Immunosuppressant Drug Level Monitoring
The Practical Side

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Objectives
- To appreciate practical issues associated with TDM of immunosuppressants including:
  - Proper specimen type
  - Timing of specimen collection
  - Analytical considerations
  - Reporting of results
  - Communication
  - Problems/Concerns

Immunosuppressants to Discuss
- Cyclosporine
- Tacrolimus
- Sirolimus
- Mycophenolate (mycophenolic acid, MPA)
Proper Specimen Type for TDM

- Cyclosporine
- Tacrolimus
- Sirolimus
- Mycophenolate - serum, plasma

WHY?

Cyclosporine Distribution

- RBCs: 41-58%
- Plasma: 33-47%
- Other cellular components (lymphocytes, granulocytes): the "rest"

Tacrolimus Distribution

- Blood Cellular components: ~80%
- Plasma: ~20%
Sirolimus Distribution

- RBCs: 95%
- Plasma: 3%
- Other cellular components: 2%


Mycophenolate Distribution

- Plasma (97% protein bound)

MICROMEDEX® Online Healthcare Series, 2009

Timing of Specimen Collection

- Cyclosporine: trough (<1-2h predose) vs C2?
- Tacrolimus: trough (<1-2h predose)
- Sirolimus: trough (<4h predose)
- MPA: trough (<1-2h predose) vs abbreviated AUC?
Correlation [Cyclosporine] with AUC_{0-6h}


- 21 kidney transplant patients on tacrolimus, steroids
- Good correlation (r) between C_{0} (trough) and trapezoidal AUC:
  - Day 3: 0.84
  - Day 14: 0.94

**BUT**

with target of 5-10 ug/L, AUC is 75-225 ugh/L or
with an AUC target of 210 ugh/L +/-10%, C_{0} is 4-20 ug/L
Tacrolimus Sampling Time

Correlation between $C_0$ and AUC$_{0-12}$ in de novo renal transplant patients

([https://pharmaco.chu-limoges.fr](https://pharmaco.chu-limoges.fr))

<table>
<thead>
<tr>
<th>Time Post-Transplant</th>
<th>Correlation ($r^2$) [AUC (mg/L) versus $C_0$ (ug/L)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 days</td>
<td>0.87</td>
</tr>
<tr>
<td>3 months</td>
<td>0.72</td>
</tr>
<tr>
<td>6 months</td>
<td>0.60</td>
</tr>
</tbody>
</table>

European Consensus Conference on Tacrolimus TDM (May 2007)

Proposed AUC targets

- 0.15 – 0.21 mg/L (first weeks post transplantation)
- 0.12 – 0.15 mg/L (long-term)

Bottom Line (as of today)

Still no evidence that another exposure index would be better than $C_0$.

Sirolimus Sampling Time

  - 150 transplant patients on sirolimus, cyclosporine, steroids
  - Good correlation between $C_0$ and AUC ($r = 0.83$)
Fig 2. Concentration-time profiles of MPA in three patients on three separate occasions (Days 3, 5, 7)
Correlation between \( C_0 \) and AUC
- MMF: \( r^2 = 0.48 \) (better with tacrolimus co-therapy)
- Enteric-coated MPA-Na: \( r^2 = 0.02 \)


**MPA Sampling Times**

- Limited sampling strategies (AUC determination) (MMF)
  - e.g. 1: \( C_0, C_{0.5h}, \) and \( C_{2.0h} \)
    - Therapy with Tacrolimus
      \[ \text{AUC (mg.h/L)} = 7.75 + 6.49 \times C_0 + 0.76 \times C_{0.5h} + 2.43 \times C_{2.0h} \]
    - Therapy with Cyclosporine or Sirolimus
      \[ \text{AUC (mg.h/L)} = 10.2 + 2.4 \times C_0 + C_{40min} + 1.7 \times C_{2.0h} \]
  - \( r^2 = 0.86, 0.78 \) [Figurski et al, Transplantation (2006): 82(1) Supp 3, 502-3]

