Pharmacokinetics and pharmacodynamics of anti-tuberculosis drugs in patients with tuberculosis

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International Reference Laboratory of Mycobacteriology, Diagnostics and Infection Control Statens Serum Institut
The PhD project was carried out at the International Reference Laboratory of Mycobacteriology, 2010-2014

Supervisors:
Consultant, professor Niels Frimodt-Møller, DMSc (principal supervisor)

Consultant, professor Åse Bengård Andersen, DMSc (project supervisor)

Laboratory manager Arieh S Cohen, MSc, PhD (project supervisor)
The thesis is based on three papers:

Simultaneous quantification of isoniazid, rifampicin, ethambutol and pyrazinamide by liquid chromatography/tandem mass spectrometry
[submitted APMIS]

Clinical significance of 2 h plasma concentrations of first-line anti-tuberculosis drugs: a prospective observational study

Pharmacokinetics of isoniazid and rifampicin in patients with pulmonary tuberculosis: population PK-modeling and Monte Carlo simulation studies
[in preparation]
### First-line TB treatment

#### Preferred regimen on a daily basis

<table>
<thead>
<tr>
<th>Phase</th>
<th>Drug</th>
<th>Dosage (children)</th>
<th>Dosage (adults)</th>
<th>Rationale</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Initial Phase</strong></td>
<td><strong>isoniazid</strong> 5 mg/kg</td>
<td>[10 mg/kg]</td>
<td>(maximum 300 mg)</td>
<td>Clinical studies on mono-therapy and early bactericidal activity (EBA)</td>
</tr>
<tr>
<td></td>
<td><strong>rifampicin</strong> 10 mg/kg</td>
<td>[15 mg/kg]</td>
<td>(maximum 600 mg)</td>
<td>Plasma concentration &gt; MIC*</td>
</tr>
<tr>
<td></td>
<td><strong>ethambutol</strong> 15 mg/kg</td>
<td>[20 mg/kg]</td>
<td></td>
<td>Optic neuritis</td>
</tr>
<tr>
<td></td>
<td><strong>pyrazinamide</strong> 25 mg/kg</td>
<td>[35 mg/kg]</td>
<td></td>
<td>Plasma concentration &gt; MIC*</td>
</tr>
<tr>
<td><strong>Continuation Phase</strong></td>
<td><strong>isoniazid</strong> 5 mg/kg</td>
<td>[10 mg/kg]</td>
<td>[300 mg]</td>
<td>Adverse effects dose related?</td>
</tr>
<tr>
<td></td>
<td><strong>rifampicin</strong> 10 mg/kg</td>
<td>[15 mg/kg]</td>
<td>(maximum 600 mg)</td>
<td></td>
</tr>
</tbody>
</table>

* MIC; minimum inhibitory concentration
First-line TB treatment

- Multi-drug treatment
- Directly observed therapy (DOT)

WHO Stop TB strategy

Issue:
- 1.5 million died from TB in 2013
- Resistance to standard TB drugs is growing and multi-drug resistant TB (MDR-TB) is present in virtually all countries
- 480,000 people developed MDR-TB in 2013

Questions:
- What levels/patterns of non-adherence is predictive of acquired drug resistance and bacteriological failure?
- What other modifiable factors are associated with acquired drug resistance and bacteriological failure?
Treatment optimisation

Drug pharmacokinetics (PK)

- serum concentration vs. time ($C_{\text{max}}$, AUC, $T_{\frac{1}{2}}$ etc.)

Drug pharmacodynamics (PD)

- serum concentration vs. drug activity (minimum inhibitory concentration (MIC))

Three key PK/PD indices:

- $C_{\text{max}}$/MIC
- T$>$/MIC
- AUC/MIC
Treatment optimisation

Strongly concentration-dependent antibiotic

AUC/MIC and $C_{\text{max}}$/MIC correlates the best to antimicrobial activity

Optimal dosage regimens;
Maximises plasma concentrations

Minimal concentration-dependent antibiotic

T>MIC correlates the best to antimicrobial activity

Optimal dosage regimens;
Maximises the duration of time above the MIC
Treatment optimisation

Strongly concentration-dependent antibiotic

AUC/MIC and $C_{\text{max}}$/MIC correlates the best to antimicrobial activity

Optimal dosage regimens; Maximises plasma concentrations

Isoniazid, rifampicin, ethambutol and pyrazinamide

Minimal concentration-dependent antibiotic

T>MIC correlates the best to antimicrobial activity

Optimal dosage regimens; Maximises the duration of time above the MIC

Does PK variability lead to low drug concentrations in some patients?
Does plasma concentration levels influence on treatment efficacy?
## Therapeutic ranges?

