Immunosuppressant Monitoring by LC-MS/MS Using Mitra™ Microsampling Devices

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Mass Spectrometry: Applications to the Clinical Lab 2016
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Disclosure

• Relevant Financial Relationships:
  • Neoteryx provided:
    • Microsampling devices
    • One night hotel/dinner for MSACL meeting
Objectives

• Determine the feasibility of measuring Tacrolimus and Cyclosporine A by high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) using a dried whole blood sample (20 µL) on a microsampling device compared to 200 µL EDTA whole blood.

• Demonstrate the importance of therapeutic drug monitoring of immunosuppressant's in transplant patients and how microsampling devices might benefit patient care and satisfaction.
Organ Transplants in the United States

30,973 organ transplants in 2015

5% increase from 2014 to 2015

Transplants performed in the U.S.

https://optn.transplant.hrsa.gov/
Organ Donor Statistics in the United States

- ~85 people receive an organ transplant each day
- ~22 people die each day waiting for an organ transplant
- 121,439 people on the waiting list (78,031 active) as of 2-22-16

http://www.organdonor.gov/about/data.html
https://optn.transplant.hrsa.gov/
Organ Donor Statistics in the United States

- ~85 people receive an organ transplant each day
- ~22 people die each day waiting for an organ
- 121,439 people on the waiting list (78,031 active)
- % of recipients still living 5-years post-transplant (Dec 4, 2012):
  - Kidney (deceased donor): 83.4%
  - Kidney (living donor): 92%
  - Heart: 76.8%
  - Liver (deceased donor): 74.3%
  - Liver (living donor): 81.3%
  - Lung: 55.2%

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Immunosuppressive Agents

• Definition:
  • Anti-rejection medications to inhibit or prevent activity of the immune system.

• Use:
  • Reduce and prevent the rejection of transplanted organs and tissues.

• Small Molecule Preparations:
  • Calcinerin inhibitors
    • Cyclosporine A (Neoral ® and Sandimmune ®)
    • Tacrolimus (Prograf ®)
  • Antiproliferative agents
    • Azothiprine
    • Mycophenolate mofetil (CellCept ® and Myfortic ®).
  • mTOR inhibitors
    • Sirolimus (Rapamune ®)
  • Corticosteroids
Calcineurin Inhibitors

• How do they work?
  • Inhibit T Cell activation and interleukin-2.
    • Interfere with the IL-2 Gene transcription essential for activation and proliferation of cytotoxic t-cells in response to alloantigens.
  • Tacrolimus is good at preventing rejection and has better long term graft survival outcomes.
Tacrolimus (Prograf®)

- Therapeutic Drug Monitoring (TDM) is important
  - Narrow Therapeutic Window:
    - Concentration too low:
      - Increased risk of rejection
    - Concentration too high:
      - Hypertension
      - Photosensitivity
      - Hyperglycemia
      - Insomnia
      - Hyperkalemia
      - Nephrotoxicity
      - Neurotoxicity
Example Trough Tacrolimus TDM Goals

- **Kidney Transplant:**
  - 1st month: Trough level at 8-10 ng/mL
  - >1st month: Trough level at 6-8 ng/mL

- **Pancreas Transplant:**
  - 1st month: Trough level at 10-12 ng/mL
  - 1-4 months: Trough level at 8-10 ng/mL
  - >4 months: Trough level at 6-8 ng/mL

- **Liver Transplant:**
  - First 60 days: Trough level at 6-12 ng/mL
  - 60-120 days: Trough level at 4-10 ng/mL
  - >120 days: Trough level at 4-8 ng/mL

- **Goal Levels are always individualized to each patient’s situation.**
Experimental Design

• Compare 200 µL EDTA whole blood to 20 µL dried blood collected on the Mitra™ microsampling device

VS.

