR.

Methods. All lung-transplanted patients 1992–2004 ($n = 390$) were included in a longitudinal analysis. Seven patients were excluded due to retransplantation. Pre- and post-transplant parameters included $^{51}\text{Cr}$-labelled EDTA clearance (mGFR) and the Cockcroft–Gault calculated clearance (cGFR). Trough cyclosporine levels (C0) and demographic and transplant information were also included in the analysis.

Results. A total of 66959 C0 and serum creatinine and 1945 mGFR measurements pertaining to 383 patients were included in the analysis. Pre-transplant mGFR was significantly lower with respect to recipient age over 60 years; and patients with a referral diagnosis of pulmonary hypertension had a lower mGFR and higher baseline serum creatinine levels than patients with emphysematous disease ($P < 0.05$). There were linear correlations between $\log_{10}$ mean interval serum creatinine and $\log_2$ mGFR at all time points pre- and post-transplantation ($P < 0.0001$, Spearman correlation coefficient $= -0.81$) and between $\log_2$ C0 and $\log_2$ mGFR ($P < 0.0001$, Spearman correlation coefficient $= 0.81$), however, the agreement between mGFR and cGFR was poor ($-2.7 \pm 38.6 \text{ml/min}$). A simplified repeated measure ANOVA model describing post-transplant GFR over time demonstrated a 54% decline in mGFR within the first 6 months post-transplant. Pre-transplant mGFR was an important determinant of 6 month post-transplantation mGFR.

Increasing mean C0, body mass index and early acute renal failure were independent risk factors for a more rapid decline in post-transplant mGFR.

Conclusion. mGFR decreases dramatically during the first 6 months after lung-transplantation. Avoidance of high dose calcineurin inhibition may postpone the onset of post-transplant end-stage renal failure.

Keywords: creatinine; cyclosporine; glomerular filtration rate; immunosuppression; lung transplantation

Introduction

The calcineurin inhibitors (CNI), cyclosporine and tacrolimus, still form the backbone of post-thoracic organ transplantation immunosuppression, with over 95% of centres using CNI as part of long-term triple drug regimens [1]. In addition to the risks of infection and malignancy associated with increased levels of immunosuppression, CNI are causally implicated in the development of nephropathy resulting in end-stage renal failure (ESRF) [2,3]. The overall incidence of renal disease in adult post-thoracic organ transplantation is estimated at 36% at 8 years, 7% of whom have clinical ESRF [1]. The development of ESRF not only has a significant impact on patient quality of life, but it is also associated with detrimental survival outcomes in non-renal organ transplantation [4].

The objective of the present study was first to investigate the rate of decline in renal function in a cohort of 390 consecutive lung-transplant patients from a single centre (1992–2004). Renal function was determined by the glomerular filtration rate (GFR), which was both measured using a plasma clearance technique and calculated by the Cockcroft–Gault method. In addition, we analysed potential risk factors for the deterioration in renal function.
Methods

Patients
All single (SLTX), double (DLTX) and combination heart-lung (HLTX) transplant recipients from the Danish National Centre for Lung Transplantation from the start of the program in 1992 to the year ending 2004 were included in this longitudinal analysis of post-transplant renal function (n = 390). Seven patients in the series have been re-transplanted and were excluded from the study. The transplantation procedure and the selection of recipients and donors have been previously described in considerable detail [5].

The parameters used to assess renal function included GFR, as measured by the 51Cr-ethylenediaminetetra acetic acid (EDTA) plasma clearance technique (mGFR) [6] and calculated by the Cockcroft-Gault formula (cGFR) and all serial serum (S-) creatinine levels performed by the hospital over the study period. GFR measurements were performed pre-operatively and then routinely at 6 months and 1 year and then annually thereafter. Results of mGFR investigations were obtained by retrospective chart review. S-creatinine measurements were performed regularly pre-operatively and routinely during admission and outpatient follow-up, post-transplantation. S-creatinine levels performed by the cardiac and cardiothoracic departments over the entire duration of the lung transplant program were obtained by matching unique patient identification numbers from the National Lung Transplant Registry with the hospital biochemistry database.