### Normal ranges in healthy volunteers

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg)</th>
<th>( T_{\text{max}} ) (h)</th>
<th>( T_{\frac{1}{2}} ) (h)</th>
<th>( C_{\text{max}} ) (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid 300 mg</td>
<td></td>
<td>0.75-2</td>
<td>3.5-4 / 1.5-2</td>
<td>3-5</td>
</tr>
<tr>
<td>Rifampicin 600 mg</td>
<td></td>
<td>2</td>
<td>2-4</td>
<td>8-24</td>
</tr>
<tr>
<td>Ethambutol 1200 mg</td>
<td></td>
<td>2-3</td>
<td>4.5 / 10-15</td>
<td>2-6</td>
</tr>
<tr>
<td>Pyrazinamide 2000 mg</td>
<td></td>
<td>1-2</td>
<td>9-11</td>
<td>20-60</td>
</tr>
</tbody>
</table>

Peloquin 1996 and 2002

### Targets for optimal efficacy in mouse studies

<table>
<thead>
<tr>
<th>Drug</th>
<th>( \text{AUC/MIC} ) (h)</th>
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<tr>
<td>Isoniazid 300 mg</td>
<td>500</td>
</tr>
<tr>
<td>Rifampicin 600 mg</td>
<td>271</td>
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</table>

Jayaram 2003, 2004

Neither normal \( C_{\text{max}} \) ranges nor \( \text{AUC/MIC} \) targets have been validated clinically.
To develop a rapid, precise and accurate method for simultaneous quantitation of all four first-line TB drugs in human plasma samples, and to study plasma concentration levels of first-line TB drugs in groups of TB patients in Denmark

Main hypothesis:
*Inter-patient pharmacokinetic variability is not recognized and standard dosing therefore leads to insufficient plasma concentration levels in some patients and consequently reduced treatment efficacy*
To study two-hour plasma concentration levels of first-line tuberculosis drugs in a cohort of patients from the eastern part of Denmark and to determine the relationship to clinical outcome.

$C_{2h} < \text{normal ranges}$

- Prevalence
- Association to therapy failure (death/relapse) at 1 year follow-up
Study I: Methods

- Clinical reasons, n=10
- As part of the study, n=25

Study group, n=35

- Prophylactic Tx, n=2
  - Diagnostic uncertainty, n=1
- Active TB, n=32

Follow up, n=28

- Lost to follow up, n=3
  - INH-resistant strain, n=1
Study I: Results

Isoniazid

Rifampicin

Ethambutol

Pyrazinamide
Study I: Results

71% Isoniazid

Median 2.1 mg/L

58% Rifampicin

Median 6.5 mg/L

46% Ethambutol

Median 2.2 mg/L

10% Pyrazinamide

Median 31.3 mg/L
## Study I: Results

### Plasma concentration in mg/L

<table>
<thead>
<tr>
<th></th>
<th>Therapy failure (n=5)</th>
<th>Successful (n=23)</th>
<th>P value for diff.*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Isoniazid</strong>, mean† (95% CI)</td>
<td>1.0 (0.4-2.2)</td>
<td>2.6 (2.0-3.3)</td>
<td><strong>0.004</strong></td>
</tr>
<tr>
<td><strong>Rifampicin</strong>, mean† (95% CI)</td>
<td>4.0 (2.2-7.6)</td>
<td>7.3 (5.1-10.5)</td>
<td>0.129</td>
</tr>
<tr>
<td><strong>Ethambutol</strong>, mean† (95% CI)</td>
<td>2.5 (0.7-9.2)</td>
<td>2.0 (1.4-2.8)</td>
<td>0.551</td>
</tr>
<tr>
<td><strong>Pyrazinamide</strong>, mean† (95% CI)</td>
<td>26.4 (14.0-50.0)</td>
<td>35.2 (28.8-43.1)</td>
<td>0.232</td>
</tr>
</tbody>
</table>