- e.g. 2: Bayesian estimator (https://pharmaco.chu-limoges.fr/abis.htm)
  - Abbreviated AUC based on 3 concentrations (30min, 1h, 3h after administration)
  - Target range: 30 – 60 mg.h/L

- free MPA level monitoring
  - altered plasma protein binding
  - better correlation to toxicity

**MPA – Comparison of Trough to AUC Levels**

UAH Experience

- Mypfortic

\[ \begin{align*}
\text{Trough Target: } & \quad 1.0 \to 3.5 \text{ mg/L} \\
\text{AUC Target: } & \quad 30 \to 60 \text{ mg.h/L}
\end{align*} \]
TDM for MPA at UAH

- Routine TDM is not warranted
  (for most patients, standard dose regimes yield excellent efficacy and safety profiles)

- What to monitor?
  - Trough levels (Ther Drug Monit, 2006; 28(2), 145-54)

  Renal:  
  - ≥ 2.0 mg/L (cyclosporine co-therapy)
  - ≥ 1.9 mg/L (tacrolimus co-therapy)

  Cardiac:  
  - ≥ 2.0 mg/L (EMIT)
  - 1.2 – 3.5 mg/L (HPLC)

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TDM for MPA at UAH

- When to Monitor?
  - suspected malabsorption (e.g., cystic fibrosis, patients on high doses with questionable clinical benefit)
  - suspected toxicity at low/moderate doses
  - suspected drug interaction(s)
  - compromised clearance
  - suspected rapid metabolizers
  - verification of compliance
  - patients with immunosuppressive minimization protocol (e.g., CNI withdrawal)

*Guideline developed in cooperation with transplant physicians*

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Immunosuppressant Analytical Techniques

- Immunoassays
- Chromatographic methods
  - HPLC, tandem MS
MPA Assays

- **HPLC, tandem MS**
  - speciation of MPA and metabolites
  - free MPA (tandem MS)

- **EMIT® 2000 Immunoassay (Dade Behring)**
  - Cross-reactivity (~100%) with acyl glucuronide metabolite
  - Proportional bias (Deming) compared to HPLC (n=99 renal transplant patients) (overall bias ~9%)
    

- **Enzyme Receptor Assay (Roche Diagnostics)**
  - automated assay for total MPA
  - Cross-reactivity with acyl glucuronide metabolite (~5%)
  - Based on inhibition of IMPDH mediated conversion of IMP to XMP in presence of MPA (patient plasma)

Immunosuppressant Analytical Techniques

Enzyme multiplied immunoassay technique (EMIT) overestimates compared to LC-MS

CsA: 23% (6 - 46%) n=38
TAC: 30% (-3 - 73%) n=41


Hope for Immunoassays?

- **Cyclosporine CMIA (Chemiluminescent Microparticle IA)**
  - 200 µL whole blood (offline pre-treatment required)
  - AMR: 40 - 1500 µg/L
  - LOQ: ~2 µg/L
  - Minimal metabolite crossreactivity (%) (AM1/AM9): <1.7/<1.9 versus:
    - FPIA (6.7/19.4); EMIT (0.3/7.3); CEDIA (4.4/20.0)
  
  - Bias compared to tandem MS:
    - ≤400 µg/L: ~ the same
    - >400 µg/L: ~ +100 µg/L
Hope for Immunoassays?

- Cyclosporine ACMIA (Chemiluminescent Microparticle IA)
  - LOQ: ~1.5 µg/L
  - Bias:
    - Schmid et al., Clin Biochem, 42 (2009): 1543-48
    - Multi-site evaluation (5): 655 patient specimens
    - Mean bias compared to LCMS/MS: 14-39% higher
    - Crossreactivity with sirolimus metabolites:
      - 11-hydroxy sirolimus: 36.9%
      - 41-O-desmethyl sirolimus: 20.3%

- Tacrolimus ACMIA (Antibody Conjugated Magnetic Immunoassay) method (Siemens)
  - Tacrolimus apparent levels up to 14.4 µg/L in patients with rheumatoid factor >100 IU/mL.
  - Tacrolimus apparent levels up to 24.0 µg/L (no RF; unidentified endogenous antibody).
  - No interference in Abbott IMx and tandem MS methods.

