Post-transplant nuclear renal scans correlate with renal injury biomarkers and early allograft outcomes

Motaz A. Obeidat1,6, Valerie A. Luyckx2,7, Scott O. Grebe3,8 Gian S. Jhangri4,9, Connor Maguire5,10, Anna Zavodni5,11, Stuart Jackson5,12 and Thomas F. Mueller2,13

1Department of Medicine, Jordan University of Science and Technology, Irbid, Jordan, 2Department of Medicine, Division of Nephrology and Immunology, University of Alberta, Edmonton, Alberta, Canada, 3Division of Nephrology, Helios-Clinics Wuppertal, University of Witten-Herdecke, Witten-Herdecke, Germany, 4Department of Public Health Sciences, School of Public Health, University of Alberta, Edmonton, Alberta, Canada, 5Present address: Department of Medicine, Division of Nephrology and Immunology, University of Alberta, Edmonton, Alberta, Canada, 6Present address: Department of Medicine, Division of Nephrology and Immunology, University of Alberta, Edmonton, Alberta, Canada, 7Present address: Department of Medicine, Division of Nephrology and Immunology, University of Alberta, Edmonton, Alberta, Canada, 8Present address: Department of Medicine, Division of Nephrology and Immunology, University of Alberta, Edmonton, Alberta, Canada, 9Present address: Department of Medicine, Division of Nephrology and Immunology, University of Alberta, Edmonton, Alberta, Canada, 10Present address: Department of Medicine, Division of Nephrology and Immunology, University of Alberta, Edmonton, Alberta, Canada, 11Present address: Department of Medicine, Division of Nephrology and Immunology, University of Alberta, Edmonton, Alberta, Canada, 12Present address: Department of Medicine, Division of Nephrology and Immunology, University of Alberta, Edmonton, Alberta, Canada, 13Present address: Department of Medicine, Division of Nephrology and Immunology, University of Alberta, Edmonton, Alberta, Canada

Correspondence and offprint requests to: Thomas F. Mueller; E-mail: tmueller@ualberta.ca

Abstract

Background. Clinical- and histopathology-based scores are limited predictors of allograft outcome. In addition, more objective markers of early transplant function are needed to identify and validate biomarkers and predictive scores. We evaluated existing scores and transcriptome biomarkers of kidney injury as predictors of early transplant function measured by renal scan.

Methods. Clinical, histopathologic and transcriptome data were collected in 143 consecutive kidney transplant recipients. A post-operative renal scan was performed within 48 h. Prediction scores for early outcomes were calculated.

Results. Patients were stratified into three groups by renal scan: normal, mild-to-moderate or severe dysfunction. Kidneys with severe dysfunction were more often from deceased donors (P < 0.001), had greater HLA antigen mismatches (P < 0.001), were transplanted into older recipients (P = 0.040), had lower urine output during the first 8 h (P < 0.001), higher Day 7 serum creatinine (P < 0.001) and higher incidence of delayed graft function (P < 0.001). Clinical- and pathology-based scores did not discriminate between scan groups. In contrast, the overall transcriptome (P < 0.001) and transcripts of preselected acute kidney injury (AKI) genes were significantly different between the groups, with kidney injury molecule 1 (P = 0.001) and neutrophil gelatinase-associated lipocalin (P = 0.002) being most highly expressed and genes associated with glutathione metabolism (GSTA1, 3 and 4) most down-regulated in kidneys with subsequent severe dysfunction.

Conclusions. Renal scans reflect early transplant function and allow for a more objective assessment of scores predicting early outcome and for identification of biomarkers. The study shows that transcript levels of AKI genes correlate better with renal scans than clinical- or histopathology-based scores.

Keywords: acute kidney injury; gene expression; graft function; nuclear scan; renal transplantation

