Agnija Kivrāne

ORCID 0000-0002-8284-2011

Efficacy and Safety of Anti-Tuberculosis Therapy: Pharmacokinetic and Pharmacogenetic Studies in Latvian Patient Population

Summary of the Doctoral Thesis for obtaining the scientific degree "Doctor of Science (*PhD*)"

Sector Group – Medical and Health Sciences

Sector – Basic Medicine

Sub-Sector – Clinical Pharmacy

Riga, 2026

The Doctoral Thesis was developed at the Molecular Microbiology Group, Latvian Biomedical Research and Study Centre, and at the Prof. Renāte Ranka Group: Pharmacogenetics and Precision Medicine, Rīga Stradiņš University, Latvia

Supervisor of the Doctoral Thesis:

Dr. biol., Professor **Renāte Ranka**,
Rīga Stradiņš University, Latvia,
Leading Researcher, Latvian Biomedical Research and Study Centre

Official Reviewers:

Dr. pharm., Associate Professor **Inga Urtāne**,
Rīga Stradiņš University, Latvia

Dr. biol., Associate Professor **Vita Rovīte**,
Faculty of Medicine and Life Sciences, University of Latvia,
Leading Researcher, Latvian Biomedical Research and Study Centre

Dr. pharm., Associate Professor **Kristina Garuolienė**,
Pharmacy and Pharmacology Center, Faculty of Medicine,
Vilnius University, Lithuania

Defence of the Doctoral Thesis will take place at the public session of the Promotion Council of Basic Medicine on 1 April 2026, at 10.00 in the Hippocrates Lecture Theatre, 16 Dzirciema iela, Rīgas Stradiņš University

The Doctoral Thesis is available in RSU Library and on RSU website:
https://www.rsu.lv/en/dissertations

FLPP
FUNDAMENTAL AND APPLIED RESEARCH PROJECTS

This research was conducted as part of the Fundamental and Applied Research project "Tuberculosis treatment: Exploring the perspectives for personalised therapy" (No lzp2020/1-0050), funded by the Latvian Council of Science

Funded by the European Union
2027 National Development Plan

This research was performed within the framework of the project "Rīga Stradiņš University internal and Rīga Stradiņš University with Latvian Academy of Sport Education external consolidation" (No 5.2.1.1.i.0/2/24/I/CFLA/005), funded by the European Union's Recovery and Resilience Mechanism and the Republic of Latvia

Secretary of the Promotion Council:

Dr. pharm., Leading Researcher **Konstantīns Logviss**

Table of Contents

Abbreviations used in the Thesis

AADAC	arylacetamide deacetylase
ABCB1	adenosine triphosphate-binding cassette subfamily B member 1
ADR	adverse drug reaction
Anti-TB DILI	anti-tuberculosis drug-induced liver injury
ATP	adenosine triphosphate
AUC	area under the time-concentration curve
BMI	body mass index
C_{max}	peak concentration 2 hours post-dose
CRP	C-reactive protein
CRP_b	C-reactive protein levels measured before initiating anti-tuberculosis therapy
$CRP_{10–12d}$	C-reactive protein levels measured 10–12 days after initiating anti-tuberculosis therapy
C_{trough}	trough concentration
CYP3A	cytochrome P450 family 3 subfamily A
CYP3A4	cytochrome P450 family 3 subfamily A member 4
DILI	drug-induced liver injury
DNA	deoxyribonucleic acid
DS-TB	drug-susceptible tuberculosis
ETB	ethambutol
HIV	human immunodeficiency virus
IL-1β	interleukin-1 beta
IL-6	interleukin-6
INH	isoniazid
LC-MS/MS	liquid chromatography-tandem mass spectrometry
MIC	minimal inhibitory concentration
M. tuberculosis	*Mycobacterium tuberculosis*
N	number of patients
NAT2	N-acetyltransferase 2
NGS	next-generation sequencing
NR1I2	nuclear receptor subfamily 1 group I member 2
OATP1B1	organic anion transporting polypeptide 1B1
OATP1B3	organic anion transporting polypeptide 1B3
Pgp	P-glycoprotein
PGx	pharmacogenetics

PK	pharmacokinetics
PXR	pregnane X receptor
PZA	pyrazinamide
RIF	rifampicin
SLCO1B1	solute carrier organic anion transporter family member 1B1
SLCO1B3	solute carrier organic anion transporter family member 1B3
TB	tuberculosis
TDM	therapeutic drug monitoring
TNF-α	tumour necrosis factor alpha
tSCC	time to sputum culture conversion
WHO	World Health Organization

Introduction

With a global incidence of 10–12 million people and a mortality of 1–2 million people annually over the past decade, tuberculosis (TB), a seemingly preventable and curable infectious disease, is an alarming public health problem even in the era of advanced medical technologies (World Health Organization [WHO], 2024). The majority of patients initially present with drug-susceptible tuberculosis (DS-TB) and accordingly receive the WHO-recommended four- or six-month treatment regimen (WHO, 2022a, 2024). In most countries within the WHO European Region, including Latvia, the latter regimen with a four-drug combination – namely rifampicin (RIF), pyrazinamide (PZA), isoniazid (INH), and ethambutol (ETB) – appears to be superior in terms of accessibility and provides a cure rate of up to 88 % (Masini et al., 2022; Günther et al., 2023; WHO, 2024).

To date, many investigators have reported substantial variability in the pharmacokinetics (PK) of the four aforementioned anti-TB drugs when administered at standard doses (Fahimi et al., 2013; Pasipanodya et al., 2013; Prahl et al., 2014; Kloprogge et al., 2020; Ramachandran et al., 2020). The importance of maintaining anti-TB drug exposure within therapeutic ranges lies in the fact that each drug exhibits a distinct mechanism of action and penetrates heterogeneous tuberculous lesions to varying extents. This enables the drugs to target *Mycobacterium tuberculosis* (*M. tuberculosis*) at different stages of replication (Sirgel et al., 2000; Prideaux et al., 2015; Strydom et al., 2019; Ignatius & Dooley, 2023). Consequently, reduced exposure to anti-TB drugs has been recognised as a reason for delayed treatment responses and treatment failures, including the development of drug-resistant forms of the disease that are difficult to treat, requiring an immediate transition to extended regimens with second-line drugs (Pasipanodya et al., 2013; Prahl et al., 2014; Kloprogge et al., 2020; Ramachandran et al., 2020).

Prahl et al. (2014) studied the effect of anti-TB drug plasma exposure from a different perspective; they established a relationship between INH exposure and the degree of TB-associated inflammation during the first two months of anti-TB therapy, i.e. the intensive treatment phase. Typically, serum C-reactive protein (CRP) levels complement the disease severity at diagnosis and begin to decline after the first weeks of treatment, reflecting the gradual clearance of *M. tuberculosis* (Djoba et al., 2008; Miranda et al., 2017; Wilson et al., 2018; Azam et al., 2022). Several authors have highlighted it as a promising biomarker for monitoring the bacteriological response and predicting adverse treatment outcomes (Djoba et al., 2008; Miranda et al., 2017; Wilson et al., 2018; Azam et al., 2022). Nevertheless, the impact of anti-TB drug exposure on the patterns of serum CRP level reduction and the clinical relevance of these findings has not been further explored.

Concerning the safety of a six-month regimen for treatment of DS-TB, nearly a third of treated patients experience drug-induced liver injury (DILI), manifesting as an asymptomatic, transient elevation of liver enzymes in mild cases or acute liver failure and death in the worst-case scenarios (Devarbhavi et al., 2013; Chen et al., 2015a; Zhuang et al., 2022). This adverse drug reaction (ADR) has been attributed to the hepatotoxic properties of RIF, PZA, and INH, whether used alone or in combination (Chen et al., 2015a; Zhuang et al., 2022). Although doses at the upper end of the recommended range and higher exposure are known to trigger anti-tuberculosis drug-induced liver injury (anti-TB DILI), the complex nature of this ADR is still poorly understood (Satyaraddi et al., 2014; Chen et al., 2015a; Zheng et al., 2021; Zhuang et al., 2022).

Apart from patient-dependent factors such as age, biological sex, and comorbidities, which are often mentioned when discussing differences in anti-TB drug exposure and the development of DILI, an important consideration is gene variants altering the function of drug-metabolising enzymes and transporters (McIlleron et al., 2006; Nijland et al., 2006; Yimer et al., 2011; Chen et al., 2015a; Zhuang et al., 2022). The impact of pharmacogenetic (PGx) variability on drug efficacy and safety has been well demonstrated in other therapeutic areas. For example, Manolopoulos et al. (2010) have estimated that variants in the genes encoding cytochrome P450 family 2 subfamily C member 9 and vitamin K epoxide reductase complex subunit 1 may account for 35–50 % of the variation in the warfarin dose. Similarly, more than 60 % of statin-induced myopathy cases are linked to variants in the solute carrier organic anion transporter family member 1B1 (*SLOC1B1*) gene encoding organic anion transporting polypeptide 1B1 (OATP1B1) (SEARCH Collaborative Group et al., 2008). In the context of the present work, only the N-acetyltransferase 2 (NAT2) encoding gene is considered of significant PGx importance due to extensive evidence of a genotype-phenotype relationship, at least in part, explaining INH-related hepatotoxicity (McDonagh et al., 2014; Chen et al., 2015a; Zhuang et al., 2022; Ulanova et al., 2024). Other findings regarding the effect of patient genetic background on variability in anti-TB drug exposure and treatment-related hepatotoxicity remain controversial (Chigutsa et al., 2011; Wang et al., 2019; Zhang et al., 2019; Weiner et al., 2021).

Aim of the Thesis

To investigate the effect of variants in RIF pharmacogenes on its plasma exposure and the development of anti-TB DILI, as well as to characterise the early changes in serum CRP levels and their influencing factors in Latvian patients with DS-TB.

Objectives of the Thesis

The following objectives were set to reach the aim of the doctoral Thesis:

1. Develop and validate a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for quantification of RIF, PZA, INH, and ETB in human plasma.
2. Design a targeted next-generation sequencing (NGS) protocol for analysing genomic regions of interest.
3. Investigate whether variants detected in genes encoding enzymes (arylacetamide deacetylase [*AADAC*] gene), transporters (*SLCO1B1* and solute carrier organic anion transporter family member 1B3 [*SLCO1B3*] genes, and adenosine triphosphate-binding cassette subfamily B member 1 [*ABCB1*] gene), and nuclear receptors (nuclear receptor subfamily 1 group I member 2 [*NR1I2*] gene) involved in RIF disposition are related to RIF plasma exposure and anti-TB treatment-related hepatotoxicity.
4. Examine the role of patient- and disease-related factors, including plasma exposure of four anti-TB drugs, in the reduction in serum CRP levels after the first 10–12 days of therapy and evaluate the potential of these early changes to predict bacteriological response.

Hypotheses of the Thesis

1. One or more variants detected across the five investigated genes encoding RIF-metabolising enzymes, transporters, and associated regulatory proteins are related to variability in RIF plasma exposure and to the development of anti-TB DILI.
2. Higher serum CRP levels 10–12 days after anti-TB treatment onset, along with less than two-fold reduction compared to baseline, are related to lower plasma exposure of one or several anti-TB drugs.

Novelty of the Thesis

This Thesis analyses anti-TB drug exposure and RIF-related genetic determinants from various perspectives and outlines their role in the efficacy and safety of DS-TB therapy.

The newly developed LC-MS/MS method is the first to offer simultaneous quantification of RIF, PZA, INH, and ETB alongside their six primary metabolites in human plasma, enabling a more comprehensive PK characterisation. This assay can be adapted for therapeutic drug monitoring (TDM) to support the implementation of personalised TB treatment strategies in clinical practice. Meanwhile, the designed NGS-based protocol for targeted genetic analysis proved suitable and readily adjustable for detecting variants dispersed

across multiple genes or gene fragments of interest. Both methodologies were applied in the two subsequent studies.

The first study characterised the impact of variants in the genes encoding enzymes (*AADAC* gene), transporters (*SLCO1B1*, *SLCO1B3*, and *ABCB1* genes), and nuclear receptors (*NR1I2* gene) involved in RIF disposition (collectively referred to as RIF pharmacogenes) on its plasma exposure and anti-TB treatment-related hepatotoxicity, providing valuable data on genotype-phenotype associations in patients of European ancestry (Caucasians). Even though the obtained results largely support the view that alterations in the function of the explored proteins caused by variants in the analysed genes play a limited role, one *NR1I2* gene variant (rs3732357) was found to be related to RIF plasma exposure. Notably, its effects have not been previously documented in patients with TB.

The second study adds new evidence on the impact of patient- and disease-related factors on the early reduction in serum CRP levels following initiation of anti-TB therapy. Importantly, it demonstrates that the assessment of this biomarker levels after 10–12 days of anti-TB drug administration appears to lack clinical relevance, as the observed changes were not predictive of bacteriological response.