* t-test. † geometric mean.
Study I: Results

- Therapy failure was observed more frequently in patients with:
  - isoniazid & rifampicin < normal ranges (5/13 vs. 0/15, p=0.013)
  - isoniazid < median value (5/14 vs. 0/14, p=0.041)
  - rifampicin < median value ((5/15 vs. 0/13, p=0.044)
  - isoniazid & rifampicin < median values (5/11 vs. 0/17, p=0.005)

- Isoniazid plasma concentrations correlated inversely with CRP at time of sampling (p<0.001, $R^2=0.35$), also when adjusted for CRP at baseline (p=0.041, $R^2=0.59$). This could indicate less treatment effect of isoniazid at lower drug concentrations
Study II: Aim

To investigate the pharmacokinetics of isoniazid and rifampicin in a group of adult TB patients in Denmark, and to determine probability of target attainment (PTA) of suggested AUC/MIC levels for optimal killing effect by use of population PK-modeling and Monte Carlo simulation.

PTA

- Isoniazid AUC/MIC $\geq 500h$
- Rifampicin AUC/MIC $\geq 271h$
Study II: Methods

20 patients; Drug susceptible pulmonary TB, standard TB therapy
2 days of study; After 1 and 4 weeks of Tx
Sampling; 0, 30, 60, 90, 120, 150, 180, 240 and 360 min. after oral intake

Total plasma concentrations; Determined by LC-MS/MS
Noncompartmental analysis; $C_{\text{max}}$, AUC, $T_{1/2}$ each patient each study day

Six PK parameter linear model fit; Population PK parameter distributions
Monte Carlo simulation; 50,000 individuals

PTA analysis; isoniazid AUC/MIC $\geq 500h$, rifampicin AUC/MIC $\geq 271h$
Fixed MIC value; INH 0.05 mg/L, RIF 1 mg/L
Isoniazid dosage regimen; 300 mg every 24 hours

Population level:
5% obtained an AUC/MIC ≥ 500 h
(MIC = 0.05mg/L)
Isoniazid dosage regimen; 300 mg every 24 hours

Population level:
5% obtained an AUC/MIC ≥ 500 h (MIC = 0.05mg/L)

Monte Carlo simulation, n_{indiv} = 50,000:
AUC/MIC ≥ 500 h (MIC = 0.05 mg/L)
PTA = 1.4%
Study II: Results

Rifampicin dosage regimen; 600 mg every 24 hours

Population level:
Non obtained an AUC/MIC ≥ 271 h
(MIC = 1.0 mg/L)

Monte Carlo simulation, $n_{\text{indiv}} = 50,000$:
AUC/MIC ≥ 271 h (MIC = 1.0 mg/L)
PTA = 0 %
Using standard dosis regimens:

- a considerable interpatient pharmacokinetic variability was observed
- $C_{2h}$ and/or $C_{\text{max}}$ lower than normal $C_{\text{max}}$ ranges were frequently observed
- AUC/MIC levels below suggested targets were frequently observed
Study I suggests:

- An association between therapy failure and isoniazid and/or rifampicin plasma concentration levels.