- Cyclosporine ACMIA
  - Cyclosporine apparent level up to 93 µg/L (tandem MS and Abbott Architect CLMI assay; <LOQ).2
  - RF interference ruled out; PEG precipitation gave [ ]<LOQ, suggesting a protein/antibody.

1 Ther Drug Monit. 2009; 31(5): 743-45.

Wyeth

Pharmaceuticals 50 Minthorn Boulevard, Markham, ON L3T 7Y2

2009-11-26

Dear Healthcare Professional:

Subject: Sirolimus Therapeutic Drug Monitoring Assay Comparison

Wyeth (a Pfizer company)* in collaboration with Health Canada, would like to bring your attention to the fact that different laboratory assays used to measure Rapamune trough concentrations generate results that are not interchangeable.

健康 Care Providers should be aware that the methods used to measure Rapamune whole blood concentration have a direct impact on the values obtained.

Several immunoassays have been developed that allow for rapid turnaround of results.

Most immunoassays, including the newer ARCHITECT assay, have a modest bias of approximately 15 - 20% relative to the reference HPLC assay, with detection by tandem mass spectrometry (HPLC/MS/MS) due to antibody cross-reactivity with sirolimus metabolites [2,3].

However, it has recently come to the attention of Wyeth that one of the more commonly used immunoassay platforms, IMx, generally yields results with a greater bias of approximately 10% relative to HPLC/MS/MS [4].

References:

* Wyeth Pharmaceuticals, 50 Minthorn Boulevard, Markham, ON L3T 7Y2

Scientific Affairs
**Tandem Mass Spectrometry (LCMS/MS)**

- Simultaneous analysis of cyclosporine, tacrolimus, sirolimus in <2 min
- 50 µL whole blood + 50 µL ZnSO₄ + 75 µL IS solution (acetoniirile), vortex, centrifuge, transfer ~120 µL supernatant, analyze ("DILUTE and SHOOT")
- AMR: Cyclosporine (70 – 2500 µg/L); tacrolimus, sirolimus (1.0 – 50.0 µg/L)
  - Good for CNS "monitoring" protocols
- **NO** metabolite cross-reactivity
- Issues: capital cost, expertise to operate/maintain; **Imprecision?**
- **CAP Immunosuppressive Drugs EF Survey (CSM-A 2009) (CV%)**
  - Cyclosporine (IA/TM): 5.1-19.8% / 9-13%
  - Tacrolimus (IA/TM): 5.5-29.5% / 8.9-10.6%
  - Sirolimus (IA/TM): 5.5-15.0% / 13.3-14.9%
  - Matrix effects, interferences (e.g. cyclosporine D)

**Immunosuppressants – Reporting of Results**

**Target Ranges:** It all depends!!!!
- Transplant type
- Time post-transplant
- Concomitant immunosuppressive therapy
# Immunosuppressants TDM

## Communication

### Test Name: cyclosporine, blood (CYCLO)
- **Test Code:** CYC
- **Performing Site:** University of Alberta Hospital Laboratory
- **Performing Dept.:** Toxicology
- **Availability:** Test performed within 24 hours of receipt of sample.
- **Tube/Container Type:** Plastic 4 ml EDTA (LAVENDER) Ref# 367861
- **Minimum Collection:** 1 mL
- **Unit of Measure:** ug/L
- **Reference Interval:** Result to be interpreted by clinician. Interpretation is dependent upon various factors such as transplant type, time post-transplant, and concomitant immunosuppressive therapy.