GFR measurements by 51Cr EDTA at our institution have a variation coefficient of 4.5% and S-creatinine measurements have a variation coefficient of 4%.

Immunosuppressive treatment
This centre has advocated the use of induction treatment where possible. The agents used were antithymocyte-globulin (ATG), antilymphocyte-globulin (ALG) and daclizumab.

A triple therapy maintenance immunosuppressive treatment regimen was continued lifelong. Primary maintenance treatment consisted of cyclosporine, one of either azathioprine or mycophenolate mofetil (MMF) and prednisolone. Trough levels of cyclosporine (C0) were maintained in the range 145–245 μg/l. Tacrolimus or sirolimus had been used as late alternatives to cyclosporine in the event of rapid deterioration in pulmonary function attributable to bronchiolitis obliterans syndrome (BOS) in a small minority of patients.

Acute rejection episodes were treated with intravenous methylprednisolone 1 g daily for 3 days, followed by prednisolone tapered to maintenance dose over a 3 week period. Recurrent or persistent acute rejection has been treated with ATG (if not given previously at induction), or by the substitution of azathioprine for MMF.

Trough levels of cyclosporin were determined as Cyclosporine Monoclonal Whole Blood (TDx-FLx, Abbott).

Antimicrobial prophylaxis and treatment
All lung-transplant procedures have been covered by broad-spectrum antibiotic prophylaxis, which was continued until the removal of all intra-thoracic lines.

Between January 1992 and September 1996, cytomegalovirus (CMV) prophylaxis consisted of acyclovir and donor-receptor CMV matching. With the subsequent introduction of ganciclovir in September 1996, CMV matching was discontinued and all patients received 3 months CMV prophylaxis irrespective of donor and recipient CMV serotype. Patients with acute CMV infection/seroconversion received a minimum of two weeks intravenous or oral ganciclovir treatment.

Antifungal prophylaxis for oropharyngeal Candida consisted of oral nystatin. In addition, all patients received life-long prophylaxis against Pneumocystis carinii and Toxoplasma gondii with combination sulphamethoxazole and trimethoprim treatment.

Data analysis
Data pertaining to recipient age, gender, height, weight, pre-transplant lung disease, type of transplant and choice of induction therapy was ascertained for all patients. Measured GFR values are given corrected for surface area and standardized to a body surface area of 1.73 m².

Data analyses were performed using Statistical Analysis Software (SAS®) version 9.1. Unless specified otherwise, continuous data is described as mean ± SD or median and inter-quartile range (IQR) for normal and skewed distributions, respectively. Group comparisons of continuous data were performed using non-parametric methods throughout. χ² or Fisher’s exact tests were used for group comparisons between categorical data where appropriate. A P value of < 0.05 was used to determine significance. Confidence intervals (CI) are quoted at 5% and 95%. The Bland–Altman method was used to determine limits of agreement between mGFR and cGFR [7].

Multivariate analyses were performed using repeated measures analyses of variance (ANOVA). Patients were entered into the model as a random effect. All other variables were entered as possible fixed effects. A general Satterthwaite approximation was chosen for the determination of denominator degrees of freedom for fixed effects. The model was reduced sequentially by backward elimination (P < 0.05). The following recipient parameters were evaluated: recipient age, gender, body mass index, pre-transplant GFR, acute renal failure (defined as a doubling of creatinine during the first 2 weeks post-transplantation) and presence of arterial hypertension or diabetes mellitus requiring treatment; type of transplantation (SLTX, DLTX or HLTX), induction therapy (ATG, Daclizumab or other) and transplant indication (emphysematous, cystic fibrosis, pulmonary fibrosis, pulmonary hypertension, or other disease).

The development of K/DOQI Stage 5 Chronic Kidney Disease and death prior to the development of K/DOQI Stage 5 are competing events, thus the cumulative incidences of both events are estimated using competing risk analyses. Time dependent Cox proportional hazards models were employed to estimate the effect of the development of K/DOQI Stage 5 on survival.