Introduction

Kidney transplantation is inevitably associated with ischemia-reperfusion and acute kidney injury (AKI) [1, 2]. Injury is more severe in deceased donor (DD) than living
donor (LD) organs due to the effect of brain death and longer ischemia times, which partly explain the higher incidence of delayed graft function (DGF) in DD kidneys [3, 4]. DGF is associated with increased risk of acute rejection and decreased graft survival [3–8]. Occurrence of DGF in an individual allograft recipient is dependent on multiple donor and/or recipient-related factors [9, 10]. Prediction of early and late allograft function is central in transplantation, as this may permit the use of potentially discarded organs, as well as tailoring of medical management to maximize organ recovery. Several prediction scores have been proposed to facilitate decisions of organ acceptance, allocation and management [9, 11–16]. Some rely largely on clinical and demographic data, others on histopathological features of donor kidney biopsies. These scores have not been integrated into widespread routine clinical application, suggesting their predictive utility for the individual patient remains questionable [17, 18]. The limited performance of these scoring tools to predict early and long-term outcome likely reflect their inability to capture the degree of acute injury and intrinsic quality of the donor organ. Subsequent studies have proposed combining clinical and histopathologic variables to enhance prediction [19–21]. We have shown that global transcriptome changes in 1-h implantation biopsies were superior in predicting occurrence of DGF compared to published clinical and pathologic scoring systems [22]. Similarly, analysis of AKI biomarkers neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule 1 (KIM1) and interleukin 18 (IL-18) were found to reflect the degree of injury at the time of transplantation and in some studies, were predictive of higher creatinines or DGF [23–28].

A major obstacle to validation of any prediction score or biomarker is the lack of objective reference markers for renal function post-transplantation. Most studies utilize clinical parameters such as creatinine, estimated glomerular filtration rate (eGFR), urine output or need for dialysis to stratify function. Each of these has its own limitations [2, 29, 30]. Renal scans using radiolabeled compounds are a more objective measure of GFR and provide a snapshot of kidney function reflecting perfusion, filtration, reabsorption and secretion [31, 32]. The aim of this study is to analyze established clinical, pathological, AKI biomarkers and transcriptome data in implantation biopsies as predictors of subsequent early renal allograft function as measured by routine post-transplant scans.

Materials and methods

Patients, clinical data and biopsy processing

The study was approved by the Health Research Ethics Board of the University of Alberta. Clinical data and implantation biopsy samples were collected in 143 consecutive renal transplants, including 76 LD and 67 DD kidneys. Implantation biopsies are taken intraoperatively within 1 h of revascularization. One core of each biopsy is sent for routine histopathology and the preserved for subsequent RNA extraction. RNA processing and microarray analysis using Affymetrix® GeneChips (human Hu133 plus 2.0) is described in detail elsewhere [22, 33].

Maintenance immunosuppression included corticosteroids, mycochelinate mofetil and tacrolimus or cyclosporine. Twelve patients received induction therapy with polyclonal rabbit Anti-Thymocyte Globulin anti-

bodies, the remaining patients were treated with anti-CD25 monoclonal antibodies Basiliximab or Dacluzimab. DGF was defined as need for dialysis within the first 7 postoperative days, as determined by the attending nephrologist. GFR at 12 months post-transplantation was estimated using the simplified Modification of Diet in Renal Disease (MDRD) equation [34].

Renal scans

A routine nuclear medicine renal scan was done within the first 24–48 h post-transplantation. Most scans are performed with 99mTc-mercaptoaceta-
tyglycine (MAG3) although emergency studies are performed utilizing 99mTc-diethylenetriaminepentaacetic acid (DTPA). Time-activity curves are generated from whole kidney and cortical regions of interest. Each scan is interpreted qualitatively by assessing the dynamic images and semiquantitatively using time-activity curve analysis [5, 35]. Scans are interpreted as normal function (N), mild-to-moderate (MM) or severe (S) dysfunction.

Risk scores and transcriptome analysis

Composite risk scores were calculated for each patient using published algorithms: the ‘Irish score’ [9], percent sclerosed glomeruli (%SG) in the biopsy [12] and the global kidney score [15, 36]. The expression profile of the whole transcriptome in each sample was analyzed using principal component analysis (PC analysis). This tool reveals PCs among the samples, i.e. main patterns in gene expression that best explain the overall variability. Most of the variability is accounted for by the first PC (PC1). Based on similarity and difference within this identified PC, each sample was ranked and given a score [37, 38]. Based on the literature, transcript levels of 18 AKI biomarker genes, represented by 33 probesets on the used Affyme-
trix® GeneChips, were analyzed in implant biopsy samples [39].

Statistical analysis

All the statistical analyses were performed using SPSS 17.0 and a P-value <0.05 was considered for statistical significance. The patient demographics, clinical markers, outcome variables, composite scores and transcriptome changes (PC1, IL-18, KIM1 and NGAL) were compared among the three scan groups using analysis of variance (ANOVA) for continuous measures and chi-square test for categorical measures. The multiple comparisons among the three scan groups were corrected using Bonferroni correction.

