Perspectives for therapeutic drug monitoring

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What is Therapeutic Drug Monitoring?

- TDM corresponds to a multi-disciplinary service aiming to individualize/optimize drug treatment (efficacy/side effects)
- Requires excellent communication with prescribers
- Requires competencies in pharmacology/toxicology
  - Pharmacodynamics/biomarkers of activity-toxicity
  - Variations in drug disposition: pharmacokinetics/drug metabolism
  - Pharmacogenetics
- Requires competencies in drug analysis
  - Preanalytical phase
  - Analytical phase
    - Chromatographic methods/Immunoassays
Basic assumption for TDM

• Since the ’70 it was recognized that plasma drug concentrations were better related to effects than the amount of drug administered

• Successful applications for digoxin, theophylline, aminoglycosides, antiepileptics, immunosuppressants…
  – Low therapeutic index, variable PK, effect difficult to quantify,…

• Largely contributes to the principles of « personalized medicine » (PK variability)
  – Identification of drug interactions
  – Identification of high or low drug clearance (accumulation)
  – Identification of non-compliance
  – …
How individualize drug treatment?

Example of immunosuppressive drugs

**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...

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

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

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

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What is not Therapeutic Drug Monitoring?

- TDM should not be « reduced to a simple » drug measurement from any blood specimen
  - Time-dependent concentrations
  - In absence of known expertise from the prescriber
  - In absence of accurate information allowing adequate interpretation
  - In absence of contact/dialogue with the prescriber or his staff
What are the criteria to justify TDM?

- Critical dose drugs (small therapeutic index)
  - Drugs with unpredictable PK (non-linear PK) or unstable pathological status (intensive care, oncology, elderly, etc.)
  - Drugs with side effects possibly misinterpreted by disease progress or symptoms
  - Absence of pharmacodynamic markers
  - Chronic treatment with risk of non-compliance

- Pharmacoeconomic and cost-effective reasons
  - Shorter treatment
  - Shorter hospital stay
What drugs?

- « Old » drugs with proved interest
  - Aminoglycosides and glycopeptides (gentamicin, vancomycin..)
  - Cardiac glycosides (digoxin)
  - Antiepileptics (carbamazepine, valproic acid, phenytoin..)
  - Methotrexate
  - Theophylline
  - Immunosuppressive drugs (cyclosporine, tacrolimus, everolimus, sirolimus, MPA..)
  - Some antiarrhythmic agents (amiodarone)
  - Some antidepressive agents (Lithium, TCA ?)
What drugs?

• « New » drugs of interest
  – Newer antiepileptics (levetiracetam, oxcarbazepine, lamotrigine..)
  – Some antiretrovirals (efavirenz, lopinavir,..)
  – Some cephalosporins (cefepime, meropenem..)
  – Some antifungals (posaconazole, itraconazole, voriconazole..)
  – Some cytotoxic agents (imatinib, irinotecan, tamoxifen, L-asparaginase, ..)

• Drugs with limited interest (?)
  – Benzodiazepines
  – SSRI
TDM challenges and issues

• But...limitations of TDM
  – Sometimes poor relationship between trough drug concentration ($C_0$) and clinical outcome
  – High dependency of sampling time accuracy
    • Difficulty to get reliable data from nursing (times, dose, interval,...)

• Difficulty to get real consensus for therapeutic ranges
  – Maybe as a consequence of erratic blood sampling, variable analytical methods,...

• As a consequence: limited and still debated success in some applications
  – Mycophenolate, antiretrovirals, cytotoxics, antidepressants,...
TDM: search for better PK-PD markers the « quest of the GRAAL »

- Since the years ’70, permanent search for optimal marker of efficacy/toxicity e.g.:
  - Plasma, whole blood, free vs total fraction
  - Bioassay (MLC, RRA, EA, MIC, …),
  - Sampling time: $C_0$, $C_2$, $C_{\text{max}}$, full AUC …