### Additional Test Information:
- Specimens must be in the laboratory by 1330 hours Monday to Friday or 1100 hours on weekends and holidays for the same day analysis.
- For trough, pre-dose: collect <= 1 hour prior to next dose.
- C2, 2h post dose: collect 2 hours (+/- 15 minutes) post dose.
- Complete therapeutic drug monitoring information on requisition.
- Can be combined with sirolimus.
- Never collect from an IV line through which Cyclosporine has been administered.

### Processing Information (Lab/Referral Facility use only):
- Do not spin or separate.
- Specimen stable 1 week at 4˚ C.
- Specimen stable > 1 week at -20˚ C.

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### Test Name: sirolimus, blood (SIRO)
- **Test Code:** Siro
- **Alternate Test Name:** rapamycin, Rapamune
- **Performing Site:** University of Alberta Hospital Laboratory
- **Performing Dept.:** Toxicology
- **Availability:** Test performed within 24 hours of receipt of sample.
- **Tube/Container Type:** Plastic 4 mL EDTA (LAVENDER) Ref# 367861
- **Minimum Collection:** 3 mL
- **Unit of Measure:** ug/L
- **Reference Interval:** Result to be interpreted by clinician. Interpretation is dependent upon various factors such as transplant type, time post-transplant, and concomitant immunosuppressive therapy.

### Critical Value:
- **Additional Test Information:**
  - Specimens must be in the laboratory by 1330 hours Monday to Friday or 1100 hours on weekends and holidays for same day analysis.
  - Steady state: 1-5 days.
  - Steady state: 10-15 days.
  - Daily level monitoring is not required.
  - Collection Information:
    - Collect <= 4 hours prior to next dose.
    - Protect from light.
  - Complete therapeutic drug monitoring information on requisition. Can be combined with cyclosporine or tacrolimus.

### Processing Information (Lab/Referral Facility use only):
- Do not spin or separate.
- Protect from light.
- Specimen stable 1 week at 4˚ C (+/- 15 minutes)

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Last Updated on: Tuesday, December 16, 2008
**Immunosuppressants TDM**

**Problems/Concerns**

- IV line contamination of specimens for TDM
  - "Rechecking tacrolimus concentration value in blood taken from a catheter used for tacrolimus administration."

- "Non-steady state" issue:
  - Time to steady state:
    - Cyclosporine: 1-2 days (at least two doses)
    - Tacrolimus: 2-6 days (at least four doses)
    - Sirolimus: 10-14 days
    - MPA: ~4 days

- Kidney Disease: Improving Global Outcomes (KDIGO) CPG
  - "We recommend measuring CNI blood levels...at least every other day during the immediate post-operative period until target levels are reached."
A Non-Steady State Case

Patient: Heart transplant, 1.5 months old
ALT: 632 (day 1), 419 (Day 0), 238, 92, 74, 68, 25 U/L (Day 9) (Ref: <50 U/L); Creatinine: below/within reference range

Immunosuppressant TDM
Problems/Concerns (continued)

- Missing drug utilization information with requests
- “Baseline” or “endogenous” level requests
- Multiple “<20” cyclo and “<1” siro/tac levels
- Decentralized testing (not in Alberta!!!)

Immunosuppressant Monitoring
The Future

- Intracellular [CNI] in lymphocytes/peripheral blood mononuclear cells
- Quantification of CNI metabolites
- CNI pharmacogenetics: CYP 3A4, CYP3A5, and p-glycoprotein
- MPA pharmacodynamic monitoring: IMPDH inhibition
(blood, mononuclear cells, CD4+ T cells)

Potential impact on clinical outcome?
Complementary to TDM, will NOT replace it
Recommended Reading

“New Insights Into the Pharmacokinetics and Pharmacodynamics Of the Calcineurin Inhibitors and Mycophenolic Acid: Possible Consequences for Therapeutic Drug Monitoring in Organ Transplantation”

H de Jonge, M Naesens, and D Kuypers

Ther Drug Monit, 2009: 31(4) 416-35

Thank You
For
Your Attention

QUESTIONS?