Abstract

**Background:**
Graft-derived cell-free DNA (GcfDNA), a promising new, noninvasive biomarker of allograft rejection and injury status, was investigated in kidney transplanted (KTx) patients.

**Method:**
In a prospective observational trial, GcfDNA was evaluated at pre-specified intervals in 88 patients. Follow-up included at least one post transplant transplantation. Relative percent (GcfDNA) and absolute quantification of GcfDNA copies (GcfDNA/cp/mL) were performed as previously described [Beck J et al., Clin Chem 2014, 43 (Suppl 3) S211-M3].

**Results:**
In patients (n=99) without subsequent rejection, infection, or interventions, GcfDNA was highly elevated (median: 1946, 4.50LOD), presumably due to ischemia-reperfusion injury, in day 1 post KTx samples (n=22). In 30 patients GcfDNA values decreased over time to a baseline median of 15.05 (0.28LOD), where it remained stable throughout the one-year observation period. In patients (n=18) with samples (n=21) drawn during biopsy-proven acute rejection (BAK) periods, median GcfDNA/cp/mL was 5-fold and median GcfDNA: 2.4-fold higher (86 cp/mL, 4.64 respectively) than the median observed in samples (n=22) in 62 clinically stable patients without rejection (17 cp/mL, 0.26LOD). These comparisons were confirmed by GcfDNA median in 5 patients with negative biopsies (14 cp/mL, 0.16LOD). Both GcfDNA/cp/mL and GcfDNA were significantly different between patients with BAK and apparently stable patients (p<0.0001). To compare the diagnostic accuracy of GcfDNA/cp/mL and GcfDNA, the area under the ROC curves (AUC) were calculated in 76 patients.

**Conclusion:**
To compare the diagnostic accuracy of GcfDNA, were significantly different between patients with BAK and apparently stable patients (p<0.0001). To compare the diagnostic accuracy of GcfDNA/cp/mL and GcfDNA, the area under the ROC curves (AUC) were calculated in 76 patients.

**Fig 1:** First year GcfDNA in stable patients and during rejection episodes

**Fig 2:** First year GcfDNA measured as copies/mL in stable KTx patients and during rejection episodes

**Table 1:** Youden index-based diagnostic sensitivity and specificity obtained from ROC curves in BAK vs. stable patient samples

<table>
<thead>
<tr>
<th>Measured</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Youden index</th>
<th>Positive</th>
<th>Negative</th>
<th>Diagnostic Odds Ratio</th>
<th>Threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td>GcfDNA/cp/mL</td>
<td>82%</td>
<td>75%</td>
<td>0.57</td>
<td>22</td>
<td>21</td>
<td>15.05</td>
<td>0.23</td>
</tr>
<tr>
<td>GcfDNA %</td>
<td>72%</td>
<td>79%</td>
<td>0.51</td>
<td>23</td>
<td>21</td>
<td>15.05</td>
<td>0.23</td>
</tr>
<tr>
<td>Plasma creatinine</td>
<td>0.27</td>
<td>0.61-0.70</td>
<td>0.27</td>
<td>0.61-0.70</td>
<td></td>
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</tbody>
</table>

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

<table>
<thead>
<tr>
<th>GcfDNA (cp/mL)</th>
<th>p-value</th>
</tr>
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<tbody>
<tr>
<td>GcfDNA (%)</td>
<td>&lt;0.0001</td>
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</tbody>
</table>

**Absolute Quantification of GcfDNA as a Marker of Rejection and Graft Injury in Kidney Transplantation – Results from a Prospective Observational Trial**

**M. Dellicher a, M. Shipkova b, V. Schauerte c, T. Asendorf d, P.D. Watson e, N. Mettennye e, M. Kabakchi e, D. Ellenberger e, T. Fried e, E. Wieland e, V. Schwenger e, E. Schiott, J. Beck**

**Dept. of Clinical Pharmacology, University Medicine, Göttingen, Germany; **

**Dept. of Medical Statistics, University Medical Center Göttingen, Göttingen, Germany; **

**Clinical Pharmacology, Göttingen, Germany**

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

**Fig 3:** Association between increased GcfDNA and under-immunosuppression, P-value calculated by Fischer Exact-Test.

- **GcfDNA** has potential to identify unrecognized under-immunosuppression in KTx patients carrying the risk of dnDNA formation, e.g. in recipients with high epitope mismatch burden and high immune competence.

- **GcfDNA** is useful to detect late silent chronic active antibody-mediated rejection.

- **Fig 4:** Plasma concentrations, GcfDNA fraction and concentration associated with acute rejection, GcfDNA identified unnecessary biopsies, which were initially triggered by elevated concentrations.

- **Fig 5:** Example patient time-course

**GcfDNA Measurement**

1. Identify informative informative set of 40 assays
2. Quantify graft-derived cell-free DNA by droplet digital PCR (ddPCR)

**GcfDNA Identified Unnecessary Biopsies**

**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**