Ethical aspects

Each of the studies described in this Thesis was carried out according to the guidelines of the Declaration of Helsinki. Where applicable, the study protocol was approved by the Central Medical Ethics Committee of Latvia (Approval Nos 01-29.1/1 and 01-29.1.2/1736), the Research Ethics Committee of Rīga Stradiņš University (Approval No 6-3/1/6), the Ethics Committee of the Riga East Clinical University Hospital (Approval No 24-A/15), and the Scientific Department of the Riga East Clinical University Hospital (Approval No ZD/08-06/01-21/187).

Discussion

Addressing the knowledge gap outlined above and the raised scientific questions, the first part of this Thesis presents an analysis of genetic factors potentially contributing to variability in RIF plasma exposure and anti-TB DILI, focusing on five RIF pharmacogenes: *AADAC*, *SLCO1B1*, *SLCO1B3*, *ABCB1*, and *NR1I2*. The second part summarises key findings from a study on early changes in inflammation caused by anti-TB treatment, with particular emphasis on the impact of anti-TB drug plasma exposure. In addition, this Thesis encompasses the development of LC-MS/MS and NGS-based methods, which were required to support both areas of research. A schematic representation of the study designs is given in Figure 1.

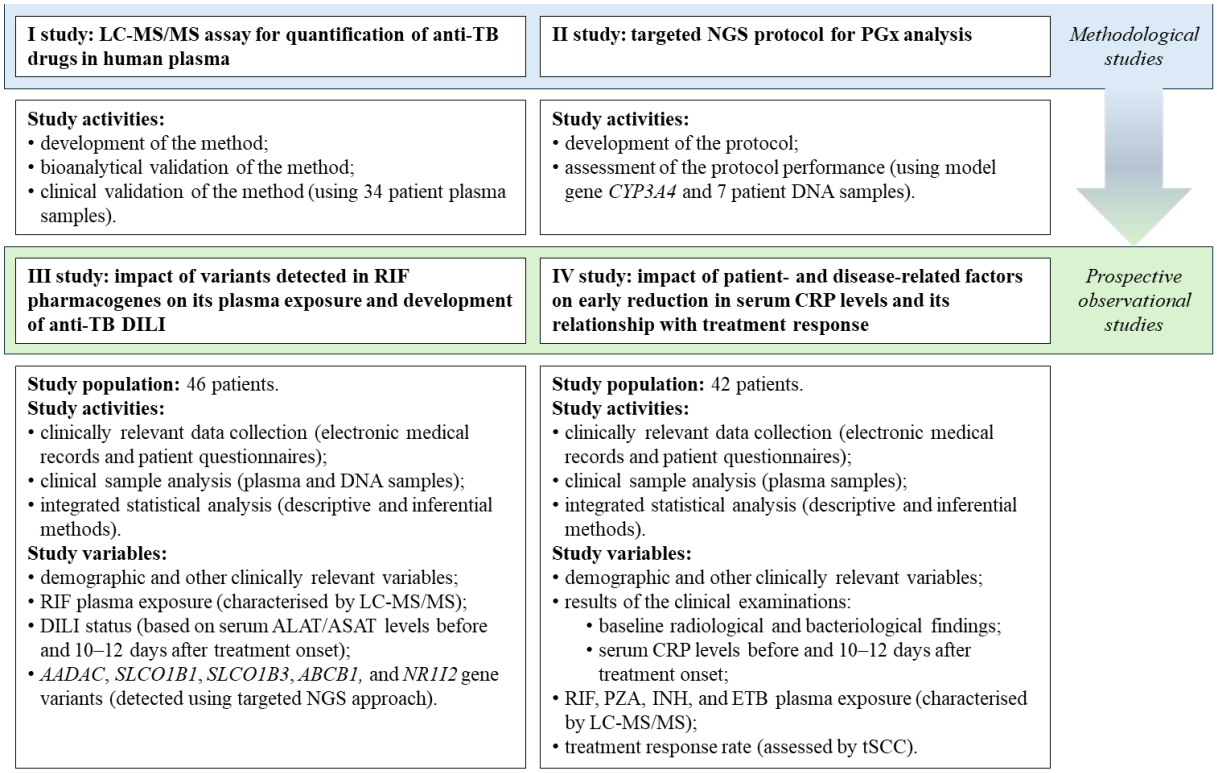

Figure 1. **Schematic representation of the study designs***

*Across all studies, a total of 64 otherwise healthy adult patients diagnosed with pulmonary DS-TB and admitted to the Riga East University Hospital, Centre of Tuberculosis and Lung Diseases (Latvia) were enrolled between April 2017 and May 2023. All patients were of European ancestry (Caucasians). At the time of clinical sample collection, the patients were in the intensive phase of the six-month treatment course, receiving RIF (8–12 mg/kg), PZA (20–30 mg/kg), INH (4–6 mg/kg), and ETB (15–25 mg/kg) for 10–12 days in accordance with the WHO guidelines for treatment of DS-TB (2022a).

Abbreviations: TB, tuberculosis; LC-MS/MS, liquid chromatography-tandem mass spectrometry; NGS, next-generation sequencing; PGx, pharmacogenetics; DNA, deoxyribonucleic acid; *CYP3A4*, cytochrome P450 family 3 subfamily A member 4 gene; RIF, rifampicin; anti-TB DILI, anti-tuberculosis drug-induced liver injury; DILI, drug-induced liver injury; ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; *AADAC*, arylacetamide deacetylase gene; *SLCO1B1*, solute carrier organic anion transporter family member 1B1 gene; *SLCO1B3*, solute carrier organic anion transporter family member 1B3 gene; *ABCB1*, adenosine triphosphate-binding cassette subfamily B member 1 gene; *NR1I2*, nuclear receptor subfamily 1 group I member 2 gene; CRP, C-reactive protein; PZA, pyrazinamide; INH, isoniazid; ETB, ethambutol; tSCC, time to sputum culture conversion.

Method development: LC-MS/MS assay for quantification of anti-TB drugs in human plasma

Overall, LC-MS/MS has become the gold standard for drug quantification in various biological matrices for research purposes and TDM in a clinical setting. As highlighted in the literature, the main challenge in the simultaneous analysis of multiple anti-TB drugs and their metabolites lies in the diversity of their physicochemical properties, which complicates the selection of a suitable sample preparation method and the optimisation of the chromatographic conditions (Zhou et al., 2013; Kim et al., 2015; Shah et al., 2016; Wang et al., 2020). During the initial experiments conducted in this work, attempts to employ existing protocols, such as those offered by Kim et al. (2015), Luyen et al. (2018), and Sundell et al. (2019), resulted in unacceptable analyte retention, poor peak shapes, and ionisation issues. Furthermore, in some cases, the quantification ranges of the reported methods did not cover the middle and upper parts of the therapeutic range, necessitating an additional dilution step during sample pretreatment to fit the calibration curves (Zhou et al., 2013; Gao et al., 2018; Wu et al., 2020). The use of advanced sample preparation techniques and separate analytical method for each analyte was not considered for practical reasons, as it would be time-consuming, require large sample volumes, and involve complex technical solutions.

These issues were resolved by developing a new method for the simultaneous quantification of RIF, PZA, INH, and ETB in human plasma, which, compared to previous assays, has the advantage of measuring their six primary metabolites – 25-desacetylrifampicin, acetylisoniazid, isonicotinic acid, pyrazine-2-carboxylic acid, 5-hydroxypyrazinamide, 5-hydroxypyrazine-2-carboxylic acid – within a single run. This approach incorporates simple single-step protein precipitation with methanol for the sample pretreatment, followed by chromatographic separation on a reverse-phase C8 column using a mobile phase gradient (0.1 % formic acid solution in water (A) and methanol (B); A from 99 % to 2 %), with a total run time of 10 minutes per sample. Analytes were detected and quantified using the multiple reaction monitoring mode. Additional sample pretreatment steps were unnecessary, as the quantification ranges for RIF, PZA, INH, and ETB were selected based on expected peak plasma concentrations 2 hours post-dose (C_{max}) (Alsultan & Peloquin, 2014).

The validation procedure was carried out in accordance with the European Medicines Agency's guidelines on bioanalytical method validation (2012), and the obtained results confirmed satisfactory performance of the developed method, thereby guaranteeing the quality and reliability of the acquired data. As part of the validation, stability tests under various storage conditions revealed limited stability of RIF, INH, and their metabolites, in agreement with findings by Sturkenboom et al. (2015), Luyen et al. (2018), and Wu et al. (2020). Therefore, to

maintain the declared performance, plasma samples should be processed within 4 hours of collection, with temporary storage at −20 °C permitted for up to 24 hours.

The clinical applicability of the method was verified by analysing plasma samples from 34 patients receiving anti-TB therapy. This preliminary assessment of anti-TB drug exposure showed that the mean C_{max} was within the therapeutic range for PZA (38.4 μg/mL *versus* 20–60 μg/mL) and ETB (2.7 μg/mL *versus* 2–6 μg/mL), but for RIF and, to a lesser extent, INH – below the expected range (2.3 μg/mL *versus* 8–24 μg/mL for RIF and 2.8 μg/mL *versus* 3–6 μg/mL for INH) (Alsultan & Peloquin, 2014). Generally, these findings are consistent with those reported in other populations and underscore the need for an in-depth analysis of factors causing variation in anti-TB drug plasma exposure and its potential clinical consequences (Fahimi et al., 2013; Prahl et al., 2014; Niward et al., 2018; Kloprogge et al., 2020; Ramachandran et al., 2020).

Although the metabolite profiling of patient plasma samples was beyond the scope of the studies included in this Thesis, data on INH and its metabolites generated during the clinical validation step are reported separately in Ulanova et al. (2024), where they were used to characterise INH PK and to assess the impact of genetic factors. Accordingly, this extended capability broadens the applicability of the developed method for future PK studies.

Method development: targeted NGS protocol for PGx analysis

Compared to conventional first-generation sequencing techniques such as Sanger sequencing, second-generation or NGS technologies have revolutionised genomic research by offering rapid, cost-effective, and high-throughput analysis of genetic variability. Given the objectives of the planned PGx research, the targeted NGS approach appeared to be more convenient than whole genome or exome sequencing, which are typically used for comprehensive genetic characterisation. At the time of designing the sequencing experiment, commercially available targeted sequencing panels covered only a limited number of variants in genes encoding enzymes, transporters, and regulatory proteins involved in the disposition of RIF and other anti-TB drugs.

The newly designed targeted NGS protocol encompasses steps from amplification and sequencing library preparation to bioinformatic analysis of the raw sequencing data. Its greatest advantage is that, technically, it can be adapted for analysing multiple genes or gene fragments of interest by modifying the amplification and, accordingly, the bioinformatic analysis step.

First, using the cytochrome P450 family 3 subfamily A member 4 (*CYP3A4*) gene as a model, it was demonstrated that the developed protocol for paired-end sequencing on the Illumina platform yielded high-quality data (e. g. the mean base quality score Q > 30, mean

read depth > 100) for the analysed patient desoxyribonucleic acid (DNA) samples (number of patients, N = 7). In parallel, 10 randomly selected *CYP3A4* gene variants were re-sequenced using the reference method, Sanger sequencing. The results obtained were consistent between the two methods, confirming the reliability of the proposed protocol.

However, in two of these samples, up to 16 % of the target sequence lacked read coverage, which was attributed to issues with the quality of individual amplicons, as no substantial variation in read depth was seen in other sequence regions. Seth-Smith et al. (2019) noted that the performance of sequencing library preparation reagents can be affected by low (< 50 %) guanine/cytosine content in the target sequence. Both factors should be considered when adapting the protocol for analysing other genes or gene fragments, thereby ensuring its robustness across different targets.

Among five patients with full target sequence coverage, one carrier of the *CYP3A4*22* (rs35599367) allele and one carrier of the *CYP3A4*1G* (rs2242480) allele were identified; the latter allele has been retired according to the recently revised *CYP3A4* allele classification (Gaedigk et al., 2021). These *CYP3A4* gene variants have been associated, for example, with variability in tacrolimus PK; nevertheless, a detailed analysis of this pharmacogene was beyond the objectives of the present work (Elens et al., 2013; Pallet et al., 2015; Liu et al., 2017; Dong et al., 2022).

Impact of variants detected in RIF pharmacogenes on its plasma exposure and development of anti-TB DILI

RIF is considered one of the most potent components of the six-month regimen for the treatment of DS-TB due to its ability to penetrate various types of tuberculous lesions and act on both metabolically active and dormant forms of *M. tuberculosis* (Dickinson & Mitchison, 1981; Prideaux et al., 2015; Rifat et al., 2018).