Transcriptome analysis was performed using GeneSpring GX 11.0.2 software. Transcript levels were compared among the three scan groups using box plots and ANOVA corrected for multiple testing using Benja-
mini Hochberg with a selected P < 0.05 (i.e. with false discovery rate <0.05).

Results

Donor and recipient characteristics

Clinical, pathology and transcriptome data in the 1-hour implantation biopsy were analyzed in 76 LD and 67 DD consecutive kidney allografts. Table 1 outlines baseline donor and recipient characteristics stratified by renal scan group. The severe dysfunction (S) group included more transplants with DD kidneys and the normal function group (N) had more LD allografts (P < 0.001). The renal scan finding of S was significantly associated with more female recipients (P = 0.042), higher donor body weight (P = 0.007), older recipients (P = 0.040) and higher degree of HLA antigen mismatch (P < 0.001). The S group also showed a trend toward longer cold ischemia and revascularization times, as well as lower postoperative blood pressures. Donor predonation serum creatinine and eGFR were not different among groups. The recipients who received kidneys from DD showed no difference in cause of death among the scan groups.
Clinical measures of early allograft outcome associated with scan results

Table 2 shows clinical outcomes stratified by scan group. Urine output within the first 8 h decreased progressively from the N to MM to S groups (P < 0.001). Similarly, creatinine reduction ratio on Day 2 (%CRR2) was lowest, and Day 7 serum creatinine was highest in the S group compared to MM and N groups (both P < 0.001). Fourteen patients (9.8%) had DGF, the incidence increased progressively from N to MM to S groups (P < 0.001). Among patients with DGF, none had N function, 14.3% had MM and 85.7% had S dysfunction. Conversely, immediate graft function occurred 27.2% in N, 58.1% in MM and 14.7% in S (P < 0.001). Allograft function at 12 months as measured by eGFR (P = 0.738) or serum creatinine (P = 0.588) was not different between the scan groups.

Pathology scores not associated with scan results

Figure 1 shows graphically that the early transplant function, as measured by renal scans, is basically independent of the severity of histopathology changes or clinical risk factors. The scans of N, MM and S dysfunction distribute similarly between groups classified according to pathology or clinical scores. Altogether, 134 biopsies with sufficient tissue material were stratified into two groups according to %SG using a 20% cut off: 125 had <20% SG and 9 had ≥20% SG [12, 15]. Transplants with a low compared to a high degree of glomerulosclerosis showed the same percentage of transplants with N function, MM and S dysfunction. The distribution of early graft function was not different between the two pathology-based groups (Figure 1a). Furthermore, all biopsies with at least 20 glomeruli (n = 51) were stratified using the global kidney score [15, 36]. Thirty-seven biopsies had a score of 0–3 and 14 had a score of ≥4, the cut-off suggested for using both mate kidneys for donation to one single recipient [15, 36]. Comparable to the findings with %SG, the pathology-based scoring according to the global kidney score does not associate with early function as measured by the renal scans (Figures 1a and b). Early function as classified according to the renal scans was not significantly different between the two pathology-based groups (Table 3). Similarly, there was no difference between kidneys with or without DGF using either score (data not shown). An additional analysis of chronic renal lesions and longer term outcome showed that neither %SG nor global

