Therapeutic drug Monitoring of Immunosuppressive drugs

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Laboratory challenges

• Since early 21st Century, with the tremendous progress in computer sciences and technologies, new biomarkers and techniques appear every year…
• With progress in life expectation and in medicine, the global “costs of health” became a real challenge for the Society
• During last decade, business has undergone fundamental changes as the world economy became more global with growing competition, affecting also clinical chemistry

As a consequence, a real gap exists currently between what is technically possible and available financial resources
Clinical Chemistry

current challenges and opportunities

• Creating new specific competencies for clinical biologists, more focused on **clinical aspects**
• Involving clinical biologists in new test development and evaluation, more on the **diagnostic value** than on analytical characteristics
• Develop stronger partnerships with industries
• Value-added knowledge service providing comprehensive **consultative support to clinicians**
  • Interpretation of complex tests
  • Listen to the needs of clinicians
  • Help for appropriate tests ordering, help in guidelines, consensus meeting…
  • Explain biological variation…
The Laboratory: a real partner for transplant clinicians

- Need to **select and optimize scientific resources** available
- Before transplantation
  - Immunohematology: HLA, screening for HLA antibody, blood typing, Cross-match between donor and potential recipient
  - Virology
  - Pharmacogenetics: CYP3A5, P-gp,… (?)
- After transplantation
  - Clinical chemistry: kidney function, liver function, cholesterol,…
  - Microbiology: bacterio + virology
  - Hematology and screening immunohematology
  - Anatomo-pathology: biopsies
  - Therapeutic drug monitoring: Antibiotics, **immunosuppressive drugs**…
Basis of Therapeutic Drug Monitoring

• Therapeutic drug monitoring (TDM) is advised for drugs
  – With narrow therapeutic window
  – With concentration-effects (PK-PD) relationship, but efficacy sometimes difficult to quantify
  – With high interpatient variability (PK, PG, drug interactions..)
  – Needing strong long term compliance. Pharmacoeconomy
  – Possible confusion between SE and pathology

• Immunosuppressants, such as cyclosporine, tacrolimus, sirolimus or MPA belong to drugs taking advantage of TDM

Drugs used to prevent organ rejection

• **Steroids** (no TDM) anti-inflammatory, rejection or maintenance
• **Antibodies** (no TDM) induction or rejection therapy
  – Anti-IL2 receptor (anti-CD25, e.g. Daclizumab, Basiliximab), anti-CD3
  – ATG or ALG
• **Azathioprine** (no TDM but need check TPMT activity), maintenance
• **Calcineurin inhibitors**
  – Cylosporine (TDM required), maintenance
  – Tacrolimus (TDM required), maintenance
• **Mycophenolate mofetil/sodium** (TDM recommended), maintenance
• **mTOR inhibitors**
  – Sirolimus/everolimus (TDM required), maintenance

Several possible combinations
Role of Immunosuppressive drugs TDM?

• Expected future progress will most likely consider patients quality of life: new challenge for TDM!
  – Reduction of side effects, rejection episodes
  – Reduction of number of drugs intake
  – Reduction of hospital stay…

• TDM is not only a drug concentration assay!
  – It should be considered as a tool for individualized therapy
  – It should be based on
    • Analytical expertise
    • Clinical pharmacokinetics including pharmacogenetics
    • Pharmacodynamics
Therapeutic drug monitoring: development of new strategies

- Since the years ’80, permanent search for an optimal marker of efficacy/toxicity e.g.:
  - plasma, whole blood, free vs total fraction
  - bioassay (MLC, radio-receptor assay, calcineurin pentamer assay…),
    - sampling time: trough, C₂, peak, full AUC, abbreviated AUC, …
- Lack of efficacy for routine C₀ monitoring for predicting rejection → need for complementary/alternative TDM approaches
### Suggested therapeutic ranges