At the same time, it demonstrates the highest PK variability among the four anti-TB drugs at standard daily doses, with reported underexposure rates ranging from 42 % to 93 % (McIlleron et al., 2006; Fahimi et al., 2013; Prahl et al., 2014; Niward et al., 2018; Ramachandran et al., 2020). In line with these findings, LC-MS/MS analysis in the present study (N = 46) confirmed a similar trend – 91 % of patients had RIF plasma concentrations at C_{max} below the recommended range (Alsultan & Peloquin, 2014). Compared to patients with optimal RIF plasma exposure, those with underexposure had higher body weight and, thus, a lower body weight-adjusted RIF dose. The impact of other factors described elsewhere was not evident, likely due to the study population's structure, i. e. otherwise healthy adult patients, 76 % of whom were male, and only 11 % were aged 60 years or older (Walubo et al., 1991; McIlleron et al., 2006; Nijland et al., 2006; Weiner et al., 2010; Milán Segovia et al., 2013).

As previously mentioned, DILI is a well-documented ADR in patients treated for DS-TB (Chen et al., 2015a; Zhuang et al., 2022). Abbara et al. (2017) estimated that symptoms of hepatotoxicity emerge in approximately half of cases within the first two weeks of anti-TB drug administration. Notably, symptoms of RIF-related hepatotoxicity may appear earlier than those linked to PZA and INH toxicity (Durand et al., 1996; Abbara et al., 2017). In this study, 13 % of patients developed DILI within 10–12 days of anti-TB therapy, aligning with other reports where incidence rates can reach up to 28 % (Chen et al., 2015a). Regarding patient characteristics, no significant differences were found between the DILI and non-DILI groups, except for elevated serum alanine aminotransferase and aspartate aminotransferase levels on the 10^{th} to 12^{th} day of treatment, reaffirming the homogeneity of the study population discussed above.

The patient's genetic background is another factor that can potentially compromise the efficacy and safety of therapy. Thus, this study further explored the contribution of 10 variants, selected from those detected in the four RIF pharmacogenes (*SLCO1B1*: rs2306283, rs11045819, and rs4149056; *SLCO1B3*: rs60140950; *ABCB1*: rs9282564 and rs1045642; and *NR1I2*: rs3814055, rs3732357, rs2276707, and rs3732359), to the variability in RIF plasma exposure, characterised by C_{max} and area under the time-concentration curve ($AUC_{0–6h}$), as well as to the development of anti-TB DILI.

Initially, *AADAC*, a gene encoding a liver microsomal enzyme responsible for hydrolysing rifamycins into non-toxic 25-desacetyl derivatives, was also considered for analysis, as AADAC enzymatic activity has been reported to be influenced by variants in the respective gene (Nakajima et al., 2011; Shimizu et al., 2012; Francis et al., 2019; Weiner et al., 2021; Ignatius & Dooley, 2023). However, evaluation of *AADAC* gene variants was not feasible due to insufficient statistical power, stemming from their inadequate frequency in the study population.

Continuing with *SLCO1B1* and *SLCO1B3*, these genes encode liver-specific membrane influx transporters, OATP1B1 and organic anion transporting polypeptide 1B3 (OATP1B3), respectively, expressed on the basolateral membrane of hepatocytes (Nie et al., 2020). OATP1B1 and OATP1B3 mediate the hepatic uptake of various endogenous and exogenous substances, including RIF, from the bloodstream (Nie et al., 2020). *In vitro* studies have shown that the investigated *SLCO1B1* exonic variant rs4149056 (T>C) impairs the transport of RIF, atorvastatin, gliclazide, and many other substrates, while the effects of exonic variants rs11045819 (C>A) and rs2306283 (A>G) vary depending on the substrate (Nie et al., 2020). In clinical practice, rs4149056 was identified as a predictor of RIF plasma trough concentration (C_{trough}) and C_{max} in the study conducted by Allegra et al. (2017). Meanwhile, the rs11045819

CA genotype, in conjunction with the African origin and male sex, was reported to be associated with lowered RIF plasma exposure (Weiner et al., 2010; Kwara et al., 2014). On the contrary, in a study involving Ghanaian children, individuals carrying the rs2306283 GG genotype demonstrated higher RIF plasma exposure estimated by $AUC_{0-\infty h}$, but the differences in other PK parameters approached significance (Dompreh et al., 2018). Despite these findings, neither this study nor other authors have confirmed the effect of these *SLCO1B1* gene variants on RIF exposure (Chigutsa et al., 2011; Huerta-García et al., 2019; Naidoo et al., 2019; Medellin-Garibay et al., 2020; Weiner et al., 2021). Similarly, the *SLCO1B3* exonic variant rs60140950 (G>C), which has been reported to adversely impact OATP1B3 expression without altering its activity *in vitro* and to influence telmisartan PK in a cohort of healthy individuals, had no effect in the present study (Schwarz et al., 2011; Hirvensalo et al., 2020; Nie et al., 2020).

Clinically, three DILI patterns are distinguished: hepatocellular, cholestatic, and mixed, all of which have been recorded in patients receiving treatment for DS-TB (David & Hamilton, 2010; Chen et al., 2015a). One proposed mechanism of cholestasis is the competitive inhibition of OATP1B1 and OATP1B3 by RIF, leading to reduced hepatic uptake of another substrate – bilirubin (Campbell et al., 2004; Nie et al., 2020). Although Li et al. (2012) and Chen et al. (2015b) described associations between the *SLCO1B1*15* (rs2306283 + rs4149056) haplotype and anti-TB treatment-related hepatotoxicity, findings from analogous works and this particular study did not confirm the effect of both variants (Yimer et al., 2011; Kim et al., 2012). Also, the third *SLCO1B1* gene variant examined in this study, rs11045819, was not found to be a significant predictor of this ADR. Interestingly, in healthy volunteers, the *SLCO1B1*15* haplotype was related to higher bilirubin levels, which even more increased after RIF initiation, albeit to a similar extent as in individuals without this haplotype (Zhang et al., 2007). This observation suggests that RIF may exacerbate pre-existing hepatobiliary disorders but is unlikely to be their primary cause. In contrast, previous studies assessing the clinical relevance of *SLCO1B3* gene variants have primarily focused on taxane toxicity without convincing evidence supporting any drug-variant combinations (Jabir et al., 2012; Mbatchi et al., 2015). Here, in patients undergoing anti-TB treatment, rs60140950 was not related to the development of hepatotoxicity; however, to the author's knowledge, this is the first report on *SLCO1B3* in this context.

Another transporter-coding gene included in the analysis, *ABCB1*, encodes adenosine triphosphate (ATP)-dependent membrane transporter P-glycoprotein (Pgp). Pgp is expressed in excretory organs and the blood-brain barrier, where it, as a natural defence mechanism, alters the disposition of xenobiotics like RIF by mediating their efflux from the intracellular space (Cascorbi, 2011). Among the *ABCB1* gene variants assessed, the exonic variant rs1045642

(A>G) has exhibited inconsistent effects on messenger ribonucleic acid and Pgp expression and the PK of its substrates (Cascorbi, 2011). In a study with Mexican patients, the rs1045642 genotype, along with biological sex and RIF dose received, was reported as a genetic determinant of RIF plasma exposure (Huerta-García et al., 2019). Regrettably, this finding was not replicated in the present study, and similar negative results have been reported by others (Weiner et al., 2010; Chigutsa et al., 2011; Allegra et al., 2017; Naidoo et al., 2019; Medellin-Garibay et al., 2020). Likewise, *ABCB1* gene variant rs2032582, which is in linkage disequilibrium with rs1045642, was shown to impair Pgp function *in vitro*, but its effect on RIF oral clearance was of marginal significance in a clinical setting (Cascorbi, 2011; Chigutsa et al., 2011). The impact on the evaluated RIF PK parameters also was not evident in the case of another *ABCB1* exonic variant within the scope of this work, rs9282564 (T>C), despite the decrease in tacrolimus plasma C_{trough} reported by Hu et al. (2018).

In the context of DILI, Yimer et al. (2011) showed that the *ABCB1* rs1045642 GG genotype increased susceptibility to this ADR in patients receiving RIF- and efavirenz-containing regimens for the treatment of TB and human immunodeficiency virus (HIV) coinfection. Conversely, a protective effect was found in patients with HIV mono-infection, whereas it had no association with INH-related hepatotoxicity in otherwise healthy patients with TB (Ritchie et al., 2006; Chan et al., 2017). In line with these earlier reports, a relationship between the *ABCB1* gene variants of interest, rs1045642 and rs9282564, and anti-TB treatment-related hepatotoxicity in patients without comorbidities was not established here.

The last of the analysed genes, *NR1I2,* encodes pregnane X receptor (PXR), a ligand-dependent transcription factor whose effects extend to numerous phases I and II drug-metabolising enzymes and transporters (Ma et al., 2008). RIF, one of the most potent PXR ligands, upregulates the transcription of PXR downstream targets and, therefore, is thought to induce its own metabolism, observed as an approximately 40 % decrease in its plasma exposure during the first weeks of anti-TB therapy (Ma et al., 2008; Smythe et al., 2012). As the *NR1I2* exonic variants were underrepresented in the enrolled patient population, this study assessed the effects of flanking intronic variants (rs2276707 (C>T) and rs3732357 (G>A)) and those located in the 5' and 3' untranslated regions (rs3814055 (C>T) and rs3732359 (G>A), respectively). Specifically, carriers of rs3732357 were found to have lower RIF plasma $AUC_{0–6h}$ under the dominant genetic model (GG *versus* GA+AA genotypes), while the difference in plasma C_{max} approached significance. A similar trend was noted for rs3732359, though it was insignificant. The potential clinical relevance of both variants has been indicated before; using midazolam as a model compound, He et al. (2006) and Oleson et al. (2010)

reported that these variants increased cytochrome P450 family 3 subfamily A (CYP3A) enzyme activity in African Americans. Nevertheless, the lack of comprehensive functional data on rs3732357 and rs3732359, particularly the impact on the enzymes and transporters directly implicated in RIF PK, precludes a mechanistic rationale for the described relationship. The two other *NR1I2* gene variants examined – earlier shown to be associated with increased *NR1I2* promoter activity *in vitro* (rs3814055) or elevated intestinal CYP3A expression and contrasting evidence about its impact on tacrolimus PK (rs2276707) – did not demonstrate a relationship with the investigated RIF PK parameters in the performed analyses (Zhang et al., 2001; Barraclough et al., 2012; Rana et al., 2017; Lu et al., 2021).

There has been considerable interest in whether *NR1I2* gene variants modulate individuals' predisposition to anti-TB treatment-related hepatotoxicity, as changes in the basal transcriptional activity of PXR upon RIF exposure are believed to contribute to increased fatty acid absorption and subsequent accumulation in the liver, aggravation of INH-induced oxidative stress due to excessive production of hepatotoxic INH metabolites, and cholestasis caused by increased accumulation of the heme biosynthesis intermediate protoporphyrin IX, all of which are implicated in the development of this ADR (Metushi et al., 2011; Zhuang et al., 2022). Regarding the four *NR1I2* gene variants analysed in this study (rs3814055, rs3732357, rs2276707, and rs3732359), none was significantly related to anti-TB DILI. Analogous studies conducted in populations of Asian ancestry have documented conflicting findings on rs3814055. Zazuli et al. (2015) described an increased susceptibility to this ADR in patients carrying the rs3814055 TT genotype, while Wang et al. (2022), in a more recent study, clarified that a combination of the rs3814055 T allele and NAT2 non-slow acetylator status might be a predisposing factor. In contrast, Zhang et al. (2019) reported a protective effect of this variant. In the case of rs2276707, Yang et al. (2020) concluded that, under the recessive genetic model, it was associated with a reduced risk of anti-TB treatment-related hepatotoxicity, though this finding has not yet been confirmed in different settings.

A considerable strength of this study is its design, which enabled the evaluation of the clinical effects of selected gene variants without interference from other health conditions, as the resulting physiological changes and concomitantly used drugs could influence the anti-TB drug PK and the occurrence of treatment-related ADRs. In parallel, given that the study was conducted in a low-endemic setting (with a provisional TB incidence of 15 cases per 100 000 population in 2024, for a total population of 1.8 million), the applied patient exclusion criteria substantially reduced the number of eligible patients (Centre for Disease Prevention and Control of Latvia, 2025). Consequently, the size of the study population, in conjunction with the utilised NGS protocol, affected the number of gene variants studied.

For instance, it was impossible to verify the finding by Chigutsa et al. (2011) that the *SLCO1B1* deep intronic variant rs4149032, located > 2 kb from the exon-intron junction, was associated with reduced RIF plasma exposure. Notably, this limitation does not extend to low-frequency gene variants such as *AADAC* stop-loss variant rs61733692, with a minor allele frequency of < 1 % in the European population. Expanding the study cohort is unlikely to increase statistical power for testing whether the decreased enzymatic activity determined by the *AADAC*3* (rs1803155 + rs61733692)/*AADAC*3* diplotype, as described in the *in vitro* study by Shimizu et al. (2012), holds clinical significance. Despite these limitations and emphasising the novelty of this work, some of the evaluated gene variants have not been previously explored in patients with TB. Finally, assessing blood biochemical parameters at a single time point within the first few weeks after initiating anti-TB therapy carries the risk of missing DILI cases that develop later. However, the primary interest was RIF-related hepatotoxicity, with its signs and symptoms typically manifesting during this period (Durand et al., 1996; Abbara et al., 2017).