- Single blood sampling: easy but sometimes weak for predicting effects (e.g. $C_0$, …)
  - Pharmacokinetic reasons
    - Drug ≠ endogenous analyte (e.g. creatinine)
    - Logistic issues (accuracy in routine setting)
    - Sometimes lack of good relationship between $C_0$ and AUC
  - Pharmacological reasons
    - Concentration- or time-related effects e.g. antibiotics
    - Blood conc not well related to target (intra-cellular) site conc,…
Evolution in Laboratory Medicine

• Important economic pressure and progress in technology
• Trend to « industrialize » laboratory testing
  – Increased automation
  – Consolidation, laboratories merging
  – Reduction of test production costs
• Need to increase the Medical expertise and the « value-added » for any laboratory tests
  – Need to optimize the value of any laboratory result
  – Knowledge service
  – Better interpretation
  – Guidance in prescription
  – Evidence based …
How could progress TDM during the coming decade?
Three main perspectives
First perspective of progress
Pharmacology:
Strengthening of the PK-PD relationship
Stronger pharmacokinetics involvement

- Better relationship between AUC and drug effects than $C_0$
- Direct access: possible but difficult
  - 8-12 blood sampling
  - Medical issue, costs, time,…
- Indirect access: prediction through mathematical approaches
  - Limited sampling strategies: 2-3 blood samples with equations to specific populations: (need strict sampling times)
  - Population pharmacokinetics with Bayesian estimates: (allow more flexibility in sampling times)
- Need standardization of TDM approaches (cfr P Marquet)
Drug Area Under the time-concentration Curve (AUC)

Population pharmacokinetics modelling with optimal sampling strategy for Bayesian estimation

Limited Sampling Models and Bayesian Estimation for Mycophenolic Acid Area under the Curve Prediction in Stable Renal Transplant Patients Co-Medicated with Ciclosporin or Sirolimus

Flora T. Mustamba,1 Annick Rousseau,2 Jean-Louis Bosmans,3 Jean-Jacques Senessael,4 Jean Cumps,1 Pierre Marquet,2 Pierre Walleniacq and Roger K. Verheeck1
Drug Area Under the time-concentration Curve (AUC)
Access portal to the websites of routine and clinical trials of the Limoges University Hospital laboratory of Pharmacology

<table>
<thead>
<tr>
<th>Access</th>
<th>TDM - Modalities</th>
<th>Available tools</th>
<th>ISBA Newsletters</th>
<th>Publications</th>
</tr>
</thead>
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Please identify yourself

Login: [Enter]
Password: [Enter]

You lost your identifier and/or your password

Delete Enter the Websites

Registration on ISBA website
Pharmacodynamic biomarkers

• Two patients exposed to similar drug blood conc may respond differently!
  – Possible different receptor-effector response
  – Individual susceptibility (genetics, environment, …)

• Urgent need to add PD biomarkers in the TDM activity
  – More difficult to implement routinely
  – Lack of automation
  – Lack of well identified PD biomakers
  – Lack of quality controls and proficiency scheme
  – Lack of standardization, etc…

• Proteomics, metabolomics…
Pharmacodynamic biomarkers

• **Antibiotics:**
  – MIC, peak/MIC, Time above MIC, AUIC…

• **Immunosuppressants**
  – 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
  – Measure of the ATP production from stimulated T-cells (Cylex ImmuKnow assay)
  – Specific enzymes activity (IMPDH, calcineurin, …)

• **Antiretrovirals**
  – Viral load,
  – CD4,
  – RNA-HIV,…
Pharmacodynamic biomarkers

• Antidepressive agents
  • Difficulty in defining the efficacy (scoring) of psychoactive drugs
  • Existence of standardized clinical rating scales to evaluate therapeutic responses and SE
    – To assess efficacy: Brief Psychiatric Rating Scale (BPRS)
    – Positive and Negative Symptom Scale for Schizophrenia (PANSS)
    – To assess extrapyramidal SE: Abnormal Involuntary Movement Scale

• Antipsychotic agents
  – Dosage titration to a target % of dopamine receptor (D$_2$) blockade by tomography (Positron Emission or PET tomography)
  – Therapeutic effect but also SE of antipsychotic drugs related to blockade of D$_2$ receptors for schizophrenia
Genetic factors