- That slightly lower threshold targets of $C_{2h}$ and/or $C_{max}$ of isoniazid and rifampicin might be more predictive of treatment outcome.
Acknowledgements

Supervisors
- Niels Frimodt-Møller (principal supervisor)
- Åse Bengaard Andersen (project supervisor)
- Arieh S Cohen (project supervisor)

Evaluation Committee
- Staff Specialist, associate professor Christian Wejse, (chairman)
- Regional Medical Officer, associate professor Håkan Miörner, Sweden
- Consultant, associate Professor, Ole Kirk, Denmark
Acknowledgements

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- Nurse Nete Olsen
- Laboratory technicians Inge Møller, Sara Olsen, Anita Jørgensen, Rehab M. El Maghrabi and Pia Nygaard Kristiansen
- Staff at International Reference Laboratory of Mycobacteriology, Department of Clinical Biochemistry, Immunology and Genetics and collaborating departments
- Colleagues and fellow PhD students

All collaborators and co-authors
Pharmacokinetics and pharmacodynamics of anti-tuberculosis drugs in patients with tuberculosis

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Statens Serum Institut
Taget ud
Considerable interpatient pharmacokinetic variability was observed, and $C_{2h}$ and/or $C_{\text{max}}$ lower than normal $C_{\text{max}}$ ranges were frequently observed.

AUC/MIC ratios above suggested targets for optimal killing effect of isoniazid and rifampicin are generally not attained.

Study I suggests an association between treatment outcome and isoniazid and/or rifampicin plasma concentration levels.
Study II: Limitations

- Small sample size
- Both patients receiving directly observed therapy (DOT) and self-administered therapy (SAT) were included
- Time of sampling during treatment was not standardized
- Patients not necessarily fasting
- Measured $C_{2h}$ and not $C_{\text{max}}$
Isoniazid PK data of one patient and rifampicin PK data of two patients were left out of the population PK-modeling.

Focus on the typical male, HIV negative, TB patient in Denmark.
Mycobacterium tuberculosis

Etiological agent of tuberculosis

Airborne

Aerobe

Intra- and extracellular

Slowly growing may even lie domant

Thick cell membrane rich on lipids

Complex cell-mediated immune response
In vitro model

Hollow fiber model
Non-adherence only leads to failure of therapy after very high cumulative non-adherence >60% of doses

Non-adherence does not lead to MDR-TB

Multidrug-Resistant Tuberculosis Not Due to Noncompliance but to Between-Patient Pharmacokinetic Variability

Shashikant Srivastava,1 Jotam G. Pasipanodya,1 Claudia Meek,2 Richard Leff,2 and Tawanda Gumbo1

The Journal of Infectious Diseases 2011;204:1951–9
## Study II: Results

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Therapy failure (n=5)</th>
<th>Successful (n=23)</th>
<th>P value for diff.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex, n(%)</td>
<td>3(60)</td>
<td>13(57)</td>
<td>1.0§</td>
</tr>
<tr>
<td>Age in years, median</td>
<td>49</td>
<td>38</td>
<td>0.401∞</td>
</tr>
<tr>
<td>BMI in kg/m², median</td>
<td>18.6</td>
<td>20.3</td>
<td>0.266∞</td>
</tr>
<tr>
<td>Inpatient, n(%)</td>
<td>4(80)</td>
<td>13(57)</td>
<td>0.620§</td>
</tr>
<tr>
<td>Focus, n(%)</td>
<td>Pulmonary</td>
<td>Extrapulmonary</td>
<td>Both</td>
</tr>
<tr>
<td></td>
<td>5(100)</td>
<td>-</td>
<td>12(52)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>10(44)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>1(4)</td>
</tr>
<tr>
<td>Diabetes mellitus, n(%)</td>
<td>2(40)</td>
<td>3(13)</td>
<td>0.207§</td>
</tr>
<tr>
<td>HIV infection, n(%)</td>
<td>0(0)</td>
<td>2(9)</td>
<td>1.0§</td>
</tr>
<tr>
<td>Excessive alcohol use, n(%)</td>
<td>4(80)</td>
<td>4(17)</td>
<td>0.015§</td>
</tr>
<tr>
<td>FDC product, n(%)</td>
<td>2(40)</td>
<td>16(70)</td>
<td>0.315§</td>
</tr>
<tr>
<td>CRP&lt;sub&gt;baseline&lt;/sub&gt;, in mg/L, median</td>
<td>155</td>
<td>44</td>
<td>0.029∞</td>
</tr>
</tbody>
</table>

§ Fischer exact test. ∞ Mann-Whitney. Abbreviations: FDC = fixed dose combination.
Study II: Results