<table>
<thead>
<tr>
<th>Variables</th>
<th>N (n = 35)</th>
<th>MM (n = 77)</th>
<th>S (n = 31)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor type</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LD</td>
<td>82.9 (29)</td>
<td>54.5 (32)</td>
<td>16.1 (5)</td>
<td>&lt;0.001a</td>
</tr>
<tr>
<td>DD</td>
<td>17.1 (6)</td>
<td>45.5 (45)</td>
<td>83.9 (26)</td>
<td></td>
</tr>
<tr>
<td>Donor gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>31.4 (11)</td>
<td>39.0 (30)</td>
<td>45.2 (14)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>68.6 (24)</td>
<td>61.0 (47)</td>
<td>54.8 (17)</td>
<td>0.521a</td>
</tr>
<tr>
<td>Donor age (year)</td>
<td>39 ± 12</td>
<td>45 ± 15</td>
<td>44 ± 16</td>
<td>0.178b</td>
</tr>
<tr>
<td>Donor weight (kg)</td>
<td>75 ± 15</td>
<td>74 ± 17</td>
<td>86 ± 23</td>
<td>0.007b</td>
</tr>
<tr>
<td>Donor serum creatinine (μmol/L)</td>
<td>73 ± 22</td>
<td>70 ± 17</td>
<td>68 ± 24</td>
<td>0.673b</td>
</tr>
<tr>
<td>Donor eGFR (MDRD) (mL/min)</td>
<td>101 ± 41</td>
<td>105 ± 46</td>
<td>118 ± 62</td>
<td>0.333b</td>
</tr>
<tr>
<td>Donor COD (in the 67 DD)</td>
<td>16.7 (1)</td>
<td>40.0 (14)</td>
<td>30.8 (8)</td>
<td>0.478a</td>
</tr>
<tr>
<td>Cerebrovascular</td>
<td>83.3 (5)</td>
<td>60.0 (21)</td>
<td>69.2 (18)</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recipient gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>20.0 (7)</td>
<td>29.9 (23)</td>
<td>48.4 (15)</td>
<td>0.042a</td>
</tr>
<tr>
<td>Male</td>
<td>80.0 (28)</td>
<td>70.1 (54)</td>
<td>51.6 (46)</td>
<td></td>
</tr>
<tr>
<td>Recipient age (year)</td>
<td>47 ± 15</td>
<td>49 ± 15</td>
<td>55 ± 11</td>
<td>0.046b</td>
</tr>
<tr>
<td>Recipient weight (kg)</td>
<td>84 ± 20</td>
<td>80 ± 21</td>
<td>79 ± 16</td>
<td>0.577b</td>
</tr>
<tr>
<td>Cold ischemia time (min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1440</td>
<td>97.1 (34)</td>
<td>96.1 (74)</td>
<td>87.1 (27)</td>
<td>0.138a</td>
</tr>
<tr>
<td>≥1440</td>
<td>2.9 (1)</td>
<td>3.9 (3)</td>
<td>12.9 (4)</td>
<td></td>
</tr>
<tr>
<td>Revascularization time (min)</td>
<td>37 ± 8</td>
<td>38 ± 8</td>
<td>39 ± 9</td>
<td>0.551b</td>
</tr>
<tr>
<td>MAP (intra-operative) (mmHg)</td>
<td>76 ± 7</td>
<td>78 ± 9</td>
<td>76 ± 7</td>
<td>0.421b</td>
</tr>
<tr>
<td>Recipient sBP (mmHg) at Day 0</td>
<td>135 ± 18</td>
<td>133 ± 18</td>
<td>126 ± 21</td>
<td>0.127b</td>
</tr>
<tr>
<td>Day 1</td>
<td>139 ± 20</td>
<td>136 ± 20</td>
<td>129 ± 21</td>
<td>0.109b</td>
</tr>
<tr>
<td>Number of HLA mm</td>
<td>65.7 (23)</td>
<td>39.5 (31)</td>
<td>32.3 (10)</td>
<td>&lt;0.001a</td>
</tr>
<tr>
<td>0–3</td>
<td>34.3 (12)</td>
<td>60.5 (46)</td>
<td>67.7 (21)</td>
<td></td>
</tr>
<tr>
<td>4–6</td>
<td>22.9 (8)</td>
<td>26.0 (20)</td>
<td>19.4 (6)</td>
<td></td>
</tr>
<tr>
<td>PRA &lt;10%</td>
<td>77.1 (27)</td>
<td>74.0 (57)</td>
<td>80.6 (25)</td>
<td>0.981a</td>
</tr>
<tr>
<td>≥10%</td>
<td>22.9 (8)</td>
<td>26.0 (20)</td>
<td>19.4 (6)</td>
<td></td>
</tr>
</tbody>
</table>

N: normal allograft function; S: severe dysfunction on the renal scan; MDRD: donor eGFR using Modification of Diet in Renal Disease equation; COD (in DD): cause of death in the DDs; MAP: average mean arterial pressure intra-operatively; sBP: mean systolic blood pressure at post-operative; HLA: HLA mismatch number; PRA: panel reactive antibodies; aP-value by chi-square test; bP-value by ANOVA.
kidney score and interstitial fibrosis/tubular atrophy correlated with 1-year eGFR (P-values > 0.05).

