Tandem Mass Spectrometry Improves Immunosuppressant Therapeutic Monitoring

**INTRODUCTION**

Effective Wednesday, April 17, 2013, PeaceHealth Laboratories will use Liquid Chromatography (HPLC) Tandem Mass Spectrometry (MS/MS) for quantitation of the immunosuppressants cyclosporine A, tacrolimus (FK506), sirolimus and mycophenolic acid.

Tandem Mass Spectrometry will significantly improve the specificity for immunosuppressants compared to commonly used immunoassays. Immunoassays are reported to have a positive bias relative to the reference assay, HPLC with detection by MS/MS. Immunoassays can run 15-40% higher than LC/MS/MS due to cross-reactivity with non-pharmacologically active metabolites.

**IMMUNOSUPPRESSANT GENERAL PRINCIPLES**

The goal of immunosuppression is to prevent allograft rejection in organ transplant patients while minimizing drug toxicity and the major sequela of immune suppression, infection and malignancy.

Immunosuppressive regimens are classified as induction, maintenance and anti-rejection. Induction regimen provides intense, early post-operative immune suppression while maintenance and anti-rejection immunosuppression regimens are used through the patient’s life to prevent both acute and chronic rejection.

Most transplant centers use a maintenance regimen consisting of triple immunosuppression therapy with a calcineurin inhibitor (cyclosporine or tacrolimus), an antimetabolite (azathioprine or mycophenolate mofetil) and prednisone. Sirolimus or everolimus are used in some transplant centers, often in place of the calcineurin inhibitor or antimetabolite, or with low dose calcineurin inhibitor without an antimetabolite.

**DRUG MONITORING POST ORGAN TRANSPLANTATION**

- Optimize dose for efficacy
  - Reduce incidence of organ rejection
- Monitor drug concentrations to minimize toxicity
  - Immunosuppressants can cause toxicity to other organs – nephrotoxicity
  - Immunosuppressants can accelerate atherosclerotic diseases, diabetes and hypertension
  - Excessive suppression of the immune system increases vulnerability to life-threatening opportunistic infections (CMV, BK virus) and malignancies (lymphoproliferative diseases, cancer)
- Compliance
  - Side effect issues
  - Cost
  - Lifelong therapy: Therapeutic drug monitoring is essential for optimal patient care

**BENEFITS**

- Significantly improve specificity for immunosuppressants when compared to commonly used immunoassay methods
- Measures four immunosuppressive drugs (cyclosporine A, tacrolimus, sirolimus, mycophenolic acid) simultaneously
- Specific for only parent compound of each drug
### IMMUNOSUPPRESSANT DRUGS

<table>
<thead>
<tr>
<th>Drug Name</th>
<th>Mechanism of Action:</th>
<th>Role</th>
<th>Adverse Effects</th>
<th>Brand Names</th>
<th>Drug Combinations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cyclosporine A</strong></td>
<td></td>
<td>Blocks activation of cytotoxic response of T-cells</td>
<td>Nephrotoxicity Decreased renal blood flow Decreased glomerular filtration rate Hypertension Opportunistic infections (over suppression)</td>
<td><strong>Sandimmune® (non-modified)</strong> Neoral® (modified) Gengraf (modified) Restasis® Cyclosporin</td>
<td>concomitantly with adrenal corticosteroids</td>
</tr>
<tr>
<td><strong>Tacrolimus (FK506)</strong></td>
<td></td>
<td>Calcineurin inhibitor Suppress acute rejection in allograft organ transplants</td>
<td>Malignancy Nephrotoxicity Abnormal liver function Hyperglycemia Opportunistic infections (over suppression)</td>
<td>Prograf® Adagraf®</td>
<td>concomitantly with cyclosporine and corticosteroids</td>
</tr>
<tr>
<td><strong>Sirolimus</strong></td>
<td></td>
<td>Calcineurin inhibitor * Prophylaxis of allograft organ rejection - Liver and kidney transplant - Prevent graft-versus-host disease in allograft stem cell transplant</td>
<td>Thrombocytopenia Leukopenia Hyperlipidemia Opportunistic infections (over suppression)</td>
<td>Rapamycin Rapamune®</td>
<td>in combination with cyclosporine, corticosteroids, OKT3, antithymocyte globulin</td>
</tr>
<tr>
<td><strong>Mycophenolic Acid</strong></td>
<td></td>
<td>Mechanism of action: Inhibit T-cell activation and proliferation and antibody production</td>
<td>Immunosuppressant used in renal, cardiac and hepatic transplantation Inhibits T-lymphocyte activation and proliferation</td>
<td>CellCept® Myfortic®</td>
<td></td>
</tr>
</tbody>
</table>

**Mechanism of action:**
- Inhibit T-cell activation and proliferation and antibody production
- Inhibit T-lymphocyte activation and proliferation