Taken together, current evidence indicates that the analysed variants in the four RIF pharmacogenes (*SLCO1B1*, *SLCO1B3*, *ABCB1*, and *NR1I2*) do not exert a fundamental impact on RIF plasma exposure or the development of anti-TB treatment-related hepatotoxicity. The observed discrepancies between *in vitro* and *in vivo* findings suggest that the biological effects of these gene variants may be mitigated by compensatory mechanisms or influenced by other patient-dependent factors. Furthermore, inconsistencies in *in vivo* findings across studies are likely attributable to variation in study design and patient characteristics. At the same time, this study identified a previously unreported relationship between the *NR1I2* gene variant rs3732357 and RIF plasma exposure. As such, the first hypothesis of this Thesis is considered partly confirmed.

Impact of patient- and disease-related factors on early reduction in serum CRP levels and its relationship with treatment response

Given the complexity and duration of anti-TB therapy required to completely eradicate the infection, monitoring of treatment response and early identification of patients at risk of adverse treatment outcomes are crucial for achieving therapeutic goals. According to WHO (2022b), patients treated for DS-TB should demonstrate clinical improvement and achieve sputum-smear and sputum-culture conversion by the end of the second month of therapy. Calderwood et al. (2021) estimated that approximately 20 % of patients fail to meet this short-term goal, experiencing a delayed bacteriological response, indicative of limited antibacterial effect and an increased risk of treatment failure or relapse in subsequent months.

Measuring serum CRP levels is a well-established approach for assessing and monitoring inflammation in various acute and chronic conditions (Sproston & Ashworth, 2018). Mechanistically, the increased production of CRP is an inherent response to infection or tissue damage mediated by macrophages (Sproston & Ashworth, 2018). Upon activation, macrophages release proinflammatory cytokines interleukin-1 beta (IL-1β), interleukin-6 (IL-6), and tumour necrosis factor alpha (TNF-α), which, in turn, stimulate the production of this acute-phase protein, primarily in human hepatocytes (Sproston & Ashworth, 2018). In the context of TB, this biomarker has recently been approved for screening of active TB in individuals living with HIV, and there is increasing interest in its potential utility for monitoring treatment response in patients receiving anti-TB therapy (Djoba et al., 2008; Miranda et al., 2017; Sigal et al., 2017; Musteikienė et al., 2021; WHO, 2021). However, given the non-specific nature of CRP expression, its levels may vary considerably over the six-month treatment period, making it more suitable for predicting short-term rather than long-term effects (Sproston & Ashworth, 2018).

This study first assessed the impact of patient characteristics on the serum CRP levels measured before initiating anti-TB therapy (CRP_b) (Wener et al., 2000; Khera et al., 2005; Ohsawa et al., 2005; Wyczalkowska-Tomasik et al., 2016). While biological sex, age, and smoking status had negligible impact, body mass index (BMI) exhibited a moderately strong negative correlation with the levels of this biomarker in the study population (N = 42). Despite marginal significance, there was a 10-fold difference in CRP_b levels between underweight patients and those categorised as overweight. It contrasts with observations in patients without TB infection, where serum CRP levels typically increase with BMI (Onwubalili, 1988; Visser et al., 1999; Lönnroth et al., 2010). Nutritional status influences both innate and adaptive immunity and, in the case of TB, modulates susceptibility to infection and the ability to clear an existing one (Gupta et al., 2009; Chandrasekaran et al., 2017; Cho et al., 2022). Among patients with TB, lack of appetite and weight loss are frequently reported symptoms, with severe wasting linked to advanced disease, delayed treatment response, and adverse outcomes (Hoyt et al., 2019; Diktanas et al., 2018; Kornfeld et al., 2020; Sinha et al., 2023).

Characterising the relationship between TB clinical features and serum CRP_b levels, Djoba et al. (2008), Furuhashi et al. (2012), and other authors have shown that levels of this biomarker reflect disease severity, mirroring the radiological and bacteriological findings at the time of diagnosis (Brown et al., 2016; Sigal et al., 2017; Azam et al., 2022). Aligning with that, radiologically confirmed cavitary disease and positive sputum-smear microscopy result, suggestive of severe lung damage with presumably higher bacillary loads, were related to higher serum CRP_b levels in this study. Although Djoba et al. (2008), Furuhashi et al. (2012), and

Musteikienė et al. (2021) reported that the levels of this biomarker may vary depending on the extent of lung involvement, this was not confirmed here, and other groups did not report such a relationship (Mendy et al., 2016; Kumar et al., 2019). The plausible explanation for this discordance is the methodological differences in estimating the TB-affected lung area.

Next, this study examined changes in serum CRP levels 10–12 days after anti-TB treatment onset and evaluated their utility in predicting bacteriological response. This timeframe was deemed appropriate, as a prior study determined that the failure to achieve a 55 % reduction in the levels of this biomarker within the first two weeks of therapy was associated with hospitalisation and death in patients with TB-HIV coinfection (Wilson et al., 2018). Moreover, it is the earliest point at which anti-TB drug exposure can be objectively assessed, ensuring steady-state plasma concentration is reached (Tostmann et al., 2013).

After 10–12 days of treatment, the median serum CRP level in the enrolled patient population decreased from 21.9 to 6.4 mg/L, reaching the reference range of < 8 mg/L utilised at the study site. These results are consistent with earlier studies, reporting a significant drop in serum CRP levels between the first and fifth weeks of anti-TB therapy (Djoba et al., 2008; Miranda et al., 2017; Wilson et al., 2018; Musteikienė et al., 2021). A more detailed analysis demonstrated that underweight patients, smokers, and those presenting with lung cavitations and positive sputum-smear microscopy result at the initial examination exhibited a significant decline compared to the baseline, yet their serum CRP levels 10–12 days after initiating anti-TB therapy ($CRP_{10–12d}$) remained above the target range. As previously discussed, low body weight and smoking potentiate TB-associated inflammation, while the aforementioned radiological and bacteriological findings, characteristic of the advanced TB, are typically accompanied by a high degree of inflammation (Djoba et al., 2008; Furuhashi et al., 2012; Chandrasekaran et al., 2017; Opolot et al., 2017; Sigal et al., 2017). These factors indeed have been associated with slower sputum bacillary clearance in the studies by Nijenbandring de Boer et al. (2014), Kanda et al. (2015), Diktanas et al. (2018), Hernandez-Romieu et al. (2019), and Kornfeld et al. (2020). Consequently, it is unsurprising that patients with a combination of these factors may require a longer time to recover from the infection and normalise their serum CRP levels. Meanwhile, the composition of the enrolled patient population, with 81 % aged < 60 years, may have limited the ability to observe the relationship between age and serum CRP levels. However, it was noticed that patients ≥ 60 years of age failed to achieve a significant decline in the levels of this biomarker within the specified period. This distinct pattern of serum CRP level reduction in older patients could be attributed to the deleterious impact of ageing on the immune system (immunosenescence) and inflammation (inflammaging) (Li et al., 2023). With age, inflammatory stimuli induce more intense and prolonged secretion of IL-1β, IL-6,

and TNF-α, leading to enhanced CRP production (Li et al., 2023). Ageing also impairs the function of immune cells, such as macrophages and CD4+ T lymphocytes, which are key components of immune defence against *M. tuberculosis*, thereby diminishing the ability to rapidly clear the infection (O'Garra et al., 2013; Li et al., 2023).

In the absence of clinical guidelines defining the reduction pattern or timeframe for serum CRP normalisation in response to anti-TB therapy, a slightly modified approach proposed by Wilson et al. (2018) was implemented here to stratify patients and categorise changes in the levels of this biomarker for subgroup analyses. Specifically, 31.7 % of patients had serum CRP levels within the reference range at both time points (Group A), 34.1 % experienced a reduction in serum CRP levels ≥ 2 times from baseline or reached the reference range (Group B), and the remaining 34.1 % did not achieve the stated goal (Group C). Multiple-group comparisons revealed that patients in Group C more frequently presented with lung cavitations or positive sputum-smear microscopy result at diagnosis compared to those in Groups A and B, underscoring the role of disease severity in the early reduction of inflammation highlighted in the primary analyses.

Another factor explored in relation to TB-associated inflammation was anti-TB drug plasma exposure, characterised by C_{max} and $AUC_{0–6h}$. The LC-MS/MS analysis indicated that a considerable proportion of patients had suboptimal anti-TB drug plasma exposure at C_{max}, with the highest underexposure rate recorded for RIF (92.5 %), followed by INH (54.8 %), ETB (26.2 %), and PZA (9.5 %). These results are comparable to those reported by others and support the notion that the currently employed therapeutic ranges proposed by Alsultan & Peloquin (2014) differ substantially from the drug plasma concentrations documented in clinical practice (Fahimi et al., 2013; Pasipanodya et al., 2013; Prahl et al., 2014; Kloprogge et al., 2020; Ramachandran et al., 2020). Interestingly, in the present study, drug exposure at the site of infection appeared sufficient to provide an early antibacterial effect, as evidenced by the significant reduction in serum CRP levels. This observation might be explained by the variation in anti-TB drug distribution across different body compartments, as described by Ziglam et al. (2002) and McCallum et al. (2021, 2022), who reported drug accumulation in epithelial lining fluid and alveolar cells to varying extents, but plasma and serum exposure, determined in parallel, tended to be lower or even below the therapeutic range.

Subsequent analyses did not confirm a relationship between the plasma exposure of any of the four anti-TB drugs and serum $CRP_{10–12d}$ levels. Additionally, when patients were stratified based on a 2-fold reduction in serum CRP levels, the assessed PK parameters were similar among Groups A, B, and C. On the contrary, Prahl et al. (2014) reported an inverse correlation between INH C_{max} and serum CRP levels. The considerable differences in study

design and patient characteristics may have led to discordant findings. Here, the period between treatment onset and PK sampling was constant, and the study population was homogeneous in terms of ethnicity, race, form of TB, comorbidities, care setting, and drug formulation used. Several of these factors have been reported to influence the PK of anti-TB drugs (Walubo et al., 1991; McIlleron et al., 2006; Nijland et al., 2006; Weiner et al., 2010; Milán Segovia et al., 2013).

Lastly, this study evaluated the utility of the serum $CRP_{10–12d}$ levels and the patterns of early changes in the serum CRP levels for predicting the bacteriological response to anti-TB treatment, assessed by time to sputum culture conversion (tSCC). The structure of the study population and absence of concomitant diseases likely determined relatively fast sputum culture conversion (median tSCC: 56 days), even among patients who did not achieve a rapid reduction in serum CRP levels within the specified period (median tSCC: 66 days in Group C *versus* 46 days in Groups A and B). While patients with delayed bacteriological response ($tSCC \geq 60$ days) had higher serum $CRP_{10–12d}$ levels compared to rapid responders ($tSCC < 60$ days), the difference was not statistically significant, and none of the examined parameters emerged as independent predictors of tSCC in the Cox regression analysis. This suggests that changes in serum CRP levels, although reflecting the effect of treatment, may be excessively dynamic due to the non-specific nature of CRP expression, and are therefore unable to reliably predict the bacteriological response to anti-TB therapy (Sproston & Ashworth, 2018). Similarly, Musteikienė et al. (2017, 2021) reported that serum CRP_b levels were higher in patients with tSCC > 30 days, yet this biomarker exhibited limited utility in predicting sputum culture status or treatment outcomes. Another group of authors found that the ratio of serum CRP levels at Week 8 to Week 0 was more strongly associated with sputum culture status at Week 8 than at Week 12, but testing of multiple biomarker combinations containing serum CRP levels failed to yield a signature with high predictive performance (Sigal et al., 2017). By comparison, Djoba et al. (2008) identified a biomarker signature encompassing serum CRP levels at baseline and Week 1 that could predict sputum culture status at Month 2 with more than 80 % accuracy. Notably, Ferrian et al. (2017) proposed a CRP-based biomarker combination for predicting delayed bacteriological response in patients with drug-resistant TB, though this finding requires confirmation in other studies.

A strength of this study is the use of various data sources to comprehensively characterise the relationship between numerous patient- and disease-related factors and TB-associated inflammation. Additionally, enrolling patients without concomitant diseases enabled a clearer assessment of serum CRP kinetics and emphasised the potential influence of other health conditions.