Clinical scores not associated with scan results

The transplants using DD samples were stratified into tertiles based on the ‘Irish score’, a tool integrating donor and recipient variables at time of transplantation as predictors of risk of DGF [9]. As shown in Figure 1c, renal scan distribution did not differ between the transplants with a low, intermediate or high risk (63–125, 126–136 and 137–161 points, respectively) to develop DGF (P low, intermediate or high risk (63–125, 126–136 and 137–161 points, respectively) to develop DGF (P

Transcript levels of AKI biomarkers differ significantly among scan groups

Using PC analysis across the whole transcriptome of the implant biopsy, the mean PC1 scores were significantly different between the renal function groups as classified by renal scans (Table 3). The overall variability among the samples, as measured by the PC1 score, is reflected in the separation into normal, mild-to-moderate and severely dysfunctional transplants during the first two postoperative days. As shown by Figure 1c, kidneys with lower PC1 scores are significantly more likely to have a poor early transplant function as measured by renal scans (P < 0.001 and P < 0.015 for N versus S and MM versus S, respectively). However, the PC1 scores between N and MM transplants are largely overlapping, suggesting only a trend toward separating these two functional groups (P = 0.05 for N versus MM).

A recent meta-analysis proposed different biomarkers measured in urine or blood as predictors of AKI [39]. These 18 genes are represented by 33 probesets on the Affymetrix microarray Hu133 plus 2.0. The individual markers and their average transcript levels in the three functional groups are listed in Supplementary Table 1. The expression of these biomarkers as measured by the relative transcript levels within the renal function groups is shown in Figure 2. The box-and-whisker plots show the most significant outliers in each function group. The greatest changes in transcript levels were seen in group S, with expression of KIM1 being highest (adjusted P = 0.010) followed by NGAL (adjusted P = 0.022) within the groups. LDHA (M-subunit of lactate dehydrogenase, LDH) was significantly more highly expressed in S compared to MM and N groups (P < 0.001). Conversely, GSTA1 and GSTA3 (glutathione-S-transferase alpha 1 and 3) were both down-regulated in S kidneys (P < 0.001) compared to their transcript levels in the MM and N groups. Expression of IL-18, alkaline phosphatase, neutrophil adhesion receptor CD11b, matrix metalloproteinase-9, N-acetyl-b-D-glucosaminidase (NAG) and sodium hydrogen exchanger isoform 3 were not different among the groups.

Discussion

Changes in the implant biopsy transcriptome as a whole, and in preselected AKI biomarker genes, correlate with early allograft function measured by renal scans better than clinical- or pathology-based scores. These findings highlight the importance of incorporating markers of renal injury to enhance prediction scores.

Better prediction of early function would facilitate individualization of peritransplant management as well as assist in determining organ quality as a criterion for acceptance of an organ for donation. Published prediction scores utilize either histologic criteria alone or clinical and demographic characteristics of donor and recipient pairs
These scores have been validated in specific populations but generalizability and applicability to the individual patient has been limited [2, 17, 18, 22]. Gaber et al. [12] reported a higher incidence of DGF in kidneys with >20% sclerosed glomeruli compared to two groups with fewer sclerosed glomeruli. Remuzzi et al. [15] developed a global kidney score incorporating histopathologic changes in the glomeruli, interstitium, tubules and vessels and found similar outcomes when single kidney with scores of 0–3 were transplanted compared to double kidneys with scores of 4–6, suggesting kidneys with worse scores would not perform well and could not be transplanted alone. Kidneys with scores ≥7 were discarded. Karpinski et al. evaluated the same scoring system in high-risk donors alone and found those with a score of ≥6 had higher risk of DGF and higher serum creatinines compared to those with a score of <6. We did not find a significant association between either score and scan group, although there was a non-significant trend toward more severe dysfunction with higher global kidney score. The lack of association of histopathologic scores with renal function is not surprising, as features of AKI may be very subtle on biopsy [40, 41]. In our cohort, however, there were fewer patients with poor scores which may have been biased toward the null.

Irish et al. developed a nomogram, based on 16 donor- and recipient-derived clinical variables, significantly associated with DGF in a registry database. Irish scores are not different among the three scan groups, although no patient in the highest tertile had a normal scan. The lack of association with scan function may have resulted from the small number of patients or the short cold ischemia time, as most donors are local at our center. Notwithstanding these potential limitations, several patients with low scores did have severe dysfunction, and some with high scores had normal function, pointing to the limited individual applicability of such database-derived nomograms [17, 18].