**Adverse Effects:**
- Nephrotoxicity
- Decreased renal blood flow
- Decreased glomerular filtration rate
- Hypertension
- Opportunistic infections (over suppression)
- Malignancy
- Nephrotoxicity
- Abnormal liver function
- Hyperglycemia
- Opportunistic infections (over suppression)
- Thrombocytopenia
- Leukopenia
- Hyperlipidemia
- Opportunistic infections (over suppression)
- GI (gastritis, diarrhea)
- Leukopenia
- Opportunistic infections
- Thrombosis
- Hepatic – abnormal LFTs
- Renal: Increased BUN/creatinine
<table>
<thead>
<tr>
<th></th>
<th>36380 Cyclosporine A</th>
<th>36381 Tacrolimus (FK506)</th>
<th>36382 Sirolimus</th>
<th>36384 Mycophenolic Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Half-life</strong></td>
<td>5-18 hours (modified); 10-27 hours (non-modified)</td>
<td>8-12 hours (average) 4-41 hours</td>
<td>46-78 hours</td>
<td>CellCept oral 18 hours IV 17 hours; Myfortic oral 8-16 hours; Mycophenolic acid glucuronide (MPAG) metabolite 13-17 hours</td>
</tr>
<tr>
<td><strong>Absorption</strong></td>
<td>1-2 hours post dose (modified) 2-6 hours (non-modified)</td>
<td>0.5-4 hours</td>
<td>1-2 hours</td>
<td>CellCept 1.0-1.5 hours Myfortic: 1.5-2.5 hours</td>
</tr>
<tr>
<td><strong>Bioavailability</strong></td>
<td>33% average</td>
<td>7-28%</td>
<td>15%</td>
<td>CellCept 94%; Myfortic 72%</td>
</tr>
<tr>
<td><strong>Volume distribution</strong></td>
<td>3-5 L/kg</td>
<td>1-2 L/kg</td>
<td>4-20 L/kg</td>
<td>CellCept 4 L/kg; Myfortic 5.4 L/kg</td>
</tr>
<tr>
<td><strong>Protein binding</strong></td>
<td>90%</td>
<td>99%, albumin and alpha-1-acid glycoprotein</td>
<td>92%, albumin</td>
<td>MPA 97%, albumin; MPAG 82%, albumin</td>
</tr>
<tr>
<td><strong>Metabolism</strong></td>
<td>CYP3A4 and p-glycoprotein: 31 known metabolites are not active; hydroxylated cyclosporine – 10% active</td>
<td>CYP3A4; 8 known metabolites are not active. 31-O-desmethyl tacrolimus are active</td>
<td>CYP3A4; 7 known metabolites are not active</td>
<td>MMF hydrolyzed in liver to MPA: MPA is glucuronidated to MPAG (not active), acyl glucuronide and 7-O-glucoside (active)</td>
</tr>
</tbody>
</table>
| **Excretion**        | Biliary primarily | Feces (~92%); urine <1% eliminated unchanged | Feces (~92%) | CellCept: MPA (urine < 1%); feces 6%; MPAG: urine 87%; Myfortic: MPA (urine 3%); MPAG (urine >60%)

Clearance of MPA is affected by glucuronidation, free fraction of MPA and enterohepatic circulation

Rate-limiting step for clearance of MPA is conversion to MPAG which is cleared via the kidneys

Drug-drug interaction:

*Antacids with Mg2+ and AlOH decrease peak concentration by 33% (AUC 17%)

*Cholestyramine – 40% decrease in MPA AUC

*Ferrous sulfate – lowers MPA AUC by 90%

*Rifampicin - lowers MPA (induced glucuronidation)

*Salicylates increase free MPA

*High MPAG increase free MPA

* continued on next page *
THERAPEUTIC DRUG MONITORING FOR IMMUNOSUPPRESSANT DRUGS

- Dose adjustments
  - Based on pre-dose (trough) after patient has reached steady state concentration
  - Trough collection – generally demonstrates good correlation with total drug exposure and therapeutic effect
- Monitor compliance and toxicity
  - Drug-drug interaction can occur with inhibitors or inducers of CYP3A4 or p-glycoprotein
- Frequency of monitoring will vary with
  - Type of transplant
  - Length of time since transplant
  - Immunocompromised patients developing infection will require a dose reduction
- Cyclosporine A
  - Dosing protocols that use low-dose cyclosporine are preferred to minimize toxicity (especially nephrotoxicity)
- Tacrolimus
  - Liver transplant patients with hyperbilirubinemia – active metabolite can accumulate due to impaired bile clearance
  - Inactive metabolites may accumulate leading to high bias immunoassay results (use LC/MS/MS to avoid bias)
- Mycophenolic acid
  - In uremic patients MPAG can accumulate several hundred fold higher in plasma and increase free MPA
  - Drug-drug interactions: antiviral drugs (ganciclovir) can increase MPAG concentration
- Sirolimus
  - Immunoassay test methods may exhibit cross-reactivity of everolimus with sirolimus reagents (use LC/MS/MS to avoid bias)

