



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
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Immunosuppressants to Discuss

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

- Cyclosporine
 - Tacrolimus
 - Sirolimus
- } whole blood (EDTA)
- Mycophenolate - serum, plasma

WHY?



Cyclosporine Distribution

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


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Tacrolimus Distribution

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


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Sirolimus Distribution

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


Yatscoff R, LeGatt D, Keenan R, et al, *Transplantation* 1993; 56:1202-6.



Mycophenolate Distribution

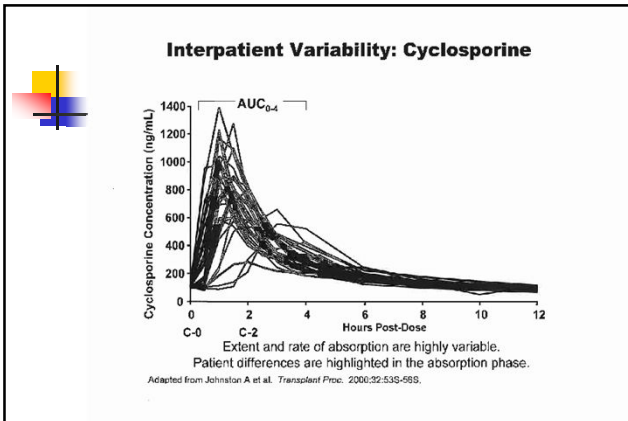
- Plasma (97% protein bound)

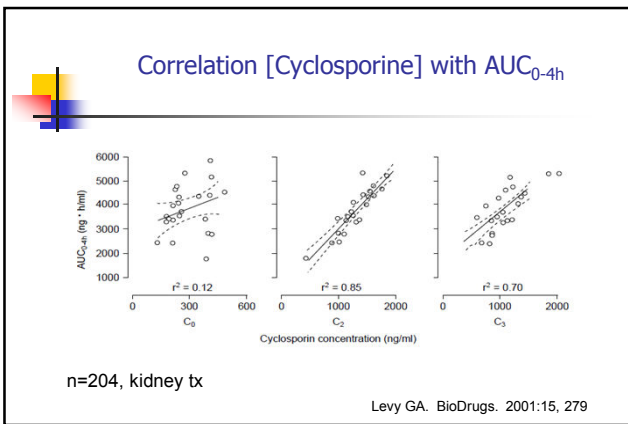
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Timing of Specimen Collection

- **Cyclosporine:** trough (<1-2h predose) vs C2?
- **Tacrolimus:** trough (<1-2h predose)
- **Sirolimus:** trough (<4h predose)
- **MPA:** trough (<1-2h predose) vs abbreviated AUC?





Tacrolimus Sampling Time

- Jorgensen et al, *Nephrol Dial Transplant* (2002) 17: 1487-90
 - 21 kidney transplant patients on tacrolimus, steroids
 - Good correlation (r) between C₀ (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%, C₀ is 4-20 ug/L
[*Clin Chem* (2010) 56(5): 732-35]

Tacrolimus Sampling Time

Correlation between C_0 and AUC_{0-12} in *de novo* renal transplant patients
(<https://pharmaco.chu-limoges.fr>)

Time Post-Transplant	Correlation(r^2) [AUC (mgh/L) versus C_0 (ug/L)]
7 days	0.87
3 months	0.72
6 months	0.60

Tacrolimus Sampling Time

European Consensus Conference on Tacrolimus TDM (May 2007)

Proposed AUC targets

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

Bottom Line (as of today)

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

Sirolimus Sampling Time

- Kahan et al, *Clin Transplantation* (2000) 14: 97-109
 - 150 transplant patients on sirolimus, cyclosporine, steroids
 - Good correlation between C_0 and AUC ($r = 0.83$)

MPA Sampling Time

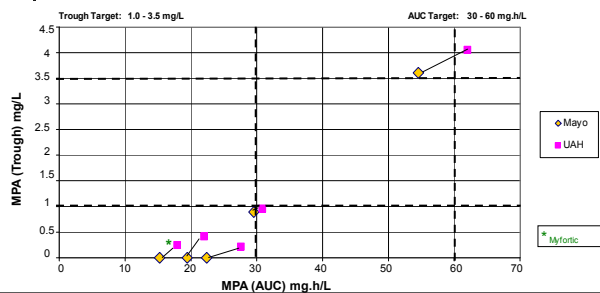
- Correlation between C_0 and AUC
 - MMF: ($r^2 \sim 0.48$) (better with tacrolimus co-therapy)
 - Enteric-coated MPA-Na: $r^2 = 0.02!!!$

Clin Pharmacokinet 2008; 47: 827-38

MPA Sampling Times

- ▶ **Limited sampling strategies (AUC determination) (MMF)**
 - e.g. 1: C_0 , $C_{0.5h}$, and $C_{2.0h}$
 - Therapy with Tacrolimus
 - AUC (mg,h/L) = $7.75 + 6.49 (C_0) + 0.76 (C_{0.5h}) + 2.43 (C_{2.0h})$
 - $r^2 = 0.86$ [Pawinski et al, Clin Chem (2002); 48(9) 1497-1504]
 - Therapy with Cyclosporine or Sirolimus
 - AUC (mg,h/L) = $10.2 + 2.4 (C_0) + C_{40min} + 1.7 (C_{2.0h})$
 - $r^2 = 0.86, 0.78$ [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 (20min,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



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)

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

Immunosuppressant Analytical Techniques

- Immunoassays
- Chromatographic methods
 - HPLC, tandem MS



Hope for Immunoassays?