Adapted from Lindpaintner
Pharmacogenetics

- Genetics polymorphisms may affect both
  - The drug disposition (e.g. transport proteins, CYP3A5, CYP2D6, etc…)
  - The receptor activity (e.g. IMPDH, BCR-ABL kinase activity,…)
- More pharmacogenetic-based recommendations at the onset of a treatment will appear
  - To select drugs
  - To adjust doses
Drug Metabolism (Degradation)

- WT/WT: AUC = 100
- WT/V: AUC = 200
- V/V: AUC = 400

Drug Receptor (Efficacy)

- Metabolism genotype
- Receptor genotype
- Response

<table>
<thead>
<tr>
<th>Metabolism genotype</th>
<th>Receptor genotype</th>
<th>Efficacy</th>
<th>Toxicity</th>
</tr>
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<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>65%</td>
<td>Low (5%)</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>32%</td>
<td>Low</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>9%</td>
<td>Low</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>79%</td>
<td>Moderate (15%)</td>
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<tr>
<td>+</td>
<td>+</td>
<td>40%</td>
<td>Moderate</td>
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<tr>
<td>+</td>
<td>+</td>
<td>10%</td>
<td>Moderate</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>80%</td>
<td>High (80%)</td>
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<tr>
<td>+</td>
<td>+</td>
<td>40%</td>
<td>High</td>
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<tr>
<td>+</td>
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<td>10%</td>
<td>High</td>
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</tbody>
</table>
Pharmacogenetics

CYP3A5 and ABCB1 Polymorphisms and Tacrolimus Pharmacokinetics in Renal Transplant Candidates: Guidelines from an Experimental Study


Introduction

Tacrolimus (Tac, FK506) is widely used to prevent acute rejection following solid-organ transplantation. Like cyclosporin (CSA), this drug is characterized by a narrow
Intracellular drug concentrations

• A similar « postulate » could be used
  – Dosis vs plasma (blood) concentration (rationale for classical TDM)
    • Blood pharmacokinetics, -genetics
  – Plasma (blood) concentration vs target intracellular concentration
    • Cellular pharmacokinetics, -genetics

• First reports for the interest of intracellular drug conc.
  – Lymphocytes Lamivudine in 1999 (Moore et al, AIDS, 1999, 13,2239-50)
Intracellular drug concentration

- Intracellular drug concentrations are regulated by passive or active processes
  - Blood or plasma free conc
  - Physicochemical factors across bilayer membranes (mw, pKa, logP)
  - Carrier-mediated transport (efflux or influx pumps, …)
  - Local biotransformation (CYP)
- Cellular specific PK and PG
- Ratio blood vs intracellular concentration not constant
Intracellular drug concentration

Drug blood concentration (ng/mL)

Time (h)

Intracellular drug concentration

Passive diffusion

Small uncharged molecules:
- O₂
- CO₂
- H₂O
- N₂

Large polar molecules and ions:
- H⁺
- Na⁺
- Cl⁻

Intracellular drug concentration

Time (h)

Drug blood concentration (ng/mL)

ABC superfamily

Efflux pump (P-gp, MRP, ...)

Carrier mediated uptake (OATP, ...)

CYP3A

CYP2B6
Intracellular drug concentration

Correlation of tacrolimus levels in peripheral blood mononuclear cells with histological staging of rejection after liver transplantation: preliminary results of a prospective study

Arnaud Capron,¹,² Jan Lerut,³ Dominique Latinne,⁴ Jacques Rahier,⁵ Vincent Haufrroid¹,² and Pierre Wallemacq¹,²
Second perspective of progress: Analytical aspects
Analytical methods improvement

• Clinicians need consistent results
• 20-50% variations among methods should not be accepted anymore
• Need of international standardization supported by a consortium of clinical labs, scientific associations, industries and health authorities
  – To limit calibration bias occurring both with IA and LC-MSMS
  – To limit interferences with endogenous compounds or metabolites
  – To improve outcome studies comparison
• Increase sensitivity adapted to clinical needs
Analytical methods improvement and standardization