<table>
<thead>
<tr>
<th>Trough level</th>
<th>Time</th>
<th>Kidney</th>
<th>Liver</th>
<th>Heart or lung</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cyclosporine</strong></td>
<td></td>
<td>150-250 (&gt;1200)</td>
<td>250-350 (&gt;1000)</td>
<td>250-350</td>
</tr>
<tr>
<td>$C_0$ (C2)</td>
<td>Initiation</td>
<td>75-150 (800)</td>
<td>100-200 (600)</td>
<td>150-250</td>
</tr>
<tr>
<td>(ng/mL)</td>
<td>maintenance</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>Tacrolimus</strong></td>
<td></td>
<td>10-15</td>
<td>10-20</td>
<td>15-20</td>
</tr>
<tr>
<td>(ng/mL)</td>
<td>Initiation</td>
<td>5-10</td>
<td>5-10</td>
<td>5-10</td>
</tr>
<tr>
<td></td>
<td>Maintenance</td>
<td>3-7</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Minimization</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>MPA</strong></td>
<td>Initiation</td>
<td>1.3-3.5 (CsA) or 1.7-4.0 (Tac)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(µg/mL)</td>
<td>Maintenance</td>
<td>Target AUC 30-60 µg.h/mL</td>
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<tr>
<td><strong>Sirolimus</strong></td>
<td>Initiation</td>
<td>5-15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(ng/mL)</td>
<td>Maintenance</td>
<td></td>
<td></td>
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</tr>
<tr>
<td><strong>Everolimus</strong></td>
<td>Initiation</td>
<td>5-15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(ng/mL)</td>
<td>Maintenance</td>
<td>3-8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Complementary possible approaches

- **Pharmacokinetics**
  - Improve AUC prediction: by Pop PK, Bayesian estimates, abbreviate AUC,…
  - Determine or predict drug concentration in target tissues (biopsies, lymphocytes,…)
  - Implement pharmacogenetics for early dosage optimisation

- **Pharmacodynamic markers**
  - Identify markers
  - Standardize methods

- **Analytical**
  - Improve robustness and standardisation
  - Improve sensitivity and specificity
How individualize drug treatment

**Pharmacokinetics**
- Drug exposure
- Drug interactions
- Distribution
- Metabolism
- Elimination
- Pharmacogenetics (CYP3A5, P-gp,...)

**Pharmacodynamics**
- Action on receptors
  - IL2
- Lymphocytes CD+4
- Cylex assay
- Pharmacogenetics
- Proteomic, metabolomics...

**Methods**
- Immunoassays
- LC-MSMS
- Analytical performances (specificity, sensitivity,...)
- Dry spot analysis,...

**Adverse events**
- Nephro-, neurotoxicity
- Hypercholesterolemia
- Overimmunosuppression

**Treatment efficacy**
- Acute rejection
- Chronic rejection
- Tolerance

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Pharmacokinetics
Variability of the pharmacokinetics

**Fig. 1** Cyclosporin concentrations versus time profiles (n = 8) obtained after Neoral administration at steady state 

\( \text{PK}_3 \) with the monoclonal whole blood FPIA method.
Clinical pharmacokinetics

• Clinical pharmacokinetics is the science describing drug absorption and disposition in patients
• It provides useful information on plasma or blood concentration
• It could provide important information on target tissue or cellular drug concentration
  • Free fraction: interpatient variability
  • Physicochemical properties (hydrophobic behavior)
  • Cellular passive diffusion and active transport (influx and efflux): interpatient variability
Pharmacokinetics

• Compliance
  – Particularly critical because IS chronic treatment causes some side effects and may result in lack of compliance
  – Relatively high percentage of patients decide to stop taking their IS drugs
  – TDM could detect such lack of compliance

• Liberation
  – The orally ingested drug needs to be solubilized in the GI tract in order to be further absorbed
  – The solubilization step is not critical for IS drugs (neutral)
  – Could be limited by ingestion of antiacid delaying the dissolution
Pharmacokinetics

- Absorption
  - The drugs solubilized or dissolved need to be absorbed by the GI tract (stomach and small intestine)
  - This step is critical because there are both some active and passive processes involved: transport proteins (P-gp, MRP2,…) and passive diffusion
  - Impact of intestinal and hepatic first pass effect
  - Bioavailability (F) variable and generally ranging from 5-40%
    - Neoral F: 20-35%
    - Tacrolimus F: 10-40%
    - Sirolimus F: 15-40%
  - Usually F increases slightly with time, with fat meals and with drugs such as metoclopramide (increase gastric emptying kinetic)
Pharmacokinetics