71% Isoniazid

58% Rifampicin

46% Ethambutol

10% Pyrazinamide
Perspectives

In order to optimise the use of TDM:

- To optimise sampling and analysis:
  - when and how to sample
  - Use of filter paper
  - determine the unbound drug concentrations

- Evidence based guidelines for TDM
  - relationship btw. doses, plasma concentrations, treatment efficacy and side effects
  - develop clinically validated target ranges of the drugs

- Individualise TB therapy:
  - measure individual MIC values
  - causes of interpatient pharmacokinetic variability
To develop a rapid, precise and accurate liquid chromatography tandem mass spectrometry (LC-MS/MS) method for simultaneous quantitation of isoniazid, rifampicin, ethambutol and pyrazinamide

- Clinically relevant ranges
- Rapid and simultaneous quantitation of all four first-line drugs
- Precision estimated by the coefficient of variation; CV <15%
- Accuracy estimated by the relative error; relative error % <15%
Mass spectrometry (MS):

- Analytical technique to quantitate each type of molecules in a sample
- Separates molecules by mass to charge ratio
- Tandem mass spectrometry (MS/MS); selects and fragments precursor ions and selects product ions

Liquid chromatography (LC):

- Method for physical separation of components in a complex mixture
- Different components are retained to varying degrees
- Compounds are introduced into the MS sequentially
Study I: Methods

**LC-MS/MS:**

For a compound to be determined it must:

- have the right retention time
- have the right mass/charge ratio
- form the right fragments

Thus, LC-MS/MS has a high degree of selectivity
Study I: Methods

**Calibration curves**
Linear plot of the analyte respons/internal standard respons (respons ratio) against the analyte concentration

- 7 calibration standards (clinically relevant ranges)
- 4 isotopically labeled internal standards

![Calibration curve graph] The analyte concentration in an unknown sample can be determined by measuring the analyte respons/internal standard respons which can be converted to a corresponding concentration.
Study I: Results

Linearity test

**Isoniazid**
- Linear range: 0.5-10 mg/L
- Equation: \( y = -0.0145 + 1.0035x \)
- \( R^2 = 0.9978 \)

**Rifampicin**
- Linear range: 0.75-30 mg/L
- Equation: \( y = 0.348 + 0.9691x \)
- \( R^2 = 0.9948 \)

**Ethambutol**
- Linear range: 0.25-10 mg/L
- Equation: \( y = 1 \times 10^{-5} + 1x \)
- \( R^2 = 0.9987 \)

**Pyrazinamide**
- Linear range: 4.0-80 mg/L
- Equation: \( y = -0.2191 + 1.0037x \)
- \( R^2 = 0.9957 \)

Coefficient of variation and relative error <15%
## Study I: Results

Quality controls at two levels, low and high:

<table>
<thead>
<tr>
<th></th>
<th>CV (%)</th>
<th>Accuracy (relative error %)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>INH</strong></td>
<td>5.1 - 7.8</td>
<td>-13.7 - -13.2</td>
</tr>
<tr>
<td><strong>RIF</strong></td>
<td>4.3 - 4.8</td>
<td>-7.7 - -3.4</td>
</tr>
<tr>
<td><strong>EMB</strong></td>
<td>1.6 - 3.5</td>
<td>8.2 - 15.0</td>
</tr>
<tr>
<td><strong>PZA</strong></td>
<td>6.0 - 11.0</td>
<td>-8.6 - -6.9</td>
</tr>
</tbody>
</table>

Data represent 6 observations

Proficiency test samples at two levels, low and high:

<table>
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<tr>
<td><strong>INH</strong></td>
<td>11.3 - 12.4</td>
<td>-2.2 - -0.6</td>
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<td>7.9 - 9.6</td>
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</table>

Data represent 12 observations
Study I: Conclusions

The developed LC-MS/MS method:

- provides a rapid and simultaneous quantitation of first-line TB drugs
- has clinically relevant quantitation ranges
- performs within accepted standards of LC-MS/MS analysis in terms of precision and accuracy
- is suitable for therapeutic drug monitoring (TDM) and PK/PD studies
Study I: Limitations

- Precision and accuracy were compromised to be able to quantitate all four TB drugs rapidly and simultaneously.
- Lack of golden standards for comparison.
**Strongly concentration-dependent antibiotic**

AUC/MIC and $C_{\text{max}}$/MIC correlates the best to antimicrobial activity

Optimal dosage regimens; Maximises plasma concentrations

*Isoniazid, rifampicin, ethambutol and pyrazinamide*

**Minimal concentration-dependent antibiotic**

T>MIC correlates the best to antimicrobial activity

Optimal dosage regimens; Maximises the duration of time above the MIC
Therapeutic ranges?