• Need to improve automation and robustness of IA and LC-MSMS
  – Automated preanalytical phase (both IA and LC-MSMS)
  – Reduce heterophilic antibodies interferences (IA)
  – Reduce risk for ion suppression effect (LC-MSMS)
  – LC-MSMS perspectives of progress
    • Not yet fully adapted to routine laboratory medicine
    • Lack of automation (e.g. User friendly software, on-line extraction, random access, bare codes...)
    • Lack of comprehensive and rapid support from MS manufacturer’s (24h hotline) as compared to major diagnostic companies
Emergence of immunosensors
« Real-time drug measurements »

- Possible for hospitalized patient to determine individual PK and dosing
- Blood drawn (< 1 µL) by intravenous microdialysis catheter (MicroEye®)
- Miniaturisation down to micro- or nanoscale
- Short intervals of measurements (e.g. few min)
- Detection by optical chip (nanotechnology) through luminescent signals
Emergence of immunosensors « Real-time drug measurements »

- Direct online (e.g. 48 h) with individual PK and AUC measurements of the drug free fraction
  - Real-time full AUC (> 500 measurements)
- Very sensitive
- Multiplexing measurements possible
Emergence of immunosensors
« Real-time drug measurements »

Figure 1. Diagram of the proposed POCT device.

Project FP7, NANODEM 2010
F Baldini et al.
Luminescent-based optical sensors

- Biochip considered as an array of biosensors (multiple analyses)
- Use of polymeric or silica nanoparticles carrying fluorophores
- Use of magnetic nanoparticles to concentrate the analyte in the sensing chip
- Possibility of large scale automation and re-use (low costs)
- Fluorescent scanners used for lecture
Optical sensors

Direct surface plasmon resonance immunosensor for in situ detection of benzylecgonine, the major cocaine metabolite

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TRENDS

Optical fibre gratings as tools for chemical and biochemical sensing

F. Baldini • M. Brenei • F. Chiavaioni • A. Giannetti • C. Trono

• Label-free
• In situ
• Real time

• In situ
• Real time monitoring
• Oral fluid
• High sensitivity (< 4 ng/mL)
• (SAMHSA oral fluid cutoff: 20 ng/mL)
• Reusable test
Optical sensors

A clinical trial for therapeutic drug monitoring using microchip-based fluorescence polarization immunoassay

Tomoya Tachi • Tetsunari Hase • Yukihiro Okamoto • Noritada Kaji • Takeshi Arima • Hiroyuki Matsumoto • Masashi Kondo • Manabu Tokeshi • Yoshinori Hasegawa • Yoshinobu Baba

- Microchip size: 100 x 40 μm
- Small volume (drops of blood)
- Rapid (65 sec)
- Lecture by optical microscope, polarizer and argon lazer
- Good correlation with CEDIA or PETINIA
Interest for increased throughput

- Important for out-patient clinics to allow direct dose adjustment during the patient visit
  - Avoiding late call, misunderstanding or even new appointment
- For the emergency room to allow more rapidly discharge or hospitalisation
- To allow more time for PK calculations
- To increase laboratory efficiency
RapidFire SPE/MSMS

Ultra-fast autosampler & online SPE system

• Replaces LC in LC/MS
• Reusable SPE cartridge
• Integrates with standard ESI MS instruments
• Cycle time = 7-13 s/sample

Compatible with biological matrices

• Serum
• Plasma
• Whole blood
• Urine
Tacrolimus correlation (n: 220)

\[ y = 0.9757x + 0.1753 \]

\[ R^2 = 0.9773 \]
Third perspective of progress
Improve patient’s quality of life
Improve patient’s quality of life

• Reducing duration of hospitalisation and frequency of visits
• Hospitalisation
  – Initiation of therapy and optimisation of a personalized dosing regimen: *In-situ* nanotechnologies
  – Reduction of blood sampling
  – Reduction of drug interactions or dose adjustments
• Out-patient follow up
  – POCT
  – Dried spot
  – Home Telehealth
Possible evolutions: the best or the worst