SPECIMEN TYPE FOR THERAPEUTIC DRUG MONITORING

Most specimens are drawn as a trough, just before dose

- Whole blood
  - Cyclosporine A – RBC binding 40-60%
  - Tacrolimus – RBC binding 80%
  - Sirolimus – RBC binding 95%
- Plasma or serum
  - Mycophenolic acid is tested

REFERENCE RANGES FOR IMMUNOSUPPRESSANTS

Refer to the table on page 6 for therapeutic range list by immunosuppressant. The optimal therapeutic range for a given patient may differ from the suggested ranges based on the following:

- Dose
- Time since transplant (induction dose or maintenance dose)
- Type of allograft
- Co-administration of other immunosuppressants
- Interaction with other drugs (CYP3A4 inducers or inhibitors)
- Preference of the transplant center or clinician
- Time of specimen collection relative to prior dose
- Side effects such as hepatotoxicity or nephrotoxicity

CONCLUSIONS

- Organ transplantation success is limited by organ rejection and immunosuppressant drug failure
- The need to prevent organ rejection has led to the discovery and implementation of immunosuppressant drugs
- Therapeutic drug monitoring is necessary for optimal immunosuppressant therapy
**ORDERING INFORMATION**

<table>
<thead>
<tr>
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<th>36382</th>
<th>36384</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclosporine</td>
<td>Tacrolimus</td>
<td>Sirolimus</td>
<td>Mycophenolic Acid</td>
</tr>
<tr>
<td>Alias</td>
<td>Cyclosporin</td>
<td>FK506, Prograf</td>
<td>CellCept</td>
</tr>
<tr>
<td></td>
<td>Cyclosporine A</td>
<td>Adagral</td>
<td>MPA</td>
</tr>
<tr>
<td></td>
<td>Gengraf</td>
<td></td>
<td>Mycophenolate</td>
</tr>
<tr>
<td></td>
<td>Neoral</td>
<td>Rapamune,</td>
<td>Myfortic</td>
</tr>
<tr>
<td></td>
<td>Restasis</td>
<td>Rapamycin</td>
<td></td>
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<tr>
<td></td>
<td>Sandimmune</td>
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</tr>
</tbody>
</table>

**Methodology**

- Liquid Chromatography Tandem Mass Spectrometry

**Performed**

- Monday-Friday

**Released**

- Within 24 hours after receipt at testing laboratory

**CPT Code**

- 80158
- 80197
- 80195
- 80299

**REFERENCES**

2. Chao, N: “Overview of Immunosuppressive Agents Used for Prevention and Treatment of Graft-Versus-Host Disease”; UpToDate; June 2012.
8. Miller, B and Brennan, D; “Maintenance Immunosuppressive Therapy in Renal Transplantation in Adults”; UpToDate, August 2012.
9. Pham, M and Valantine, H; “Induction and Maintenance of Immunosuppressive Therapy in Cardiac Transplantation”; UpToDate, April 2012.
10. Sussman, N and Vierling, J; “Overview of Immunosuppression in Adult Liver Transplantation,” UpToDate, January 2012.

**QUESTIONS?**

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<table>
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<tr>
<th>SPECIMEN REQUIREMENTS</th>
<th>36380 Cyclosporine</th>
<th>36381 Tacrolimus</th>
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<th>36384 Mycophenolic Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collect</td>
<td>One 4 mL lavender top tube (EDTA). Draw blood specimen pre-dose (trough)</td>
<td>One 5 mL red top tube. Also acceptable one 4 mL lavender top tube (EDTA). Draw blood specimen pre-dose (trough).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Handling</td>
<td>Submit whole blood, do not centrifuge; transfer 1 mL well-mixed whole blood to plastic vial</td>
<td>Allow red tube blood to clot, centrifuge and immediately separate serum (or EDTA-plasma) into a plastic vial.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stability</td>
<td>24 hours ambient; 7 days refrigerated; 3 months frozen</td>
<td>6 weeks ambient; 6 weeks refrigerated; 11 months frozen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard Volume</td>
<td>1 mL EDTA whole blood</td>
<td>1 mL serum or plasma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minimum Volume</td>
<td>0.5 mL EDTA whole blood</td>
<td>0.5 mL serum or plasma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transport</td>
<td>Refrigerated or frozen on dry ice</td>
<td>Refrigerated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rejection Criteria</td>
<td>Serum, plasma, clotted whole blood, or specimen left ambient longer than 24 hours</td>
<td>Use of SST or gel-barrier tube, unspun whole blood specimen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Comment</td>
<td>Therapeutic range, trough specimen at steady state</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0-3 months post-transplant: 150-400 ng/mL ≥3 months (maintenance): 75-300 ng/mL Critical value: &gt;700 ng/mL</td>
<td>0-2 months post-transplant: 5-20 ng/mL ≥3 months (maintenance): 5-15 ng/mL Critical value: &gt;25 ng/mL</td>
<td>Maintenance (with cyclosporine or tacrolimus): 4-15 ng/mL Critical value: &gt;25 ng/mL</td>
<td>Maintenance: 1.0-3.5 µg/mL</td>
</tr>
</tbody>
</table>