Results
Seven patients were excluded from the analysis due to retransplantation. A total of 66959 C0 and S-creatinine
and 1945 mGFR values pertaining to 383 patients were included in all subsequent analyses. There were a median number of 70 (IQR, 46–109) C0, 63 (IQR, 40–103) S-creatinine and 4 (IQR, 2–7) mGFR measurements per patient.

Table 1 illustrates the distribution of pre-transplantation S-creatinine and mGFR with respect to recipient demography, mGFR was significantly lower with respect to recipient age over 60 years compared with patients aged between 40–60 years (P < 0.05). Pulmonary hypertension associated with lower pre-transplant GFR than emphysematous and bronchiectatic disease groups and higher pre-transplant serum creatinine than emphysema patients (P < 0.05). HLTX associated with lower GFR and higher serum creatinine pre-transplant than SLTX and DLTX patients (P < 0.05).

The median and inter-quartile range of mean interval S-creatinine and mGFR are shown in Figure 1A and B. Both figures suggest a steep decline in renal function, which occurred during the first 6 months, with stabilization in renal function thereafter. The figures illustrate that by 6 months after transplantation the median mGFR had halved and the median of the mean interval S-creatinine had doubled relative to pre-transplant values. The steep reduction in renal function coincided with peak mean interval C0, which was highest during the first 6 months and decreased thereafter (Figure 1C).

There were strong linear correlations between log₁₀ mean interval S-creatinine and log₂ mGFR at all time points pre- and post-transplantation (P < 0.0001, Spearman correlation coefficient = –0.81) and between log₂ cGFR (derived from the closest serum creatinine measurement time in relation to the date of mGFR measurement (n = 1701), according to the Cockcroft–Gault formula) and log₂ mGFR (P < 0.0001, Spearman correlation coefficient = 0.81). The Bland–Altman plot, however, showed a relatively poor agreement between mGFR and cGFR, with a mean difference of –2.7 ml/min/1.73 m², and limits of agreements of ±38.4 ml/min (precision ± 0.9 ml/min)/1.73 m² (Figure 2). The predictive performance (accuracy) of cGFR as an estimate of mGFR ±30% was 60%.

The cumulative incidence of K/DOQI Stage 5 Chronic Kidney Disease was 1.4% at 6 months and 2.2, 6.8, 13.5 and 22.0% at 1, 3, 5 and 10 years, respectively (Figure 3, Table 2). The percentage of patients dying prior to the development of K/DOQI Stage 5 at 6 months was 15.8%. The onset of K/DOQI Stage 5 was associated with a significant increase in the hazard of death (HR 2.0, CI 1.2–3.3, P = 0.01).

In addition to the 15.8% of patients that died before the date of the first mGFR, 4 patients had not completed at least 6 months follow-up and 25 patients had an mGFR performed at a later time post-transplantation at a median 1 (range 1–7) year. The 21/25 patients with an mGFR at 1 year had a mean GFR of 40 ± 17 (29–45) ml/min, which was not significantly different to the 1 year mGFR of patients included in the 6 month analysis (P = 0.2). No mGFR could be identified for an additional 12 patients (two of whom originated from the Faroe Islands and received care locally).

A repeated measures ANOVA model described a 54% decline in mGFR during the first 6 months post-transplant, in all patients surviving at least 6 months with available mGFR measurements (n = 282). The results of backward elimination multivariate
For the analysis of the aforementioned recipient, transplant and early post-transplant variables, the results are shown in Table 3. Increasing mean C0 and recipient age were independent risk factors for a more rapid decline in GFR at 6 months. In addition, patients with cystic fibrosis (CF) experienced a more rapid decline in renal function compared with patients with chronic obstructive pulmonary disease (COPD) and alpha-1-antitrypsin (A1AT) deficiency. Patients with pulmonary fibrosis or sarcoidosis had a significantly smaller decline in mGFR compared with patients with emphysematous or bronchiactatic respiratory disease.

Thus, the model predicts that an increase in the mean C0 by 100 ng/ml would result in a 17 ml/min further reduction in GFR during the first 6 months, after having made allowances for pre-transplant GFR, recipient age and pre-transplant diagnosis.