However, the present work shares limitations previously discussed concerning the sample size when exploring the role of PGx in RIF PK and anti-TB DILI. The impact of sample size was evident in the high data skewness and unequal distribution across subcategories. Complex statistical approaches were employed to overcome this limitation. Another point to mention is that there were no cases of treatment failure or serious adverse events detected in the study population, so it was impossible to test the predictive value of the examined CRP parameters in such contexts. After all, this study lacked data on the patients' *M. tuberculosis* isolates, including minimal inhibitory concentration (MIC), which would have enabled consideration of strain-specific effects and isolate drug susceptibility. Other authors have demonstrated the utility of combined indices, such as C_{max}/MIC, in predicting bacteriological response and treatment outcomes in patients with DS-TB (Chigutsa et al., 2011; Fahimi et al., 2013; Zheng et al., 2021).

To summarise, this study identified patient- and disease-related factors influencing the reduction in serum CRP levels shortly after the initiation of anti-TB therapy. The absence of a measurable effect of suboptimal anti-TB drug plasma exposure within the given timeframe did not exclude the possibility of negative clinical implications emerging at later stages of therapy. Finally, in this setting, neither serum $CRP_{10–12d}$ levels nor the fold change between serum CRP_b and $CRP_{10–12d}$ levels could predict bacteriological response to treatment, questioning the clinical relevance of the early assessment of the levels of this biomarker, at least in otherwise healthy adult patients with TB. Consequently, the second hypothesis of this Thesis was not confirmed.

Conclusions

1. According to the analytical and clinical validation results, the developed LC-MS/MS method is suitable for the simultaneous analysis of RIF, PZA, INH, and ETB in human plasma samples, with the added capability to quantify six primary metabolites within the same run, thereby enabling expanded PK profiling.
2. The designed NGS-based protocol for targeted genetic analysis produced reliable, high-quality data suitable for PGx applications, as demonstrated by analyses of the *CYP3A4* gene and five RIF pharmacogenes.
3. Among the 10 evaluated variants detected across four RIF pharmacogenes (*SLCO1B1*, *SLCO1B3*, *ABCB1*, and *NR1I2*), the intronic variant rs3732357 in the *NR1I2* gene was found to influence RIF plasma exposure. In contrast, the study results did not support the contribution of any of these variants to the development of anti-TB DILI in the enrolled population.
4. Several patient characteristics and disease severity at diagnosis, but not the anti-TB drug plasma exposure, were identified as affecting the pattern of serum CRP level reduction during the first 10–12 days of therapy. Early changes in the serum CRP levels could not predict the bacteriological response to anti-TB treatment.

Proposals

1. Based on the findings from the study exploring the role of genetic determinants in RIF plasma exposure and anti-TB DILI, further studies should consider:
   a) *in vitro* functional characterisation of the *NR1I2* intronic variant rs3732357 to provide a mechanistic rationale for the observed impact on RIF PK;
   b) evaluation of additional liver-specific biomarkers, such as alkaline phosphatase, to better characterise the pattern of liver injury caused by the concomitant use of RIF, PZA, and INH, and clarify whether genetic factors contribute to liver injury primarily induced by RIF.
2. The study characterising early changes in serum CRP levels after anti-TB treatment initiation indicated that, while measuring levels of this biomarker after 10–12 days of anti-TB drug administration may be useful for assessing dynamics of TB-associated inflammation, these early reduction patterns cannot predict bacteriological response to treatment. Further research should aim to identify robust biomarker or multimodal biomarker signatures linked to relevant clinical, radiological, and/or bacteriological endpoints, enabling the timely identification of patients at risk of treatment failure.
3. The considerable rates of anti-TB drug underexposure observed across the conducted studies, together with the absence of a clear relationship between plasma concentrations and early changes in TB-associated inflammation, underline the importance of investigating how plasma exposure reflects drug concentrations at the site of infection. Addressing this aspect could support the refinement of therapeutic ranges, which currently appear inadequately high. Moreover, defining clinically relevant thresholds would enhance the utility of TDM in assessing the efficacy and safety of anti-TB therapy.

List of publications and reports on the topic of the Thesis

Publications:

1. **Kivrane, A.**, Grinberga, S., Sevostjanovs, E., Igumnova, V., Pole, I., Viksna, A., Bandere, D., Krams, A., Cirule, A., Pugovics, O., & Ranka, R. (2021). LC-MS/MS method for simultaneous quantification of the first-line anti-tuberculosis drugs and six primary metabolites in patient plasma: Implications for therapeutic drug monitoring. *Journal of chromatography. B, Analytical technologies in the biomedical and life sciences*, *1185*, 122986. https://doi.org/10.1016/j.jchromb.2021.122986

2. **Kivrane, A.**, Igumnova, V., Kimsis, J., Freimane, L., Sadovska, D., Viksna, A., Pole, I., & Ranka, R. (2021). Implementation of a next-generation sequencing-based targeted approach for full-length CYP3A4 gene sequencing. *Pharmacogenomics*, *22*(9), 519–527. https://doi.org/10.2217/pgs-2020-0128

3. **Kivrane, A.**, Ulanova, V., Grinberga, S., Sevostjanovs, E., Viksna, A., Ozere, I., Bogdanova, I., Zolovs, M., & Ranka, R. (2024). Exploring Variability in Rifampicin Plasma Exposure and Development of Anti-Tuberculosis Drug-Induced Liver Injury among Patients with Pulmonary Tuberculosis from the Pharmacogenetic Perspective. *Pharmaceutics*, *16*(3), 388. https://doi.org/10.3390/pharmaceutics16030388

4. **Kivrane, A.**, Ulanova, V., Grinberga, S., Sevostjanovs, E., Viksna, A., Ozere, I., Bogdanova, I., Simanovica, I., Norvaisa, I., Pahirko, L., Bandere, D., & Ranka, R. (2024). Identification of Factors Determining Patterns of Serum C-Reactive Protein Level Reduction in Response to Treatment Initiation in Patients with Drug-Susceptible Pulmonary Tuberculosis. *Antibiotics*, *13*(12), 1216. https://doi.org/10.3390/antibiotics13121216

Reports and theses at international congresses and conferences:

1. **Kivrane, A.**, Igumnova, V., Kimsis, J., Freimane, L., Sadovska, D., Viksna, A., Pole, I., & Ranka, R. (2021, March 24–26). *Next-generation sequencing-based targeted-sequencing approach for the full-length CYP3A4 gene sequencing* [Poster abstract]. RSU International Conference on Medical and Health Care Sciences: Knowledge for Use in Practice, Riga, Latvia. Hybrid event. Abstract book, 442.

2. **Kivrane, A.**, Igumnova, I., Grinberga, S., Sevostjanovs, E., Viksna, A., Ozere, I., Bandere, D., & Ranka, R. (2021, October 19–22). *First-line anti-tuberculosis drug exposure in newly diagnosed TB patients: Latvian perspective* [Poster abstract]. 52nd World Conference on Lung Health of the International Union Against Tuberculosis and Lung Diseases: TBScience 2021. Virtual event. In: *Int J Tuberc Lung Dis*, *25*(S2), S425.

3. **Kivrane, A.**, Igumnova, V., Viksna, A., Simanovica, I., Ozere, I., Pole, I., Bandere, D., & Ranka, R. (2022, March 25–26). *Assessment of complete blood count-derived marker association with pulmonary tuberculosis severity and treatment response* [Oral presentation]. International Scientific Conference on Medicine organized within the frame of the 80th International Scientific Conference of the University of Latvia. Virtual event. In: *Medicina (Kaunas)*, *58*(S1), 48.

4. **Kivrane, A.**, Igumnova, V., Viksna, A., Simanovica, I., Ozere, I., Pole, I., Bandere, D., & Ranka, R. (2022, July 9–14). *The utility of complete blood count-derived markers for characterising pulmonary tuberculosis severity and prediction of treatment response* [Poster abstract]. The Biochemistry Global Summit Lisbon, The 46th FEBS congress, Lisbon, Portugal. In: *FEBS Open Bio*, *12*(S1), 160.

5. **Kivrane, A.**, Ulanova, V., Grinberga, S., Sevostjanovs, E., Viksna, A., Pole, I., Ozere, I., Bogdanova, I., Zolovs, M., Bandere, D., & Ranka R. (2023, March 27–31). *Assessment of rifampicin exposure in Latvian pulmonary tuberculosis patients in the context with the AADAC genetic polymorphisms* [Poster abstract]. RSU International Conference on Medical and Health Care Sciences: Knowledge for Use in Practice, Riga, Latvia. In: *Medicina (Kaunas)*, *59*(S2), 351.

6. **Kivrane, A.**, Ulanova, V., Grinberga, S., Sevostjanovs, E., Viksna, A., Pole, I., Ozere, I., Bogdanova, I., Zolovs, M., Bandere, D., & Ranka, R. (2023, June 14–16). *Assessment of four first-line anti-tuberculosis drug exposure in pulmonary tuberculosis patients with drug-induced hepatotoxicity* [Poster abstract]. Second Nordic Conference Personalized Medicine, Turku, Finland. In: *Basic and Clinical Pharmacology and Toxicology*, *132*(S1), 19–20.

7. **Kivrane, A.**, Ulanova, V., Grinberga, S., Sevostjanovs, E., Viksna, A., Bogdanova, I., Pahirko, L., Bandere, D., & Ranka, R. (2024, June 8–11). *Identification of factors determining patterns of serum CRP level reduction in response to anti-tuberculosis treatment initiation* [Poster abstract].16th congress of the European Association for Clinical Pharmacology and Therapeutics: WHO Step 6. Monitoring outcome and compliance, Rotterdam, Netherlands. Abstract book, 273–274.

8. **Kivrane, A.**, Ulanova, V., Sadovska, D., Viksna, A., Ozere, I., Bogdanova, I., Simanovica, I., Norvaisa, I., & Ranka, R. (2025, June 22–15). *Exploring patterns of serum CRP level reduction in response to treatment initiation in Latvian patients with pulmonary tuberculosis: the impact of Mycobacterium tuberculosis genotype* [Poster abstract]. 45th annual congress of the European Society of Mycobacteriology, Lisbon, Portugal. Abstract book, 59.

References

1. Abbara, A., Chitty, S., Roe, J. K., Ghani, R., Collin, S. M., Ritchie, A., Kon, O. M., Dzvova, J., Davidson, H., Edwards, T. E., Hateley, C., Routledge, M., Buckley, J., Davidson, R. N., & John, L. (2017). Drug-induced liver injury from antituberculous treatment: a retrospective study from a large TB centre in the UK. *BMC infectious diseases*, *17*(1), 231. https://doi.org/10.1186/s12879-017-2330-z

2. Allegra, S., Fatiguso, G., Calcagno, A., Baietto, L., Motta, I., Favata, F., Cusato, J., Bonora, S., Di Perri, G., & D'Avolio, A. (2017). Role of vitamin D pathway gene polymorphisms on rifampicin plasma and intracellular pharmacokinetics. *Pharmacogenomics*, *18*(9), 865–880. https://doi.org/10.2217/pgs-2017-0176

3. Alsultan, A., & Peloquin, C. A. (2014). Therapeutic drug monitoring in the treatment of tuberculosis: an update. *Drugs*, *74*(8), 839–854. https://doi.org/10.1007/s40265-014-0222-8

4. Azam, K., Khosa, C., Viegas, S., Massango, I., Bhatt, N., Jani, I., Heinrich, N., Hoelscher, M., Gillespie, S. H., Rachow, A., & Sabiiti, W. (2022). Reduction of blood C-reactive protein concentration complements the resolution of sputum bacillary load in patients on anti-tuberculosis therapy. *Frontiers in immunology*, *13*, 1005692. https://doi.org/10.3389/fimmu.2022.1005692

5. Barraclough, K. A., Isbel, N. M., Lee, K. J., Bergmann, T. K., Johnson, D. W., McWhinney, B. C., Ungerer, J. P., Campbell, S. B., Leary, D. R., Bialasiewicz, S., Rockett, R. J., & Staatz, C. E. (2012). NR1I2 polymorphisms are related to tacrolimus dose-adjusted exposure and BK viremia in adult kidney transplantation. *Transplantation*, *94*(10), 1025–1032. https://doi.org/10.1097/TP.0b013e31826c3985

6. Brown, J., Clark, K., Smith, C., Hopwood, J., Lynard, O., Toolan, M., Creer, D., Barker, J., Breen, R., Brown, T., Cropley, I., & Lipman, M. (2016). Variation in C - reactive protein response according to host and mycobacterial characteristics in active tuberculosis. *BMC infectious diseases*, *16*, 265. https://doi.org/10.1186/s12879-016-1612-1

7. Calderwood, C. J., Wilson, J. P., Fielding, K. L., Harris, R. C., Karat, A. S., Mansukhani, R., Falconer, J., Bergstrom, M., Johnson, S. M., McCreesh, N., Monk, E. J. M., Odayar, J., Scott, P. J., Stokes, S. A., Theodorou, H., & Moore, D. A. J. (2021). Dynamics of sputum conversion during effective tuberculosis treatment: A systematic review and meta-analysis. *PLoS medicine*, *18*(4), e1003566. https://doi.org/10.1371/journal.pmed.1003566