- Sirolimus CMIA (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-hydroxysirolimus: 36.8%
 - 41-O-desmethylsirolimus: 20.3%



Hope for Immunoassays?

- Tacrolimus ACMA (Antibody Conjugated Magnetic ImmunoAssay) method (Siemens)
 - Tacrolimus apparent levels up to **14.4 µg/L** in patients with rheumatoid factor >100 IU/mL¹
 - Tacrolimus apparent levels up to **24.0 µg/L** (no RF; ? Unidentified endogenous antibody)²
 - No interference in Abbott IMx and tandem MS methods
- Cyclosporine ACMA
 - Cyclosporine apparent level up to **492 µg/L** (tandem MS and Abbott Architect CLMI assays <LOQs)³
 - RF interference ruled out; PEG precipitation gave [] <LOQ, suggesting a protein/antibody?

¹ *Ther Drug Monit.* 2009; 31(6): 743-45.
² *Ther Drug Monit.* 2010; 32(2): 228-31.
³ *Ther Drug Monit* 2010; 32(5): 529-31.

Wyeth

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

Scientific Affairs

Health Canada Endorsed Important Safety Information on Rapamune® (sirolimus)

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.

- Health 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 **positive** 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 **negative** bias of approximately 10% relative to HPLC/MS/MS [4].

2. IMx Sirolimus Assay Package Insert. Abbott Diagnostics Division. Abbott Park, IL. September, 2006.
 3. Architect System Sirolimus Assay Package Insert. Abbott Laboratories Diagnostics Division; Abbott park, IL. January, 2009.
 4. Analytical Services International; London, UK. www.biomatrix.co.uk/rd/csbtes_and_results/sirol_08ens009.html (August, 2009)



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 (acetonitrile), vortex, centrifuge, transfer ~120 µL supernatant, analyze ("**DILUTE and SHOOT**")
- AMR: Cyclosporine (20 – 2500 µg/L); tacrolimus, sirolimus (1.0 – 50.0 µg/L)
 - Good for CNI "minimization" protocols
- **NO** metabolite cross-reactivity
- **Issues:** capital cost, expertise to operate/maintain; **imprecision?**
 - CAP Immunosuppressive Drugs EP 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): 6.5-15.8% / 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



Regional Laboratory Services



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 Volume: 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. Steady state: 1-5 days.

Collection Information:
 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.

Last Updated on: Tuesday, December 16, 2008



Regional Laboratory Services



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 Volume: 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: > 30.0 **Additional**

Test Information:
 Specimen must be in the laboratory by 1330 hours Monday to Friday or 1100 hours weekends and holidays for same day analysis.
 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 (>1 week at -20°C)

Last updated on: Thursday, May 21st, 2009



Immunosuppressants TDM

Problems/Concerns

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29/Oct/2009      CAPITAL HEALTH/DYNACARE KASPER MED LABORATORIES      INTERIM REPORT
08:56           Edmonton, Alberta                                   Page      2


NAME: *****
H# *****      LOC: SA2 (UAR)   ROOM: SA2 ***   AGE: ****   SEX:  M
ACCT: 015012141107      DR: *****
                        PT HOME PHONE#: (780) *****

***** COLL: 28/Oct/2009 05:25 REC: 28/Oct/2009 05:49  PHYS: *****

TACROLIMUS (FK506)      (CONTINUED)
TACROLIMUS (FK506)
Tacrolimus (FK506)

                        6.7                               ug/L
Results to be interpreted by clinician.
Interpretation is dependant upon various factors such as
transplant type, time post transplant, and concomitant
immunosuppressive therapy.

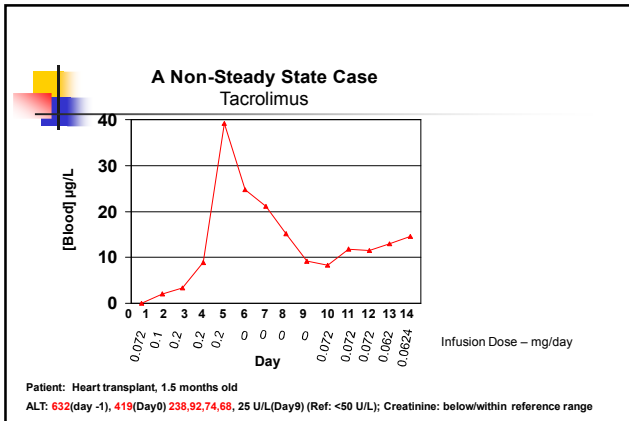
Specimen collected      8.5                               hours post dose
                        Ideal sampling time is predose.
  
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Immunosuppressant TDM

Problems/Concerns (continued)

- IV line contamination of specimens for TDM
 - Am J Health-System Pharm 2008; 65(3):226-8,
"Misleading tacrolimus concentration value in blood taken from a catheter used for tacrolimus administration".
- "Non-Steady state" Issue:
 - Time to steady state:
 - Cyclosporine: 1-5 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
[Am J Transplant 2009; 9 (Suppl 3): S19-20]
"We recommend measuring CNI blood levels.....at least every other day during the immediate post-operative period until target levels are reached".



- ### 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?