• Measure Tacrolimus and Cyclosporine A by LC-MS/MS
  • 20 µL tip + 150 µL CLWR w/ IS; Vortex 20 min @ 2500 rpm
  • 150 µL 0.1 M ZnSO4; Vortex 5 min @ 2500 rpm
  • 150 µL MeOH; Vortex 5 min @ 2500 rpm;
  • Centrifuge; Transfer supernatant to new plate for analysis
Microsampling Precision: Tacrolimus

• Intra-assay \((n=10)\)

<table>
<thead>
<tr>
<th>Tacrolimus</th>
<th>Level I</th>
<th>Level II</th>
<th>Level III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (ng/mL)</td>
<td>6.1</td>
<td>16.8</td>
<td>35.1</td>
</tr>
<tr>
<td>SD</td>
<td>0.44</td>
<td>1.50</td>
<td>4.02</td>
</tr>
<tr>
<td>% CV</td>
<td>7.3%</td>
<td>8.9%</td>
<td>11.5%</td>
</tr>
</tbody>
</table>

• Inter-assay \((n=3 \text{ days})\)

<table>
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<tr>
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<th>Level III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (ng/mL)</td>
<td>5.4</td>
<td>16.0</td>
<td>32.1</td>
</tr>
<tr>
<td>SD</td>
<td>0.49</td>
<td>1.59</td>
<td>2.66</td>
</tr>
<tr>
<td>% CV</td>
<td>9.1%</td>
<td>9.9%</td>
<td>8.3%</td>
</tr>
</tbody>
</table>
Microsampling Linearity: Tacrolimus

y = 1.2274x - 1.4256
R² = 0.9712
Microsampling Accuracy: Tacrolimus

$n=45$ patients

Tacrolimus, ng/mL

$y = 1.083x - 0.816$

$R^2 = 0.947$
Microsampling Precision: Cyclosporine

• Intra-assay \((n=10)\)

<table>
<thead>
<tr>
<th>Cyclosporine</th>
<th>Level I</th>
<th>Level II</th>
<th>Level III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (ng/mL)</td>
<td>138</td>
<td>375</td>
<td>646</td>
</tr>
<tr>
<td>SD</td>
<td>7.9</td>
<td>35.1</td>
<td>65.3</td>
</tr>
<tr>
<td>% CV</td>
<td>5.7%</td>
<td>9.4%</td>
<td>10.1%</td>
</tr>
</tbody>
</table>

• Inter-assay \((n=3\) days\)

<table>
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<th>Cyclosporine</th>
<th>Level I</th>
<th>Level II</th>
<th>Level III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (ng/mL)</td>
<td>120</td>
<td>342</td>
<td>564</td>
</tr>
<tr>
<td>SD</td>
<td>9.8</td>
<td>33.3</td>
<td>53.8</td>
</tr>
<tr>
<td>% CV</td>
<td>8.1%</td>
<td>9.7%</td>
<td>9.5%</td>
</tr>
</tbody>
</table>
Microsampling Linearity: Cyclosporine

Cyclosporine, ng/mL

\[ y = 1.0771x - 26.658 \]

\[ R^2 = 0.9668 \]
Microsampling Accuracy: Cyclosporine

$n=45$ patients
## Microsampling Carryover

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Average Blank % LLOQ Area following High sample</th>
<th>Criteria: Must be &lt;50% LLOQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tacrolimus</td>
<td>43%</td>
<td>Pass</td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>10%</td>
<td>Pass</td>
</tr>
</tbody>
</table>
Conclusions

• Preliminary validation data of Mitra™ microsampling device used to measure both Tacrolimus and Cyclosporine showed acceptable:
  • Precision (Intra- and Inter-day)
  • Linearity
  • Accuracy
  • Carryover

• Future studies with patient self-collected samples will be performed to fully characterize variability in TDM results
Potential Benefits

1. Reduced blood draw (20 µL vs. 4 mL)
   A. Especially important for pediatric transplant patients

2. Ability to collect sample at home
   A. Improved patient satisfaction

3. Centralized TDM of immunosuppressant's
   A. Decreased shipping costs (DBS vs. tube)
   B. Immunosuppressant testing is NOT standardized/harmonized
We Still Need to Standardize Immunosuppressant Testing

Fig. 1. Box-and-whisker plots of Architect, LC-MS, and Dade Dimension test values for samples P-1 (A), P-4 (B), P-6 (C), and P-9 (D).

Vertical solid line across the plots, reference value determined by EM-IDMS; dashed lines, associated expanded uncertainty; values in parentheses, range of tacrolimus concentration values obtained by each tacrolimus test method.

Questions & Discussion