The lack of simple and reliable outcome measures of kidney function after injury is a major obstacle to studying AKI and early allograft function. Serum creatinine and GFR estimations have recognized limitations, well highlighted in a study where group differences in the measured GFR were not detected by eGFR calculations [42]. Similarly, DGF is often used as a hard outcome, but definitions are not consistent and may be subjective [2, 10, 30]. DGF also does not capture kidneys with slow graft function, which may be incorrectly categorized as ‘immediate’ graft function, i.e. non-DGF. More refined assessment of renal function after injury is required. Nuclear medicine renal scans are used to assess renal dysfunction in transplant recipients [31, 32, 43]. We have shown a strong association between the three categories of graft function (N, MM and S), as measured by routine MAG3 or DTPA scanning on postoperative day 1 or 2, and clinical measures of early allograft function, with the S group having the worst clinical parameters. These findings are supported by others, demonstrating clinical validation and relevance of such qualitative scan results [44]. Renal scans can provide more subtle and objective evaluation of early allograft function than creatinine-based methods or DGF alone and may provide more reliable end points for validation of prediction scores and biomarker performance.

In contrast to the clinical- and pathology-based scores, the transcriptome changes in the implant biopsies differed significantly among scan groups. Both unsupervised analysis using the overall transcriptome changes and analysis of preselected transcript levels of AKI biomarkers derived from the literature showed significant differences between groups, consistent with our previous results [22]. Association between global gene expression and renal function strongly underscores the importance of intrinsic kidney quality in early function. Plausibly, AKI biomarker gene expression changed progressively with worsening renal function. The S group had more DD transplants and a trend toward longer ischemia times, both likely contributors to greater kidney injury. Simultaneous analysis of 18 AKI biomarkers identified genes being up-regulated and

![Fig. 1. Distribution of kidneys with normal function (N), mild-to-moderate (MM) and severe dysfunction (S) in relation to degree of histopathology changes and clinical risk scores. (a) Donor kidneys grouped according to %SG [12]. (b) Donor kidneys grouped according to the global kidney score [15]. (c) DD transplants grouped according to the ‘Irish score’ [9]. Transplants with better scores (left half) show a similar distribution of function from N to MM to S compared to those with poorer scores (right half).]
downregulated, with deviation from baseline becoming progressively greater with increasing severity of renal dysfunction, supporting their clinical relevance. The best performing genes were KIM1 and NGAL, whose expression is known to increase with degree of kidney injury [23–26, 28, 39, 45–48]. These results support the diagnostic and predictive values of these biomarkers at the time of transplantation, which has been shown in some, but not all recent studies [23–27, 49]. The time courses of up-regulation of NGAL and KIM1 are somewhat different with KIM1 rising later than NGAL [45]. It is possible that KIM1 expression may reflect earlier kidney injury, i.e. associated with brain death, as suggested by others [25]. Interestingly, IL-18 which has also been shown to be a good predictive urinary marker for DGF was not changed significantly in our study [26, 45]. This finding may reflect that urinary IL-18 is not derived from local synthesis but may be derived from circulating IL-18. Similarly, with the other AKI biomarker genes, protein detectable in the urine may not derive from gene transcription or change in expression may occur at

### Table 3. Performance of clinical prediction scores and transcript levels in relation to renal function groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SD or % (n)</th>
<th>N (n = 35)</th>
<th>MM (n = 77)</th>
<th>S (n = 31)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prediction scores&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sclerosed glomeruli</td>
<td>94.1 (32)</td>
<td>93.1 (67)</td>
<td>92.9 (26)</td>
<td>0.975&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>&lt;20%</td>
<td>5.9 (2)</td>
<td>6.9 (5)</td>
<td>7.1 (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥20%</td>
<td>84.6 (11)</td>
<td>73.1 (19)</td>
<td>58.3 (7)</td>
<td>0.352&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Global kidney score</td>
<td>0–3</td>
<td>15.4 (2)</td>
<td>26.9 (7)</td>
<td>41.7 (5)</td>
<td></td>
</tr>
<tr>
<td>≥4</td>
<td>115 ± 27</td>
<td>132 ± 16</td>
<td>129 ± 16</td>
<td>0.097&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Transcriptome changes&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PC1</td>
<td>14.99 ± 19.80</td>
<td>3.49 ± 24.72</td>
<td>−10.68 ± 23.26</td>
<td>&lt;0.001&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>IL-18</td>
<td>0.027 ± 0.526</td>
<td>0.067 ± 0.509</td>
<td>0.273 ± 0.787</td>
<td>0.178&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>KIM1</td>
<td>−0.135 ± 0.589</td>
<td>0.385 ± 1.051</td>
<td>0.774 ± 1.176</td>
<td>0.001&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>NGAL</td>
<td>−0.082 ± 0.686</td>
<td>0.230 ± 0.731</td>
<td>0.703 ± 1.286</td>
<td>0.002&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