8. Campbell, S. D., de Morais, S. M., & Xu, J. J. (2004). Inhibition of human organic anion transporting polypeptide OATP 1B1 as a mechanism of drug-induced hyperbilirubinemia. *Chemico-biological interactions*, *150*(2), 179–187. https://doi.org/10.1016/j.cbi.2004.08.008

9. Cascorbi I. (2011). P-glycoprotein: tissue distribution, substrates, and functional consequences of genetic variations. *Handbook of experimental pharmacology*, (201), 261–283. https://doi.org/10.1007/978-3-642-14541-4_6

10. Centre for Disease Prevention and Control of Latvia. (2025). Tuberculosis Incidence, Absolute Numbers and per 100,000 Population (Years 2005–2024). Retrieved from https://www.spkc.gov.lv/lv/tuberkuloze-0#saslimstiba-ar-tuberkulozi-absolutais-skaits-un-uz-100-000-iedzivotaju-2005-2024-gads (accessed 25 September 2025)

11. Chan, S. L., Chua, A. P. G., Aminkeng, F., Chee, C. B. E., Jin, S., Loh, M., Gan, S. H., Wang, Y. T., & Brunham, L. R. (2017). Association and clinical utility of NAT2 in the prediction of isoniazid-induced liver injury in Singaporean patients. *PloS one*, *12*(10), e0186200. https://doi.org/10.1371/journal.pone.0186200

12. Chandrasekaran, P., Saravanan, N., Bethunaickan, R., & Tripathy, S. (2017). Malnutrition: Modulator of Immune Responses in Tuberculosis. *Frontiers in immunology*, *8*, 1316. https://doi.org/10.3389/fimmu.2017.01316

13. Chen, R., Wang, J., Zhang, Y., Tang, S., & Zhan, S. (2015a). Key factors of susceptibility to anti-tuberculosis drug-induced hepatotoxicity. *Archives of toxicology*, *89*(6), 883–897. https://doi.org/10.1007/s00204-015-1473-1

14. Chen, R., Wang, J., Tang, S., Zhang, Y., Lv, X., Wu, S., Xia, Y., Deng, P., Ma, Y., Tu, D., Chen, D., & Zhan, S. (2015b). Association of polymorphisms in drug transporter genes (SLCO1B1 and SLC10A1) and anti-tuberculosis drug-induced hepatotoxicity in a Chinese cohort. *Tuberculosis (Edinburgh, Scotland)*, *95*(1), 68–74. https://doi.org/10.1016/j.tube.2014.11.004

15. Chigutsa, E., Visser, M. E., Swart, E. C., Denti, P., Pushpakom, S., Egan, D., Holford, N. H., Smith, P. J., Maartens, G., Owen, A., & McIlleron, H. (2011). The SLCO1B1 rs4149032 polymorphism is highly prevalent in South Africans and is associated with reduced rifampin concentrations: dosing implications. *Antimicrobial agents and chemotherapy*, *55*(9), 4122–4127. https://doi.org/10.1128/AAC.01833-10

16. Cho, S. H., Lee, H., Kwon, H., Shin, D. W., Joh, H. K., Han, K., Park, J. H., & Cho, B. (2022). Association of underweight status with the risk of tuberculosis: a nationwide population-based cohort study. *Scientific reports*, *12*(1), 16207. https://doi.org/10.1038/s41598-022-20550-8

17. David, S., & Hamilton, J. P. (2010). Drug-induced Liver Injury. *US gastroenterology & hepatology review*, *6*, 73–80.

18. Devarbhavi, H., Singh, R., Patil, M., Sheth, K., Adarsh, C. K., & Balaraju, G. (2013). Outcome and determinants of mortality in 269 patients with combination anti-tuberculosis drug-induced liver injury. *Journal of gastroenterology and hepatology*, *28*(1), 161–167. https://doi.org/10.1111/j.1440-1746.2012.07279.x

19. Dickinson, J. M., & Mitchison, D. A. (1981). Experimental models to explain the high sterilizing activity of rifampin in the chemotherapy of tuberculosis. *The American review of respiratory disease*, *123*(4 Pt 1), 367–371. https://doi.org/10.1164/arrd.1981.123.4.367

20. Diktanas, S., Vasiliauskiene, E., Polubenko, K., Danila, E., Celedinaite, I., Boreikaite, E., & Misiunas, K. (2018). Factors Associated with Persistent Sputum Positivity at the End of the Second Month of Tuberculosis Treatment in Lithuania. *Tuberculosis and respiratory diseases*, *81*(3), 233–240. https://doi.org/10.4046/trd.2017.0096

21. Djoba Siawaya, J. F., Bapela, N. B., Ronacher, K., Veenstra, H., Kidd, M., Gie, R., Beyers, N., van Helden, P., & Walzl, G. (2008). Immune parameters as markers of tuberculosis extent of disease and early prediction of anti-tuberculosis chemotherapy response. *The Journal of infection*, *56*(5), 340–347. https://doi.org/10.1016/j.jinf.2008.02.007

22. Dompreh, A., Tang, X., Zhou, J., Yang, H., Topletz, A., Adu Ahwireng, E., Antwi, S., Enimil, A., Langaee, T., Peloquin, C. A., Court, M. H., & Kwara, A. (2018). Effect of Genetic Variation of *NAT2* on Isoniazid and *SLCO1B1* and *CES2* on Rifampin Pharmacokinetics in Ghanaian Children with Tuberculosis. *Antimicrobial agents and chemotherapy*, *62*(3), e02099-17. https://doi.org/10.1128/AAC.02099-17

23. Dong, Y., Xu, Q., Li, R., Tao, Y., Zhang, Q., Li, J., Ma, Z., Shen, C., Zhong, M., Wang, Z., & Qiu, X. (2022). CYP3A7, CYP3A4, and CYP3A5 genetic polymorphisms in recipients rather than donors influence tacrolimus concentrations in the early stages after liver transplantation. *Gene*, *809*, 146007. https://doi.org/10.1016/j.gene.2021.146007

24. Durand, F., Jebrak, G., Pessayre, D., Fournier, M., & Bernuau, J. (1996). Hepatotoxicity of antitubercular treatments. Rationale for monitoring liver status. *Drug safety*, *15*(6), 394–405. https://doi.org/10.2165/00002018-199615060-00004

25. Elens, L., Capron, A., van Schaik, R. H., De Meyer, M., De Pauw, L., Eddour, D. C., Latinne, D., Wallemacq, P., Mourad, M., & Haufroid, V. (2013). Impact of CYP3A4*22 allele on tacrolimus pharmacokinetics in early period after renal transplantation: toward updated genotype-based dosage guidelines. *Therapeutic drug monitoring*, *35*(5), 608–616. https://doi.org/10.1097/FTD.0b013e318296045b

26. European Medicines Agency. (2012). Guideline on bioanalytical method validation, EMEA/CHMP/EWP/192217/2009 Rev. 1 Corr. 2**. Retrieved from https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-bioanalytical-method-validation_en.pdf. (accessed 16 July 2024)

27. Fahimi, F., Tabarsi, P., Kobarfard, F., Bozorg, B. D., Goodarzi, A., Dastan, F., Shahsavari, N., Emami, S., Habibi, M., & Salamzadeh, J. (2013). Isoniazid, rifampicin and pyrazinamide plasma concentrations 2 and 6 h post dose in patients with pulmonary tuberculosis. *The international journal of tuberculosis and lung disease: the official journal of the International Union against Tuberculosis and Lung Disease*, *17*(12), 1602–1606. https://doi.org/10.5588/ijtld.13.0019

28. Ferrian, S., Manca, C., Lubbe, S., Conradie, F., Ismail, N., Kaplan, G., Gray, C. M., & Fallows, D. (2017). A combination of baseline plasma immune markers can predict therapeutic response in multidrug resistant tuberculosis. *PloS one*, *12*(5), e0176660. https://doi.org/10.1371/journal.pone.0176660

29. Francis, J., Zvada, S. P., Denti, P., Hatherill, M., Charalambous, S., Mungofa, S., Dawson, R., Dorman, S., Gupte, N., Wiesner, L., Jindani, A., Harrison, T. S., Olagunju, A., Egan, D., Owen, A., & McIlleron, H. M. (2019). A Population Pharmacokinetic Analysis Shows that Arylacetamide Deacetylase (AADAC) Gene Polymorphism and HIV Infection Affect the Exposure of Rifapentine. *Antimicrobial agents and chemotherapy*, *63*(4), e01964-18. https://doi.org/10.1128/AAC.01964-18

30. Furuhashi, K., Shirai, T., Suda, T., & Chida, K. (2012). Inflammatory markers in active pulmonary tuberculosis: association with Th1/Th2 and Tc1/Tc2 balance. *Kekkaku: [Tuberculosis]*, *87*(1), 1–7.

31. Gaedigk, A., Casey, S. T., Whirl-Carrillo, M., Miller, N. A., & Klein, T. E. (2021). Pharmacogene Variation Consortium: A Global Resource and Repository for Pharmacogene Variation. *Clinical pharmacology and therapeutics*, *110*(3), 542–545. https://doi.org/10.1002/cpt.2321

32. Gao, S., Wang, Z., Xie, X., You, C., Yang, Y., Xi, Y., & Chen, W. (2018). Rapid and sensitive method for simultaneous determination of first-line anti-tuberculosis drugs in human plasma by HPLC-MS/MS: Application to therapeutic drug monitoring. *Tuberculosis (Edinburgh, Scotland)*, *109*, 28–34. https://doi.org/10.1016/j.tube.2017.11.012

33. Günther, G., Guglielmetti, L., Leu, C., Lange, C., van Leth, F., & Tuberculosis Network European Trials group (2023). Availability and costs of medicines for the treatment of tuberculosis in Europe. *Clinical microbiology and infection: the official publication of the European Society of Clinical Microbiology and Infectious Diseases*, *29*(1), 77–84. https://doi.org/10.1016/j.cmi.2022.07.026

34. Gupta, K. B., Gupta, R., Atreja, A., Verma, M., & Vishvkarma, S. (2009). Tuberculosis and nutrition. *Lung India: official organ of Indian Chest Society*, *26*(1), 9–16. https://doi.org/10.4103/0970-2113.45198

35. He, P., Court, M. H., Greenblatt, D. J., & von Moltke, L. L. (2006). Human pregnane X receptor: genetic polymorphisms, alternative mRNA splice variants, and cytochrome P450 3A metabolic activity. *Journal of clinical pharmacology*, *46*(11), 1356–1369. https://doi.org/10.1177/0091270006292125

36. Hee, K. H., Seo, J. J., & Lee, L. S. (2015). Development and validation of liquid chromatography tandem mass spectrometry method for simultaneous quantification of first line tuberculosis drugs and metabolites in human plasma and its application in clinical study. *Journal of pharmaceutical and biomedical analysis*, *102*, 253–260. https://doi.org/10.1016/j.jpba.2014.09.019

37. Hernandez-Romieu, A. C., Little, B. P., Bernheim, A., Schechter, M. C., Ray, S. M., Bizune, D., & Kempker, R. (2019). Increasing Number and Volume of Cavitary Lesions on Chest Computed Tomography Are Associated With Prolonged Time to Culture Conversion in Pulmonary Tuberculosis. *Open forum infectious diseases*, *6*(6), ofz232. https://doi.org/10.1093/ofid/ofz232

38. Hirvensalo, P., Tornio, A., Launiainen, T., Paile-Hyvärinen, M., Tapaninen, T., Neuvonen, M., Backman, J. T., & Niemi, M. (2020). UGT1A3 and Sex Are Major Determinants of Telmisartan Pharmacokinetics-A Comprehensive Pharmacogenomic Study. *Clinical pharmacology and therapeutics*, *108*(4), 885–895. https://doi.org/10.1002/cpt.1928

39. Hoyt, K. J., Sarkar, S., White, L., Joseph, N. M., Salgame, P., Lakshminarayanan, S., Muthaiah, M., Vinod Kumar, S., Ellner, J. J., Roy, G., Horsburgh, C. R., Jr, & Hochberg, N. S. (2019). Effect of malnutrition on radiographic findings and mycobacterial burden in pulmonary tuberculosis. *PloS one*, *14*(3), e0214011. https://doi.org/10.1371/journal.pone.0214011

40. Hu, R., Barratt, D. T., Coller, J. K., Sallustio, B. C., & Somogyi, A. A. (2018). CYP3A5*3 and ABCB1 61A>G Significantly Influence Dose-adjusted Trough Blood Tacrolimus Concentrations in the First Three Months Post-Kidney Transplantation. *Basic & clinical pharmacology & toxicology*, *123*(3), 320–326. https://doi.org/10.1111/bcpt.13016