- DTC (Direct To Consumer testing)
  - Certified labs, but…
  - No need for doctor’s order
  - Without any medical supervision and interpretation

- POCT testing
  - Increasing number of tests
  - Quality control
  - Interpretation
Possible evolutions: the best or the worst

- **Transdermal sensors**
  - e.g. transdermal alcohol sensors bracelet device recording in real time alcohol consumption (wireless radiofrequency signal transmission to a modem)
    

- **Reverse iontophoresis could be used to extract some drugs from the skin**
  - e.g. phenytoin, amikacin, lithium…
    

- **Need for improved standardization among analytical methods**
Possible evolutions: the best or the worst

- « Online » or « real-time » ultra-fast measurements
  - Emergence of nanotechnologies, nano-sensors…
  - Useful for immediate decision-making at bed-side
- Home Telehealth (e-health, telemedicine)
  - Anticoagulation
  - Diabetes
  - Cardiac monitoring
- Home blood sampling
  - Dried Blood Spot

B Keevil, Clin Biochem 2011, 44, 110-18
Dried Blood Spot

- Still limited use in TDM but broad perspectives
- Advantages
  - Small volume of blood
    - Ethics: children, preclinical studies for industry on animals etc…
  - Flexibility and comfort for the patient (could be done at home)
  - No need to go to an out-patient clinic
  - Stability and shipment easier
- Challenges
  - Hct & blood viscosity effects \textit{(Denniff et al Bioanalysis, 2010, 2, 1385-95)}
  - Analyte nature
  - Spotting technique
    - Quality of paper used
    - Temperature or humidity
Dried Blood Spot

- **Applications**
  - Anti-HIV drugs
  - Drugs of abuse
  - Benzodiazepines
  - Immunosuppressants
  - SSRI
  - Antiepileptics, etc…

*B Keevil, Clin Biochem 2011,44,110-18*
Dried Blood Spot

Quantitative Analysis of Therapeutic Drugs in Dried Blood Spot Samples by Paper Spray Mass Spectrometry: An Avenue to Therapeutic Drug Monitoring

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⁵Center for Analytical Instrumentation Development, Purdue University, West Lafayette, IN, USA

• Generation of gas phase ions directly from blood card
• Reduced sample preparation: 30 sec
• LOD around 1 ng/mL
Dried Blood Spot

EBF recommendation on the validation of bioanalytical methods for dried blood spots

Over the last few years bioanalysts, pharmacokineticists and clinical investigators have rediscovered the technique of dried blood spots. The revival has provided pharmaceutical R&D a wealth of opportunities to optimize the drug-discovery and development process with respect to animal and patient ethics, new scientific insights and costs savings. On the bioanalytical front, multiple experiments have been performed and a lot of experience has been gained. Nevertheless, the technique still has a number of bioanalytical challenges. The European Bioanalysis Forum discussed the advantages and hurdles of the technique and summarized their current thinking in a recommendation on the validation of bioanalytical methods for dried blood spots, which can be used as a cornerstone for further discussions and experiments.

Bioanalysis (2011) 3(14), 1567–1575

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Steve White\textsuperscript{2},
Susanne Globig\textsuperscript{1},
Silke Lüdtke\textsuperscript{4},
Leonarda Brunet\textsuperscript{5} &
John Smeraglia\textsuperscript{6}
Take home message (1)

- TDM still has to face various challenges limiting its widespread use: different perspectives of progress
- Reinforcement of the PK-PD relationship
  - PopPK
  - Pharmacodynamic biomarkers
  - Pharmacogenetics
  - Intracellular drug concentration
  - Standardization of the TDM approaches
Take home message (2)

• Analytical methods development
  – Standardization of the calibrators and methods
  – Automatisation/robustness
  – Emergence of nanotechnologies (Lab-on-Chip), online measurement
  – Increased throughput or TAT (few seconds per test)

• Patient’s quality of life
  – Reduction of hospital stay and frequency of visits and blood drawing
  – Promotion of Dried Blood Spot testing
  – e-Health
Thank you for your attention