N: normal allograft function, MM: mild to moderate dysfunction, S: severe dysfunction on the renal scan

<sup>a</sup>P-value by chi-square test

<sup>b</sup>P-value by ANOVA

<sup>c</sup>Calculated according to the published algorithms [9,12,15]

<sup>d</sup>Calculated according to the relative intensity levels on the microarrays [22]; PC1: score calculated by PC analysis for the PC one.
different time points not captured in the 1-h implantation biopsy; therefore, their potential utility cannot be determined in our study [50].

Importantly, timing of the donor kidney biopsy, i.e. during harvesting, after cold ischemia or after revascularization, may hamper generalizability of results from one AKI biomarker study to another [22, 25, 27]. We suggest that the 1-h implantation biopsy encompasses all potential ‘hits’ sustained by the donor organ and therefore may be an optimal predictor of early function, although these data cannot be utilized for decisions whether to accept or not accept a graft for transplantation. The renal scans, as well as AKI markers, histology and clinical scores, did not predict longer term outcomes as measured by eGFR at 12 months. This might be partly due to the overall good organ quality of the transplants used in this study. A limitation of our study is the relatively small number of patients; however, being a single center, it is likely that there was some uniformity in patient care, scan interpretation or biopsy reading that may be a strength. In addition, we had detailed clinical and histopathologic data on each donor-recipient pair strengthening the clinical validation of our outcome measures. Ideally, exact measurement of GFR in each individual patient would be desirable, but GFR measurements need steady-state conditions. We retrospectively evaluated routine scans as a semiquantitative measure of renal function. The scans associated strongly with clinical parameters and this supports clinical relevance and strength of the underlying study. A potential weakness of our analysis is inclusion of scans at 24 or 48 h. This is an uncontrollable variable depending on day and time of transplantation. Again, clinical measures associated with scan results makes this an unlikely confounder. The inability of the renal scan to determine the cause of renal dysfunction, e.g. acute tubular necrosis, medication toxicity etc., may have contributed to some bias. However, gene expression did associate with renal function, suggesting some uniformity within groups.

Improving early and late renal allograft outcomes remains a challenge in transplantation. Reliable predictive markers or scores applied early in the transplantation process would allow tailoring of management to prevent or limit kidney damage, as well as potentially increase organ utilization. Existing clinical and histopathologic scores capture donor and recipient demographic data as well as evidence of chronic renal injury. We suggest that incorporation of changes in gene expression into prediction scores will also capture dynamic acute injury and provide a more comprehensive assessment of organ quality and potential performance.

**Acknowledgement.** Drs T.F.M. and V.A.L. were supported by grants from the University Hospital Foundation and the Transplant Value Added Fund of the University of Alberta. Dr T.F.M. was a co-investigator in the Genome Canada Project. We are grateful for the generous participation of our patients, the cooperation of the HOPE Program and collaboration of Dr Philip Halloran. This work was presented in part at American Society of Transplantation, May 2009.

**Author contributions:**

1. **Motaz Obeidat:** data collection, data analysis and manuscript writing
2. **Valerie A. Luyckx:** study design, data analysis, manuscript writing
3. **Scott O. Grebe:** data analysis and manuscript writing
4. **Gian S. Jhangri:** statistical analysis, interpretations and writing of statistical results
5. **Connor Maguire:** data collection and interpretation of renal scans

6. **Anna Zavodni:** data collection and interpretation of renal scans
7. **Stuart Jackson:** interpretation of renal scans
8. **Thomas F. Mueller:** sample collection, study design, microarray analysis, data analysis, manuscript writing

**Grant support:** Drs Mueller and Luyckx were supported by grants from the University Hospital Foundation and the Transplant Value Added Fund of the University of Alberta. Dr Mueller was co-investigator in the Genome Canada Project.

**Conflict of interest statement:** None declared.

**References**