Normal ranges in healthy volunteers

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<thead>
<tr>
<th>Drug</th>
<th>Dose</th>
<th>$T_{\text{max}}$ (h)</th>
<th>$T_{1/2}$ (h)</th>
<th>$C_{\text{max}}$ (mg/L)</th>
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Peloquin 1996 and 2002

Targets for optimal efficacy in mouse studies

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<td>Rifampicin 600 mg</td>
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</table>

Jayaram 2003, 2004
Study II: Conclusions

Two-hour plasma concentrations of rifampicin and isoniazid below normal values were:

- frequently observed in adult TB patients
- associated with an increased risk of death or relapse
Study III: Results

Monte Carlo simulation; Evaluation of model fit on the population level

Simulated concentrations: mean values (solid curves) 95% CI (dotted curves)

♦ observed concentrations

Simulated mean value (solid curves)

♦ observed mean concentrations ±SEM
Study III: Results

Isoniazid

95%

Cmax plasma concentration mg/L

Rifampicin

50%

Cmax plasma concentration mg/L
Monte Carlo simulation, \( n_{\text{indiv}} = 50.000: \)

Isoniazid \( C_{2h} > 3.0 \text{ mg/L} \)

PTA = 5 %

(95% CI: 0.4 - 3.4 mg/L)

Rifampicin \( C_{2h} > 8.0 \text{ mg/L} \)

PTA = 42 %

(95% CI: 1.7 - 13.6 mg/L)

For both isoniazid and rifampicin:

\( T_{\text{max}} < 2 \text{ h} \)

therefore

\( C_{2h} \) did not equal \( C_{\text{max}} \)
Study III: Conclusions

Standard doses of 300 mg isoniazid and 600 mg rifampicin are not sufficient to attain the suggested AUC/MIC targets of:

- 500 h for isoniazid
- 271 h for rifampicin
Agenda

Background

Study I-III

Summary

Perspectives
Agenda

Background

Study I-III

Summary

Perspectives
Rester
The LC-MS/MS method developed provides simultaneous, precise and accurate quantitation of all four first-line TB drugs

- Only few other LC-MS/MS methods use isotopically labeled internal standards and most only quantitate a few drugs simultaneous

Considerable interpatient pharmacokinetic variability was observed, and $C_{2h}$ and/or $C_{max}$ lower than normal $C_{max}$ ranges were frequently observed

- $C_{2h}$ levels lower than normal $C_{max}$ ranges have previously been observed [Tappero 2005]

AUC/MIC ratios above suggested targets for optimal killing effect of isoniazid and rifampicin are generally not attained

- One study has previously investigated PTA of an AUC/MIC $\geq 271$ h for rifampicin [Goutelle 2009]
Study II: Results

Isoniazid

Rifampicin

Ethambutol

Pyrazinamide
Monte Carlo simulation, $n_{\text{indiv}} = 50,000$:
Isoniazid $C_{2h} > 3.0 \text{ mg/L}$
PTA = 5 %
(95% CI: 0.4 - 3.4 mg/L)

Rifampicin $C_{2h} > 8.0 \text{ mg/L}$
PTA = 42 %
(95% CI: 1.7 - 13.6 mg/L)
## Study II: Methods