Discussion

This study represents the largest longitudinal study of renal function after lung transplantation using an accurate and precise isotope method for determination of GFR. The principle findings of this study were...
A 54% decline in measured GFR during the first 6 months after transplantation in patients surviving 6 months; (ii) increasing mean C0, increasing recipient age, early acute renal failure and a pre-transplant diagnosis of CF were independent predictors of a more rapid decline in GFR during this period; (iii) there was poor agreement between Cockcroft–Gault estimated and 51Cr EDTA measured GFR and (iv) that the onset of K/DOQI Stage 5 was associated with a significant increase in the risk of death post-transplantation. To our knowledge, this is the first study validating the Cockcroft–Gault formula for the estimation of GFR in lung-transplanted patients and the first study attempting to quantify the influence of cyclosporine exposure on the decline in measured GFR.

The inclusion of all patients, in conjunction with such a large number of repeated pre- and post-transplant measurements of renal function measured by a precise isotope method, adds to the validity of the presented findings; however, as with all retrospective studies, it was not possible to account for all potential sources of bias. One of the principal limitations to repeated measures analyses of consecutive measurements is patient drop out, especially in high-risk populations such as lung transplantation. Patient drop out due to early mortality and morbidity may have been an important source of bias. In order to describe the extent of this potential problem, competing risk analyses were employed to estimate the cumulative incidence of K/DOQI Stage 5 Chronic Kidney Disease and graft failure. As retransplanted patients were excluded from this analysis, lung allograft failure was equivalent to patient death.

The decline in renal function after lung transplantation and other solid organ transplantation is previously

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**Table 2.** The cumulative incidence of K/DOQI stage 5 chronic kidney disease (GFR < 15 ml/min/1.73 m² or dialysis) and death prior to the development of K/DOQI Stage 5, as estimated by competing risk analysis

<table>
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<th>Incidence</th>
<th>6 months</th>
<th>1 year</th>
<th>3 years</th>
<th>5 years</th>
<th>10 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>K/DOQI Stage 5</td>
<td>1.4%</td>
<td>2.2%</td>
<td>6.8%</td>
<td>13.5%</td>
<td>22.0%</td>
</tr>
<tr>
<td>Death</td>
<td>15.8%</td>
<td>18.3%</td>
<td>29.3%</td>
<td>34.9%</td>
<td>49.7%</td>
</tr>
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**Fig. 2.** Bland–Altman plot that showed variability (± 1.96SD-dotted lines) between Cockcroft–Gault calculated GFR and 51Cr EDTA measured GFR at all time points pre- and post-transplantation.

**Fig. 3.** Cumulative incidence of K/DOQI Stage 5 Chronic Kidney Disease (GFR < 15 ml/min/1.73 m² or dialysis) and death prior to the development of K/DOQI Stage 5, as estimated by competing risk analysis.
describe [4,8–11] but the majority of studies have relied on serum creatinine or calculated GFR. In patients with reduced muscle mass as seen in lung patients, indirect estimations of kidney function may be inaccurate and as such, probably underestimate the true prevalence and importance of post-transplant renal dysfunction. Furthermore, Broekroelofs et al. [12,13] described a time effect on the predictive performance of creatinine based indices, which should be considered using these. Our study supports this premise. Besides real changes in GFR, fluctuations in serum creatinine following thoracic organ transplantation may also occur due to usual substantial increases in lean body mass, as well as due to increases in the active tubular secretion of creatinine as a result of calcineurin induced nephropathy or direct interference in creatinine tubular secretion by trimethoprim, which is administered to all patients as part of a lifelong pneumocystis prophylaxis regimen.

