Abstract

Background:
GcfDNA, a biomarker for acute rejection and graft dysfunction, was measured in kidney transplantation (KTx) patients. Method:
In a prospective observational trial, GcfDNA was evaluated at pre-specified visits in 98 KTx patients, followed over at least one year post transplantation. Relative percentage (GcfDNA) and absolute quantification of GcfDNA copies (GcfDNAcopies/mL) were performed as previously described [Lee et al., Clin Chem 2014, 60 (Suppl. 1):S196-S1965]. Biopsies were obtained upon clinical suspicion of acute rejection and compared to GcfDNA. Data were analyzed using R 3.4.3 (packages: base, graphics). Pearson correlation analyses were performed on log-transformed data.

Results:
In patients (N=68) without subsequent rejection, active infection, or intervention, GcfDNA was highly elevated (median: 0.42, IQR: 0.05), possibly due to chronic-perfusion damage, in day 3 and KTx samples (N=18). In all 98 patients, GcfDNA values decreased over the first 90 days to a baseline median of 10 copies/mL (IQR: 3.3, 3.9). In patients with biopsy-proven acute rejection (N=14) with samples (N=22) drawn during biopsy-proven acute rejection (BPAR) periods, median GcfDNA was 0.55 and median GcfDNA (1.04 higher than N=5 control samples, 0.588 respectively) than the median observed in samples (N=22) from 62 clinically stable patients without rejection (77 copies/mL, 0.98). These comparisons were confirmed by GcfDNA medians in 5 patients with acute biopsies (14 copies/mL, 4.6IQR). Both GcfDNAcounts/ml and GcfDNA were significantly different between patients with BPAR and apparently stable patients [p<0.001]. To compare the diagnostic accuracy of GcfDNAcopies/mL and GcfDNA, the area under the ROC curve (AUC) were calculated in 76 patients, GcfDNAcopies/mL (AUC: 0.60, 95% CI: 0.52-0.67) better discriminated between patients with BPAR and clinically stable patients than all GcfDNA (AUC: 0.81, 95% CI: 0.75-0.87). Plasma creatinine was not an independent marker, as it was used clinically as an indicator for biopsies. Receiver-operated (ROC)-based diagnostic sensitivity was 90% for GcfDNAcopies/mL and 64% for GcfDNAobtained from ROC curves. Diagnostic specificity was 70% for GcfDNAcopies/mL, and 76% for GcfDNA. The threshold at maximum Y was 37 for GcfDNAcopies/mL and 0.67 for GcfDNA. The correlation between GcfDNAcopies/mL and GcfDNA was r=0.86. Correlations showed a moderate correlation with GcfDNA (r=0.86; r=0.66; r=0.69). In a selected patient subgroup (N=13) without clinically suspected rejection a change of transplant function concentrations (+40%, in samples (N=78) collected at 13 consecutive visits than was a negative correlation (r=0.68) between transplant function and GcfDNAcopies/mL. This suggests that GcfDNA may detect early graft damage due to under-immunosuppression which might increase the risk of de novo DSA formation and subsequent graft loss.

Conclusion:
This is the first systematic comparison of GcfDNAcopies/mL with GcfDNA. Absolute GcfDNA quantification allowed for a better discrimination than GcfDNA of KTx patients with acute rejection and graft injury, due to less influence of resident cytokine variations, and may facilitate personalized immunosuppression.

First year GcfDNA in Stable Patients and During Rejection Episodes

Fig. 1: First year GcfDNA measured as copies/mL in stable KTx patients and during rejection episodes

Table 1: Nonvalued indexed-based diagnostic sensitivity and specificity obtained from ROC curves in BPAR

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive Predictive Value</th>
<th>Negative Predictive Value</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>70%</td>
<td>0.90</td>
<td>0.70</td>
<td>0.90</td>
<td>0.70</td>
<td>0.90</td>
</tr>
</tbody>
</table>

Table 2: Correlation between GcfDNA (copies/mL) and GcfDNA (% or plasma creatinine)

<table>
<thead>
<tr>
<th>GcfDNA copies/mL</th>
<th>GcfDNA %</th>
<th>Plasma creatinine</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.67 (0.52-0.79)</td>
<td>0.27 (0.12-0.41)</td>
<td>&lt;p-value&lt;/0.001</td>
</tr>
</tbody>
</table>

Absolute Quantification of GcfDNA

1. DNA extraction (non-human DNA as internal standard to control extraction efficiency)
2. Determination of total cDNA (cp/mL) using ddPCR
3. Determination of GcfDNA % by ddPCR
4. Calculation of GcfDNA concentration (cp/mL): total cDNA (cp/mL) x GcfDNA %

GcfDNA Identified Unnecessary Biopsies

Fig. 2: Plasma creatinine, GcfDNA fraction and concentration associated with rejection biopsies. GcfDNA identified unnecessary biopsies, which were initially triggered by elevated creatinine.

GcfDNA as Marker of Graft Injury

- Directly interrogates graft health allowing comprehensive monitoring
- Detects rejection early at an actionable stage
- Reveals degree of graft cell injury
- Complements histology findings
- Helps to avoid unnecessary biopsies
- Indicates response to rejection treatment
- Detects under-immunosuppression
- Facilitates personalized immunosuppression
- Shifts emphasis from reaction to prevention

Patient Example

Fig. 5: Exemplary patient time-course

*Reference range: 80-180 μmol/L, CI: confidence interval.