<table>
<thead>
<tr>
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<th>Group A (n=10)</th>
<th>Group B (n=25)</th>
<th>P value for diff.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid $C_{2h}$ in mg/L, median</td>
<td>2.0</td>
<td>2.2</td>
<td>0.32*</td>
</tr>
<tr>
<td>Rifampicin $C_{2h}$ in mg/L, median</td>
<td>6.5</td>
<td>6.6</td>
<td>0.98*</td>
</tr>
<tr>
<td>Ethambutol $C_{2h}$ in mg/L, median</td>
<td>2.4</td>
<td>1.9</td>
<td>0.40*</td>
</tr>
<tr>
<td>Pyrazinamide $C_{2h}$ in mg/L, median</td>
<td>37.8</td>
<td>31.1</td>
<td>0.42*</td>
</tr>
<tr>
<td>CRP baseline in mg/L, median</td>
<td>74</td>
<td>57</td>
<td>0.62*</td>
</tr>
<tr>
<td>CRP sampling time in mg/L, median</td>
<td>39</td>
<td>14</td>
<td>0.37*</td>
</tr>
<tr>
<td>Therapy failure</td>
<td>3/8</td>
<td>2/20</td>
<td>0.12†</td>
</tr>
</tbody>
</table>

*Mann-Whitney, † Fisher exact*
### Study III: Results

#### Pharmacokinetics of isoniazid and rifampicin, median value (range)

<table>
<thead>
<tr>
<th></th>
<th>Isoniazid</th>
<th>Rifampicin</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>One week</td>
<td>Four weeks</td>
<td>One week</td>
</tr>
<tr>
<td>$C_{2h}$ (mg/L)</td>
<td>1.7 (0.6-3.4)</td>
<td>1.6 (0.5 - 3.2)</td>
<td>0.49</td>
</tr>
<tr>
<td>$C_{max}$ (mg/L)</td>
<td>3.0 (1.4-8.1)</td>
<td>3.1 (0.8- 6.6)</td>
<td>0.28</td>
</tr>
<tr>
<td>AUC (mg*h/L)</td>
<td>11.4 (4.0-26.1)</td>
<td>9.2 (2.8 - 23.6)</td>
<td>0.08</td>
</tr>
<tr>
<td>$T_{max}$ (h)</td>
<td>0.5 (0.5 - 3.0)</td>
<td>1 (0.5 - 2.5)</td>
<td>0.19</td>
</tr>
<tr>
<td>$T_{1/2}$ (h)</td>
<td>3.3 (1.3 - 6.5)</td>
<td>2.4 (0.5 - 6.7)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

* wilcoxon signed rank test for difference
Study III: Results

Prediction of the observed concentrations when simulations are made with individual model fit (PK parameters) for each patient.

Isoniazid
19 patients, 171 data points

Rifampicin
18 patients, 162 data points
Therapy failure was observed more frequently in patients with:

- isoniazid & rifampicin < normal ranges (5/13 vs. 0/15, p=0.013)
- isoniazid < median value* (5/14 vs. 0/14, p=0.041)
- rifampicin < median value* (5/14 vs. 0/14, p=0.041)
- isoniazid & rifampicin < median values *(5/10 vs. 0/18, p=0.003)

*median amongst the 28 follow-up patients
## Perspectives

### When to sample?

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Consideration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment start</td>
<td>No significant difference between plasma concentration measured the two days of study on population level (study III)</td>
</tr>
<tr>
<td>Single vs. multiple days</td>
<td>To eliminate day-to-day variation (study III)</td>
</tr>
<tr>
<td>Ingestion of medication</td>
<td>Quantitating $C_{2h}$ we risk underestimating $C_{\text{max}}$ in patients with rapid or delayed absorption (study III)</td>
</tr>
<tr>
<td>Single vs. multiple sample points</td>
<td>Multiple sample points (study III) or a limited sample strategy provides a more reliable estimate of $C_{\text{max}}$ and AUC</td>
</tr>
<tr>
<td>Depends on indication</td>
<td>Non-adherence? TDM and dose adjustment? PK/PD study?</td>
</tr>
</tbody>
</table>
Study II: Results

Associations:

Rifampicin plasma concentrations

- Decrease with increasing age (p=0.006, $R^2=0.23$)
- Lower in patients with low hemoglobin at baseline (p=0.004)