• Distribution
  – The fraction of drug absorbed will be distributed in the systemic circulation
  – The extent of distribution in deep compartments may depend on
    • The free fraction (generally low for IS drugs)
    • The liposolubility of the drugs (generally high for IS drugs)
    • The presence of transport proteins in tissues
  – Volume of distribution (Vd) variable but generally > 1 L/kg
    • Cyclosporine Vd: 3.5 L/kg
    • For drugs with Vd > 1 L/kg one could expect poor removal extent by dialysis…
    • Vd > 1 L/kg → generally lower relationship blood conc vs effects
  – Important distribution of these drugs within red blood cells
    • Ratio plasma/whole blood conc for Cyclosporine: ¼, Tacrolimus:1/12, Sirolimus: 1/30,…
Pharmacokinetics

• Metabolism
  – Cyclosporine, Tacrolimus, sirolimus, everolimus are oxidized by CYP3A4 and CYP3A5 in more than 20-30 metabolites (hydroxylation, N-demethylation, combination of both…)
  – Occurs in the intestinal membrane and in the liver
  – Important hepatic first pass effect
  – Hepatic extraction coefficient > 0.6 (flow limited)
  – Cl CsA will decrease in case of reduction of hepatic blood flow
  – Cl CsA: 5mL/min/kg
  – MPA undergoes mainly glucuronidation (MPAG) eliminated in urine (86%), but also some oxidation
Variability of the pharmacokinetics

- CsA, TAC, Siro and Evero are highly lipophilic agents characterized by variable level of absorption
- They are metabolized by CYP3A subfamily enzymes and are substrate of P-glycoprotein
  - subject of *interactions and ontogeny*
  - characterized by *genetic polymorphism*
- Substrates for many drug-drug (food) interactions
- Chronic diarrhea
- As a consequence: large intra- and interpatients variability in the blood levels
- Need to predict AUC and/or drug concentration at the target site
Variability of the pharmacokinetics
## Drug interactions

### Drugs that may increase tacrolimus blood concentrations

<table>
<thead>
<tr>
<th><strong>Calcium</strong></th>
<th><strong>Antifungal Agents</strong></th>
<th><strong>Macrolide Antibiotics</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Channel Blockers</td>
<td>clotrimazole</td>
<td>clarithromycin</td>
</tr>
<tr>
<td>diltiazem</td>
<td>fluconazole</td>
<td>erythromycin</td>
</tr>
<tr>
<td>nicardipine</td>
<td>itraconazole</td>
<td>troleandomycin</td>
</tr>
<tr>
<td>nifedipine</td>
<td>ketoconazole</td>
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</tr>
<tr>
<td>verapamil</td>
<td>voriconazole</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Gastrointestinal Prokinetic Agents</strong></th>
<th><strong>Other Drugs</strong></th>
<th><strong>Drink</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>cisapride</td>
<td>bromocriptine</td>
<td>grapefruit juice</td>
</tr>
<tr>
<td>metoclopramide</td>
<td>chloramphenicol</td>
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<tr>
<td></td>
<td>cimetidine</td>
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<td></td>
<td>cyclosporine</td>
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<td></td>
<td>danazol</td>
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<tr>
<td></td>
<td>methylprednisolone</td>
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<td></td>
<td>magnesium-aluminum-hydroxide</td>
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<td></td>
<td>fluoroquinolones</td>
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</tbody>
</table>

### Drugs that may decrease tacrolimus blood concentrations

<table>
<thead>
<tr>
<th><strong>Anticonvulsants</strong></th>
<th><strong>Antimicrobials</strong></th>
<th><strong>Other Drugs</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>carbamazepine</td>
<td>rifabutin</td>
<td>sirolimus</td>
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<tr>
<td>phenobarbital</td>
<td>caspofungin</td>
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</tr>
<tr>
<td>phenytoin</td>
<td>rifampin</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Herbal Preparations</strong></th>
<th><strong>Other Drugs</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>St. John’s Wort</td>
<td></td>
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</tbody>
</table>

These tables are not all inclusive.
How better predict AUC?