41. Huerta-García, A. P., Medellín-Garibay, S. E., Salazar-González, R. A., Ortiz-Álvarez, A., Magaña-Aquino, M., Rodríguez-Pinal, C. J., Portales-Pérez, D. P., Romano-Moreno, S., & Milán-Segovia, R. D. C. (2019). Anthropometric and Genetic Factors Associated With the Exposure of Rifampicin and Isoniazid in Mexican Patients With Tuberculosis. *Therapeutic drug monitoring*, *41*(5), 648–656. https://doi.org/10.1097/FTD.0000000000000631

42. Ignatius E.H., & Dooley K.E. (2023). Chemotherapy of tuberculosis and nontuberculous mycobacteria, including leprosy. Brunton L.L., & Knollmann B.C.(Eds.), Goodman & Gilman's: *The Pharmacological Basis of Therapeutics, 14th Edition*. McGraw-Hill Education. https://accessmedicine.mhmedical.com/content.aspx?bookid=3191&sectionid=269172224

43. Jabir, R. S., Naidu, R., Annuar, M. A., Ho, G. F., Munisamy, M., & Stanslas, J. (2012). Pharmacogenetics of taxanes: impact of gene polymorphisms of drug transporters on pharmacokinetics and toxicity. *Pharmacogenomics*, *13*(16), 1979–1988. https://doi.org/10.2217/pgs.12.165

44. Kanda, R., Nagao, T., Tho, N. V., Ogawa, E., Murakami, Y., Osawa, M., Saika, Y., Doi, K., & Nakano, Y. (2015). Factors Affecting Time to Sputum Culture Conversion in Adults with Pulmonary Tuberculosis: A Historical Cohort Study without Censored Cases. *PloS one*, *10*(11), e0142607. https://doi.org/10.1371/journal.pone.0142607

45. Khera, A., McGuire, D. K., Murphy, S. A., Stanek, H. G., Das, S. R., Vongpatanasin, W., Wians, F. H., Jr, Grundy, S. M., & de Lemos, J. A. (2005). Race and gender differences in C-reactive protein levels. *Journal of the American College of Cardiology*, *46*(3), 464–469. https://doi.org/10.1016/j.jacc.2005.04.051

46. Kim, S. H., Kim, S. H., Lee, J. H., Lee, B. H., Kim, Y. S., Park, J. S., & Jee, Y. K. (2012). Polymorphisms in drug transporter genes (ABCB1, SLCO1B1 and ABCC2) and hepatitis induced by antituberculosis drugs. *Tuberculosis (Edinburgh, Scotland)*, *92*(1), 100–104. https://doi.org/10.1016/j.tube.2011.09.007

47. Kim, H. J., Seo, K. A., Kim, H. M., Jeong, E. S., Ghim, J. L., Lee, S. H., Lee, Y. M., Kim, D. H., & Shin, J. G. (2015). Simple and accurate quantitative analysis of 20 anti-tuberculosis drugs in human plasma using liquid chromatography-electrospray ionization-tandem mass spectrometry. *Journal of pharmaceutical and biomedical analysis*, *102*, 9–16. https://doi.org/10.1016/j.jpba.2014.08.026

48. Kloprogge, F., Mwandumba, H. C., Banda, G., Kamdolozi, M., Shani, D., Corbett, E. L., Kontogianni, N., Ward, S., Khoo, S. H., Davies, G. R., & Sloan, D. J. (2020). Longitudinal Pharmacokinetic-Pharmacodynamic Biomarkers Correlate With Treatment Outcome in Drug-Sensitive Pulmonary Tuberculosis: A Population Pharmacokinetic-Pharmacodynamic Analysis. *Open forum infectious diseases*, *7*(7), ofaa218. https://doi.org/10.1093/ofid/ofaa218

49. Kornfeld, H., Sahukar, S. B., Procter-Gray, E., Kumar, N. P., West, K., Kane, K., Natarajan, M., Li, W., Babu, S., & Viswanathan, V. (2020). Impact of Diabetes and Low Body Mass Index on Tuberculosis Treatment Outcomes. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America*, *71*(9), e392–e398. https://doi.org/10.1093/cid/ciaa054

50. Kumar, N. P., Moideen, K., Bhootra, Y., Nancy, A., Viswanathan, V., Shruthi, B. S., Sivakumar, S., Natarajan, M., Kornfeld, H., & Babu, S. (2019). Elevated circulating levels of monocyte activation markers among tuberculosis patients with diabetes co-morbidity. *Immunology*, *156*(3), 249–258. https://doi.org/10.1111/imm.13023

51. Kwara, A., Cao, L., Yang, H., Poethke, P., Kurpewski, J., Tashima, K. T., Mahjoub, B. D., Court, M. H., & Peloquin, C. A. (2014). Factors associated with variability in rifampin plasma pharmacokinetics and the relationship between rifampin concentrations and induction of efavirenz clearance. *Pharmacotherapy*, *34*(3), 265–271. https://doi.org/10.1002/phar.1388

52. Li, L. M., Chen, L., Deng, G. H., Tan, W. T., Dan, Y. J., Wang, R. Q., & Chen, W. S. (2012). SLCO1B1 *15 haplotype is associated with rifampin-induced liver injury. *Molecular medicine reports*, *6*(1), 75–82. https://doi.org/10.3892/mmr.2012.900

53. Li, X., Li, C., Zhang, W., Wang, Y., Qian, P., & Huang, H. (2023). Inflammation and aging: signaling pathways and intervention therapies. *Signal transduction and targeted therapy*, *8*(1), 239. https://doi.org/10.1038/s41392-023-01502-8

54. Liu, M. Z., He, H. Y., Zhang, Y. L., Hu, Y. F., He, F. Z., Luo, J. Q., Luo, Z. Y., Chen, X. P., Liu, Z. Q., Zhou, H. H., Shao, M. J., Ming, Y. Z., Xin, H. W., & Zhang, W. (2017). IL-3 and CTLA4 gene polymorphisms may influence the tacrolimus dose requirement in Chinese kidney transplant recipients. *Acta pharmacologica Sinica*, *38*(3), 415–423. https://doi.org/10.1038/aps.2016.153

55. Lönnroth, K., Williams, B. G., Cegielski, P., & Dye, C. (2010). A consistent log-linear relationship between tuberculosis incidence and body mass index. *International journal of epidemiology*, *39*(1), 149–155. https://doi.org/10.1093/ije/dyp308

56. Lu, Y., Xu, L., Cui, J., Shen, S., & Li, X. (2021). Effects of Postoperative Day and NR1I2 on Tacrolimus Clearance in Chinese Liver Transplant Recipients-A Population Model Approach. *Clinical pharmacology in drug development*, *10*(11), 1385–1394. https://doi.org/10.1002/cpdd.971

57. Luyen, L.T., Hung, T.M., Huyen, L.T., Tuan, L.A., Huong, D.T.L., Duc, H.V., Tung, B.T. Simultaneous determination of Pyrazinamide, Rifampicin, Ethambutol, Isoniazid and Acetyl Isoniazid in human plasma by LC-MS/MS method. (2018). *Journal of Applied Pharmaceutical Science*, *8*(09), 061–073. https://doi.org/10.7324/JAPS.2018.8910

58. Ma, X., Idle, J. R., & Gonzalez, F. J. (2008). The pregnane X receptor: from bench to bedside. *Expert opinion on drug metabolism & toxicology*, *4*(7), 895–908. https://doi.org/10.1517/17425255.4.7.895

59. Manolopoulos, V. G., Ragia, G., & Tavridou, A. (2010). Pharmacogenetics of coumarinic oral anticoagulants. *Pharmacogenomics*, *11*(4), 493–496. https://doi.org/10.2217/pgs.10.31

60. Masini, T., Kanchar, A., Mirzayev, F., Viney, K., Yedilbayev, A., Zignol, M., & Falzon, D. (2022). Wider access to quality-assured rifapentine-based regimens is needed to accelerate tuberculosis prevention and care globally. *The European respiratory journal*, *60*(2), 2201227. https://doi.org/10.1183/13993003.01227-2022

61. Mbatchi, L. C., Schmitt, A., Thomas, F., Cazaubon, Y., Robert, J., Lumbroso, S., Brouillet, J. P., Pourquier, P., Chatelut, E., Boyer, J. C., & Evrard, A. (2015). Polymorphisms in SLCO1B3 and NR1I2 as genetic determinants of hematotoxicity of carboplatin and paclitaxel combination. *Pharmacogenomics*, *16*(13), 1439–1450. https://doi.org/10.2217/pgs.15.84

62. McCallum, A. D., Pertinez, H. E., Else, L. J., Dilly-Penchala, S., Chirambo, A. P., Sheha, I., Chasweka, M., Chitani, A., Malamba, R. D., Meghji, J. Z., Gordon, S. B., Davies, G. R., Khoo, S. H., Sloan, D. J., & Mwandumba, H. C. (2021). Intrapulmonary Pharmacokinetics of First-line Anti-tuberculosis Drugs in Malawian Patients With Tuberculosis. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America*, *73*(9), e3365–e3373. https://doi.org/10.1093/cid/ciaa1265

63. McCallum, A. D., Pertinez, H. E., Chirambo, A. P., Sheha, I., Chasweka, M., Malamba, R., Shani, D., Chitani, A., Mallewa, J. E., Meghji, J. Z., Ghany, J. F., Corbett, E. L., Gordon, S. B., Davies, G. R., Khoo, S. H., Sloan, D. J., & Mwandumba, H. C. (2022). High Intrapulmonary Rifampicin and Isoniazid Concentrations Are Associated With Rapid Sputum Bacillary Clearance in Patients With Pulmonary Tuberculosis. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America*, *75*(9), 1520–1528. https://doi.org/10.1093/cid/ciac228

64. McDonagh, E. M., Boukouvala, S., Aklillu, E., Hein, D. W., Altman, R. B., & Klein, T. E. (2014). PharmGKB summary: very important pharmacogene information for N-acetyltransferase 2. *Pharmacogenetics and genomics*, *24*(8), 409–425. https://doi.org/10.1097/FPC.0000000000000062

65. McIlleron, H., Wash, P., Burger, A., Norman, J., Folb, P. I., & Smith, P. (2006). Determinants of rifampin, isoniazid, pyrazinamide, and ethambutol pharmacokinetics in a cohort of tuberculosis patients. *Antimicrobial agents and chemotherapy*, *50*(4), 1170–1177. https://doi.org/10.1128/AAC.50.4.1170-1177.2006

66. Medellin-Garibay, S. E., Huerta-García, A. P., Rodríguez-Báez, A. S., Magaña-Aquino, M., Ortiz-Álvarez, A., Portales-Pérez, D. P., Milán-Segovia, R. D. C., & Romano-Moreno, S. (2020). A population approach of rifampicin pharmacogenetics and pharmacokinetics in Mexican patients with tuberculosis. *Tuberculosis (Edinburgh, Scotland)*, *124*, 101982. https://doi.org/10.1016/j.tube.2020.101982

67. Mendy, J., Togun, T., Owolabi, O., Donkor, S., Ota, M. O., & Sutherland, J. S. (2016). C-reactive protein, Neopterin and Beta2 microglobulin levels pre and post TB treatment in The Gambia. *BMC infectious diseases*, *16*, 115. https://doi.org/10.1186/s12879-016-1447-9

68. Metushi, I. G., Cai, P., Zhu, X., Nakagawa, T., & Uetrecht, J. P. (2011). A fresh look at the mechanism of isoniazid-induced hepatotoxicity. *Clinical pharmacology and therapeutics*, *89*(6), 911–914. https://doi.org/10.1038/clpt.2010.355

69. Milán Segovia, R. C., Domínguez Ramírez, A. M., Jung Cook, H., Magaña Aquino, M., Vigna Pérez, M., Brundage, R. C., & Romano Moreno, S. (2013). Population pharmacokinetics of rifampicin in Mexican patients with tuberculosis. *Journal of clinical pharmacy and therapeutics*, *38*(1), 56–61. https://doi.org/10.1111/jcpt.12016

70. Miranda, P., Gil-Santana, L., Oliveira, M. G., Mesquita, E. D., Silva, E., Rauwerdink, A., Cobelens, F., Oliveira, M. M., Andrade, B. B., & Kritski, A. (2017). Sustained elevated levels of C-reactive protein and ferritin in pulmonary tuberculosis patients remaining culture positive upon treatment initiation. *PloS one*, *12*(4), e0175278. https://doi.org/10.1371/journal.pone.0175278

71. Musteikienė, G., Miliauskas, S., Zaveckienė, J., Žemaitis, M., & Vitkauskienė, A. (2017). Factors associated with sputum culture conversion in patients with pulmonary tuberculosis. *Medicina (Kaunas, Lithuania)*, *53*(6), 386–393. https://doi.org/10.1016/j.medici.2018.01.005