In this select population of patients, the eGFR was found to be inaccurate and consistently overestimated the mGFR by a mean of 2.7 ml/min (bias), but there was considerable variability between the two measures as indicated by the limits of agreement (defined as ±2 SD of the mean difference). Due to the large number of measurements in this study, the limits of agreement are precise to within ±0.9 ml/min. Agreement appeared to improve at lower levels of GFR. To some extent, this may have been related to treatment with trimethoprim. At lower levels of GFR, tubular excretion of creatinine may result in an overestimation of GFR using non-isotope methods. Since trimethoprim is known to reduce the tubular excretion of creatinine, one would expect a reduction in the discrepancy between the calculated and measured GFR. However, improved agreement between calculated and measured GFR at low levels of GFR is not useful in everyday clinical practice as one of the main goals of determining GFR is to detect early loss of renal function in order to permit therapeutic intervention before the development of ESRF.

The evaluation of previously reported pre-transplant hypertension and diabetes mellitus [8,14], as risk factors for the decline in GFR was restricted to the small numbers of patients receiving pre-transplant antihypertensive (n = 47) or insulin therapy (n = 24) and is complicated by the fact that 18 (75%) of the patients receiving insulin were CF patients. Thus, the association with CF may have masked the importance of pre-transplant diabetes as a risk factor for more rapid decline in renal function. Interestingly, both Broekroelofs et al. [15] and Hmiel et al. [16] concluded an increased risk of post-transplant renal dysfunction in patients with CF. It is possible that this may be related to frequent use of additional nephrotoxic agents including aminoglycosides and amphotericin used in the treatment of acute pulmonary exacerbations in patients with a background of known pseudomonal or aspergillus colonization, respectively.

Despite the accepted association between CNIs and the development of nephropathy [17], few studies investigating post-transplant renal dysfunction have been able to demonstrate statistically significant associations between nephropathy and cyclosporine dose/concentration [8]. In this study, we demonstrate that the risk of early increasing mean C0 was important in predicting a more rapid decline in renal function. Strategies to avert or slow the progression to post-transplant renal disease include CNI dose reduction or withdrawal, with or without the substitution of other immunosuppressive agents. MMF, which is known to have stronger anti-proliferative effects compared with azathioprine, has been employed successfully to buffer CNI dose reduction to levels permitting renal recovery [18,19]. Gonzalez et al. [20] presented evidence to support CNI concentration independent effects of MMF on the reduction in deterioration of post-transplant renal function. Interestingly, the use of MMF in this study was not associated with demonstrable renal improvement.

An association between the occurrence of an acute renal injury in the early post-operative phase of transplantation and subsequent lower long-term renal function at 6 months was also observed. Acute renal injury (as indicated by a doubling of serum creatinine in the first 2 weeks after transplantation) may account for a variety of renal aggressions including large volume fluid shifts, sepsis and antibiotic and immunosuppressive nephrotoxicity. These findings suggest that early renal injury may increase the sensitivity of the kidney to cyclosporine toxicity.

The high prevalence of post-transplant nephropathy is a major challenge to the nephrologists and early referral should be considered. We propose an individual risk assessment based on pre-transplant
GFR and measured by a reliable and precise method.
Patients with CF, and patients with pre-transplant or early post-transplant exposure to additional nephrotoxic agents, such as aminoglycosides and amphoterocin, should also be considered high risk. We also recommend vigilant monitoring of blood CNI levels and subsequent dose tailoring in order to achieve the lowest effective CNI concentration. We look forward to further developments in early or complete CNI avoidance strategies, but understand that the risk of renal dysfunction must be offset by the risk of acute cellular rejection, infection and toxicity of alternative immunosuppressive regimens.

Conclusion

Glomerular filtration rate as measured by $^{51}$Cr EDTA clearance decreases dramatically during the first 6 months following lung transplantation. The Cockcroft–Gault estimation of GFR is an imprecise measure of renal function in patients after lung transplantation. Pre-transplant GFR and C0 appeared to be the most important factors in determining post-transplant renal function. Patients with CF, either due to exposure to additional nephrotoxic agents, or due to a high prevalence of insulin dependent diabetes mellitus, are at particularly high risk of more rapid decline in GFR than patients with other indications for lung transplantation. Avoidance of high dose cyclosporine and other nephrotoxic agents during this early period may help to postpone the onset of end-stage renal disease.

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References


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