- **Tacrolimus AUC0-12** (full or abbreviated: 5-12 samples)
  - Probably the best estimate for exposure but difficult to obtain
    
    Undre N et al. Transplant Proc, 31, 296-8, 1999
    Uchida K et al. Transplant Proc, 34, 1736-7, 2002

- **Limited Sampling Strategies** (Ting LSL et al. TDM, 28, 419-30, 2006)
  - Most studies proposed LSS using sampling within 4h (C2, C4) or (C1, C4, C8) with multiple regression analysis
  - Promising results obtained, but need proper validation before clinical use

- **Influence of covariates on AUC0-12**
  - Various performances
    
    Staatz CE et al. Liver Transplant, 9, 130-7, 2003

- **Pop PK and AUC0-12 Bayesian estimation using LSS**
  - Need accurate PK model
    
    Saint-Marcoux F, Clin Pharmacokinet, 44, 1317-28, 2005
Unpredictability of the drug concentration at the target site

- Target sites are the Lymphocytes or indirectly (surrogate m.)
  - Transplant tissues (liver biopsies, …)
  - Peripheral Blood Mononuclear Cells (PBMC)
- Absence of clear relationship between blood concentration and tissue or Lymphocytes concentration

Capron A et al. TDM 2008, Epub ahead of print
Tacrolimus concentration in liver biopsies
Relationship with histologic rejection score

- Choice of alternative biological matrix
  - Better correlation between Tac tissue levels (hepatic biopsies) and score for rejection than with whole blood
    
    Capron A et al. TDM 29, 240-8, 2007
  
  - Interest in intra-lymphocytes determination of Tac
    
    Capron A et al. TDM, Epub ahead of print, 2009

\[
y = 594.8e^{-0.5358x} \\
R^2 = 0.986
\]
Role of pharmacogenetics
Clinical impact of genetic polymorphism

- Kidney Transpl pop expressor: 2.3 fold difference in dose requirement
  - Proposed guidelines: different prospective loading Tac dose based on CYP3A5 GP: 0.15 vs 0.075mg/kg/12h (in expr vs non-expr) 

- Liver Transpl pop: need to consider both donor and recipient
- Useful to reach rapidly efficient drug exposure from 1st d
- No incidence of CYP3A5 expression on acute rejection,
  - Most likely due to TDM action during the 1st week
    Hesselinck DA et al, Pharmacogenet Genomics.18, 339-48, 2008

- Lack of studies analysing the incidence of the prospective loading dose based on GP, on acute rejection rate TacTic trial?
Clinical impact of genetic polymorphism

- Significant relationship between hepatic tissue Tac levels and expression of P-gp activity
  

- Significant relationship between lymphocytes Tac levels and expression of P-gp activity (ABCB1 exon 11 polymorphism, G1199A)

  Capron A al. submitted, 2009
Pharmacodynamic biomarkers
Need for Pharmacodynamic biomarkers

- Measurement of the immune status and not anymore of a particular drug
- Could better predict efficacy/safety than drug blood levels, including the risk for infection
- PD biomarkers could better monitor the resulting efficacy
  - Monitor all active xenobiotics (multiple drugs therapies, metabolites)
- Is the level of required immunosuppression the same for all patients?
  - The immune function levels of the Cylex assay appear lower in healthy children <12 yrs than in healthy adults
  
  Hooper E et al, Clin Transplant, 19, 834-9, 2005
Need for Pharmacodynamic biomarkers

• Most likely combination of different PD biomarkers
  – Lymphocytes proliferation (Proliferating Cell Nuclear Antigen)
  – Expression of surface antigens of T-cell
  – IFN-γ ELISPOT assay
  – Quantification of intracellular IL-2 in CD8+ T cells
    Appears a useful PD marker in liver transplantation to predict
    organ rejection (p=0.003), better than calcineurin activity
    Boleslawski E et al. Transplantation, 77, 1815-20, 2004
  – Measure of the ATP production from stimulated T-cells (Cylex
    ImmuKnow assay)
    Kowalski RJ, et al. Transplantation, 82, 663-8, 2006
  – Specific enzymes activity (IMPDH, calcineurin, …)