No associations were found between plasma concentrations of isoniazid, rifampicin, ethambutol or pyrazinamide and:

- Gender
- Hypoalbuminaemia
- Dose (mg/kg)
- Drug formulation
- Hospital status
- Number of days on treatment at the time of sampling
Univariate logistic regression analysis:

- Therapy failure inversely associated with plasma concentrations of isoniazid (p=0.021)
- Therapy failure inversely associated with CRP at baseline (p=0.056)
- Including both, plasma concentration of isoniazid remained borderline significant (p=0.072) whereas the effect of CRP disappeared (p=0.763)
Study II: Results

- Risk of therapy failure decreased with increasing plasma concentrations of isoniazid (p=0.021)
- Risk of therapy failure tended to increase with increasing CRP levels at baseline (p=0.056)
- Including both, the effect of isoniazid levels remained borderline significant (p=0.072) whereas the effect of CRP disappeared (p=0.763)
Study I: Methods

Precision determined by calculating the coefficient of variation:

\[
CV (\%) = \frac{\text{standard deviation}}{\text{mean concentration}} \times 100 \%
\]

Accuracy estimated by the relative error calculated as:

\[
\text{Relative error (\%)} = \frac{\text{back calculated concentration} - \text{nominal concentration}}{\text{nominal concentration}} \times 100 \%
\]
## Study I: Results

<table>
<thead>
<tr>
<th></th>
<th>Intraday (n=6)</th>
<th>Interday (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CV (%)</td>
<td>relative error (%)</td>
</tr>
<tr>
<td>Isoniazid 0.5 - 10 mg/L</td>
<td>3 - 8</td>
<td>-7.6 - 17.7</td>
</tr>
<tr>
<td>Rifampicin 0.75 - 30 mg/L</td>
<td>1.9 - 7.9</td>
<td>-4.6 - 11.1</td>
</tr>
<tr>
<td>Ethambutol 0.5 - 10 mg/L</td>
<td>2 - 4.3</td>
<td>-0.6 - 5.1</td>
</tr>
<tr>
<td>Pyrazinamide 4 - 80 mg/L</td>
<td>2.5 - 13.7</td>
<td>-4.1 - 1.5</td>
</tr>
</tbody>
</table>

CV, coefficient of variation
Summary

- Using standard dose regimens a considerable interpatient pharmacokinetic variability was observed, and $C_{2h}$ and/or $C_{\text{max}}$ lower than normal $C_{\text{max}}$ ranges were frequently observed
  - $C_{2h}$ levels lower than normal $C_{\text{max}}$ ranges have previously been observed [Tappero 2005]

- Using standard dose regimens AUC/MIC levels below suggested target ranges were frequently observed
  - One study has previously investigated PTA of an AUC/MIC $\geq 271$ h for rifampicin [Goutelle 2009]
Study II suggests an association between therapy failure and isoniazid and/or rifampicin plasma concentration levels

- Isoniazid and/or rifampicin plasma concentration levels have been suggested to influence on treatment efficacy/outcome in two resent prospective studies [Pasipanodya 2013, Ramachandran 2013]

- Lower therapeutic $C_{2h}$ and /or $C_{\text{max}}$ targets for isoniazid and rifampicin might be more predictive of treatment outcome
  - New rifampicin $C_{\text{max}}$ and isoniazid $C_{2h}$ threshold targets lower than the normal $C_{\text{max}}$ ranges have been suggested to be more predictive of therapy failure [Pasipanodya 2013, Donald 2007]
Day-to-day variability $C_{\text{max}}$ and $C_{2h}$

- $C_{\text{max}}$ INH day 2 vs day 1: $R^2=0.26$, $p=0.036$
- $C_{2h}$ INH day 2 vs day 1: $R^2=0.44$, $p=0.003$
Day-to-day variability AUC

AUC/MIC INH day2 vs day 1

R^2 = 0.73, p < 0.0001
C2h vs Cmax

C2h vs Cmax INH

R2=0.32, p=0.01
AUC INH vs NAT2

Graph showing the relationship between AUC/MIC INH day 1 and NAT2.
C2h vs Cmax