• Metabolomic, proteomic studies…?
  Kurian S et al, Int Immunopharmacol, 7, 1948-60, 2007
Analytical methods
How monitor?
Analytical methods

- Apparent simple matter… In fact full of questions
- Immunoassays or LC-MS(MS)?
- Large domination of immunoassays until recently, alternative methods appearing
  - LC-MS users 2% (1999) → >20% (2008)
    (ELISA), MEIA, EMIT, ACMIA, CMIA, CEDIA…
  
  [D Holt Europ Consensus Conf on Tac Optimisation, Brussels, 2007]

- Existence of significant differences between methods
- Important concern when performing PK studies or when comparing clinical trials and outcome studies
  - Need to describe properly the method and understand the differences among methods
How monitor?
Analytical methods

• Immunoassays
  – Fast, easy, robust (standard laboratory technician)
  – (semi)-automated, random-access, consolidation in Corelabs
  – Specificity/sensitivity/accuracy? Possible metabolites cross-reactivity and/or calibration bias.
  – Reagent costs?

• LC-MS(MS)
  – Need highly qualified technicians, home-made validation, ion sup
  – Non-automated, required specialized laboratory
  – Highly specific and sensitive
  – Equipment costs?

• Choice between IA or LC-MS(MS)?
  – Depends on each lab characteristics (nbre of samples, staff qualif)
  – Choice less important than clinical experience
Expected analytical progress?

• Clinicians need consistent results
• Need of international standardization
  – To limit calibration bias occurring both with IA and LC-MSMS
  – To limit interferences with endogenous compounds or metabolites
  – To improve outcome studies comparison
• Need to improve automation and robustness of IA and LC-MSMS
  – Automated preanalytical phase
  – Deuterated IS
Expected analytical progress?

- Trend to use smaller drug dose
  - Drugs minimization
  - Drugs combination, e.g. SYMPHONY multicentre trial
- Need to reach lower functional sensitivity with good imprecision performance at lower concentration
  - European Consensus Conference on tacrolimus optimization
    - LOQ: 1 ng/mL
  - Discriminate lack of compliance from low blood concentration
Tacrolimus International Proficiency Testing Scheme

March – August 2008: precision data
Improvement of the LC-MS(MS)
Good results of the CMIA

August 2008

- Architect (n = 24)
- ACMIA (n = 54)
- Others (n = 14)
- Abbott Imx (n = 119)
- EMIT (n = 59)
- HPLCMS (n = 92)

Tac-free blood spiked with 3 µg/L

CV%
Perspectives

Need to improve the value added of laboratory medicine
Conclusions and general perspectives

- **TDM: major support to patient management**
  - Compliance and side effects prevention (less clear for efficacy)
  - New TDM challenge: quality of life

- **Keep aware of causes of variability**
  - Pharmacogenetics (CYP3A5 expressors need higher doses)
  - Chronic diarrhea alters P-gp and causes increased Tac levels
  - Paediatrics (higher dosage requirements)
  - Drug interactions, liver function,…

- **TDM should not anymore be considered as a simple blood concentration numerical result!!**
  - It should include all complementary approaches helping tailoring individually optimal drug dosage (PK, PG, PD…)
Conclusions and general recommendations

- Clinical pharmacokinetics perspective of progress
  - AUC prediction could be improved by
    - Pop PK with Bayesian estimates to assess AUC (LSS): most likely the best marker of exposure, after multi-centre validation
    - Pharmacogenetics
      - Prediction and measurement of drug conc at target sites (PBMC?)
- Pharmacodynamic perspective of progress
  - Should better correlate with treatment efficacy
  - Need to identify the best combination of PD markers
  - Need to standardize protocols and methods
- Analytical perspective of progress
  - International standardization (inter-method bias,…)
  - Improvement of automation (preanalytical step,…)
  - Rapid adjustment according to sensitivity needs
  - Provide easier access to TDM
Thank you for your attention