72. Musteikienė, G., Miliauskas, S., Zaveckienė, J., Urbonienė, D., Vitkauskienė, A., Žemaitis, M., & Naudžiūnas, A. (2021). Is analysis of inflammatory biomarkers and lymphocyte subpopulations useful in prediction of tuberculosis treatment outcomes?. *Journal of clinical tuberculosis and other mycobacterial diseases*, *25*, 100275. https://doi.org/10.1016/j.jctube.2021.100275

73. Naidoo, A., Chirehwa, M., Ramsuran, V., McIlleron, H., Naidoo, K., Yende-Zuma, N., Singh, R., Ncgapu, S., Adamson, J., Govender, K., Denti, P., & Padayatchi, N. (2019). Effects of genetic variability on rifampicin and isoniazid pharmacokinetics in South African patients with recurrent tuberculosis. *Pharmacogenomics*, *20*(4), 225–240. https://doi.org/10.2217/pgs-2018-0166

74. Nakajima, A., Fukami, T., Kobayashi, Y., Watanabe, A., Nakajima, M., & Yokoi, T. (2011). Human arylacetamide deacetylase is responsible for deacetylation of rifamycins: rifampicin, rifabutin, and rifapentine. *Biochemical pharmacology*, *82*(11), 1747–1756. https://doi.org/10.1016/j.bcp.2011. 08.003

75. Nie, Y., Yang, J., Liu, S., Sun, R., Chen, H., Long, N., Jiang, R., & Gui, C. (2020). Genetic polymorphisms of human hepatic OATPs: functional consequences and effect on drug pharmacokinetics. *Xenobiotica; the fate of foreign compounds in biological systems*, *50*(3), 297–317. https://doi.org/10.1080/00498254.2019.1629043

76. Nijenbandring de Boer, R., Oliveira e Souza Filho, J. B., Cobelens, F., Ramalho, D.deP., Campino Miranda, P. F., Logo, K.d, Oliveira, H., Mesquita, E., Oliveira, M. M., & Kritski, A. (2014). Delayed culture conversion due to cigarette smoking in active pulmonary tuberculosis patients. *Tuberculosis* (Edinburgh, Scotland), *94*(1), 87–91. https://doi.org/10.1016/j.tube.2013.10.005

77. Nijland, H. M., Ruslami, R., Stalenhoef, J. E., Nelwan, E. J., Alisjahbana, B., Nelwan, R. H., van der Ven, A. J., Danusantoso, H., Aarnoutse, R. E., & van Crevel, R. (2006). Exposure to rifampicin is strongly reduced in patients with tuberculosis and type 2 diabetes. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America*, *43*(7), 848–854. https://doi.org/10.1086/507543

78. Niward, K., Davies Forsman, L., Bruchfeld, J., Chryssanthou, E., Carlström, O., Alomari, T., Carlsson, B., Pohanka, A., Mansjö, M., Jonsson Nordvall, M., Johansson, A. G., Eliasson, E., Werngren, J., Paues, J., Simonsson, U. S. H., & Schön, T. (2018). Distribution of plasma concentrations of first-line anti-TB drugs and individual MICs: a prospective cohort study in a low endemic setting. *The Journal of antimicrobial chemotherapy*, *73*(10), 2838–2845. https://doi.org/10.1093/jac/dky268

79. O'Garra, A., Redford, P. S., McNab, F. W., Bloom, C. I., Wilkinson, R. J., & Berry, M. P. (2013). The immune response in tuberculosis. *Annual review of immunology*, *31*, 475–527. https://doi.org/10.1146/annurev-immunol-032712-095939

80. Ohsawa, M., Okayama, A., Nakamura, M., Onoda, T., Kato, K., Itai, K., Yoshida, Y., Ogawa, A., Kawamura, K., & Hiramori, K. (2005). CRP levels are elevated in smokers but unrelated to the number of cigarettes and are decreased by long-term smoking cessation in male smokers. *Preventive medicine*, *41*(2), 651–656. https://doi.org/10.1016/j.ypmed.2005.02.002

81. Oleson, L., von Moltke, L. L., Greenblatt, D. J., & Court, M. H. (2010). Identification of polymorphisms in the 3'-untranslated region of the human pregnane X receptor (PXR) gene associated with variability in cytochrome P450 3A (CYP3A) metabolism. *Xenobiotica; the fate of foreign compounds in biological systems*, *40*(2), 146–162. https://doi.org/10.3109/00498250903420243

82. Onwubalili, J. K. (1988). Malnutrition among tuberculosis patients in Harrow, England. *European journal of clinical nutrition*, *42*(4), 363–366.

83. Opolot, J. O., Theron, A. J., MacPhail, P., Feldman, C., & Anderson, R. (2017). Effect of smoking on acute phase reactants, stress hormone responses and vitamin C in pulmonary tuberculosis. *African health sciences*, *17*(2), 337–345. https://doi.org/10.4314/ahs.v17i2.7

84. Pallet, N., Jannot, A. S., El Bahri, M., Etienne, I., Buchler, M., de Ligny, B. H., Choukroun, G., Colosio, C., Thierry, A., Vigneau, C., Moulin, B., Le Meur, Y., Heng, A. E., Subra, J. F., Legendre, C., Beaune, P., Alberti, C., Loriot, M. A., & Thervet, E. (2015). Kidney transplant recipients carrying the CYP3A4*22 allelic variant have reduced tacrolimus clearance and often reach supratherapeutic tacrolimus concentrations. *American journal of transplantation: official journal of the American Society of Transplantation and the American Society of Transplant Surgeons*, *15*(3), 800–805. https://doi.org/10.1111/ajt.13059

85. Pasipanodya, J. G., McIlleron, H., Burger, A., Wash, P. A., Smith, P., & Gumbo, T. (2013). Serum drug concentrations predictive of pulmonary tuberculosis outcomes. *The Journal of infectious diseases*, *208*(9), 1464–1473. https://doi.org/10.1093/infdis/jit352

86. Prahl, J. B., Johansen, I. S., Cohen, A. S., Frimodt-Møller, N., & Andersen, Å. B. (2014). Clinical significance of 2 h plasma concentrations of first-line anti-tuberculosis drugs: a prospective observational study. *The Journal of antimicrobial chemotherapy*, *69*(10), 2841–2847. https://doi.org/10.1093/jac/dku210

87. Prideaux, B., Via, L. E., Zimmerman, M. D., Eum, S., Sarathy, J., O'Brien, P., Chen, C., Kaya, F., Weiner, D. M., Chen, P. Y., Song, T., Lee, M., Shim, T. S., Cho, J. S., Kim, W., Cho, S. N., Olivier, K. N., Barry, C. E., 3rd, & Dartois, V. (2015). The association between sterilizing activity and drug distribution into tuberculosis lesions. *Nature medicine*, *21*(10), 1223–1227. https://doi.org/10.1038/nm.3937

88. Ramachandran, G., Chandrasekaran, P., Gaikwad, S., Agibothu Kupparam, H. K., Thiruvengadam, K., Gupte, N., Paradkar, M., Dhanasekaran, K., Sivaramakrishnan, G. N., Kagal, A., Thomas, B., Pradhan, N., Kadam, D., Hanna, L. E., Balasubramanian, U., Kulkarni, V., Murali, L., Golub, J., Gupte, A., Shivakumar, S. V. B. Y., … Cohort for Tuberculosis Research by the Indo-US Partnership (CTRIUMPh) Team (2020). Subtherapeutic Rifampicin Concentration Is Associated With Unfavorable Tuberculosis Treatment Outcomes. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America*, *70*(7), 1463–1470. https://doi.org/10.1093/cid/ciz380

89. Rana, M., Coshic, P., Goswami, R., & Tyagi, R. K. (2017). Influence of a critical single nucleotide polymorphism on nuclear receptor PXR-promoter function. *Cell biology international*, *41*(5), 570–576. https://doi.org/10.1002/cbin.10744

90. Rifat, D., Prideaux, B., Savic, R. M., Urbanowski, M. E., Parsons, T. L., Luna, B., Marzinke, M. A., Ordonez, A. A., DeMarco, V. P., Jain, S. K., Dartois, V., Bishai, W. R., & Dooley, K. E. (2018). Pharmacokinetics of rifapentine and rifampin in a rabbit model of tuberculosis and correlation with clinical trial data. *Science translational medicine*, *10*(435), eaai7786. https://doi.org/10.1126/scitranslmed.aai7786

91. Ritchie, M. D., Haas, D. W., Motsinger, A. A., Donahue, J. P., Erdem, H., Raffanti, S., Rebeiro, P., George, A. L., Kim, R. B., Haines, J. L., & Sterling, T. R. (2006). Drug transporter and metabolizing enzyme gene variants and nonnucleoside reverse-transcriptase inhibitor hepatotoxicity. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America*, *43*(6), 779–782. https://doi.org/10.1086/507101

92. Satyaraddi, A., Velpandian, T., Sharma, S. K., Vishnubhatla, S., Sharma, A., Sirohiwal, A., Makharia, G. K., Sinha, S., Biswas, A., & Singh, S. (2014). Correlation of plasma anti-tuberculosis drug levels with subsequent development of hepatotoxicity. *The international journal of tuberculosis and lung disease: the official journal of the International Union against Tuberculosis and Lung Disease*, *18*(2), 188–iii. https://doi.org/10.5588/ijtld.13.0128

93. Schwarz, U. I., Meyer zu Schwabedissen, H. E., Tirona, R. G., Suzuki, A., Leake, B. F., Mokrab, Y., Mizuguchi, K., Ho, R. H., & Kim, R. B. (2011). Identification of novel functional organic anion-transporting polypeptide 1B3 polymorphisms and assessment of substrate specificity. *Pharmacogenetics and genomics*, *21*(3), 103–114. https://doi.org/10.1097/FPC.0b013e328342f5b1

94. SEARCH Collaborative Group, Link, E., Parish, S., Armitage, J., Bowman, L., Heath, S., Matsuda, F., Gut, I., Lathrop, M., & Collins, R. (2008). SLCO1B1 variants and statin-induced myopathy – a genomewide study. *The New England journal of medicine*, *359*(8), 789–799. https://doi.org/10.1056/NEJMoa0801936

95. Seth-Smith, H. M. B., Bonfiglio, F., Cuénod, A., Reist, J., Egli, A., & Wüthrich, D. (2019). Evaluation of Rapid Library Preparation Protocols for Whole Genome Sequencing Based Outbreak Investigation. *Frontiers in public health*, *7*, 241. https://doi.org/10.3389/fpubh.2019.00241

96. Shah, P. A., Sharma, P., Shah, J. V., Sanyal, M., & Shrivastav, P. S. (2016). An improved LC-MS/MS method for the simultaneous determination of pyrazinamide, pyrazinoic acid and 5-hydroxy pyrazinoic acid in human plasma for a pharmacokinetic study. *Journal of chromatography. B, Analytical technologies in the biomedical and life sciences*, 1017–1018, 52–61. https://doi.org/10.1016/j.jchromb.2016.02.036

97. Shimizu, M., Fukami, T., Kobayashi, Y., Takamiya, M., Aoki, Y., Nakajima, M., & Yokoi, T. (2012). A novel polymorphic allele of human arylacetamide deacetylase leads to decreased enzyme activity. *Drug metabolism and disposition: the biological fate of chemicals*, *40*(6), 1183–1190. https://doi.org/10.1124/dmd.112.044883

98. Sigal, G. B., Segal, M. R., Mathew, A., Jarlsberg, L., Wang, M., Barbero, S., Small, N., Haynesworth, K., Davis, J. L., Weiner, M., Whitworth, W. C., Jacobs, J., Schorey, J., Lewinsohn, D. M., & Nahid, P. (2017). Biomarkers of Tuberculosis Severity and Treatment Effect: A Directed Screen of 70 Host Markers in a Randomized Clinical Trial. *EBioMedicine*, *25*, 112–121. https://doi.org/10.1016/j.ebiom.2017.10.018

99. Sinha, P., Ponnuraja, C., Gupte, N., Prakash Babu, S., Cox, S. R., Sarkar, S., Mave, V., Paradkar, M., Cintron, C., Govindarajan, S., Kinikar, A., Priya, N., Gaikwad, S., Thangakunam, B., Devarajan, A., Dhanasekaran, M., Tornheim, J. A., Gupta, A., Salgame, P., Christopher, D. J., … Hochberg, N. S. (2023). Impact of Undernutrition on Tuberculosis Treatment Outcomes in India: A Multicenter, Prospective, Cohort Analysis. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America*, *76*(8), 1483–1491. https://doi.org/10.1093/cid/ciac915

100. Sirgel, F. A., Donald, P. R., Odhiambo, J., Githui, W., Umapathy, K. C., Paramasivan, C. N., Tam, C. M., Kam, K. M., Lam, C. W., Sole, K. M., & Mitchison, D. A. (2000). A multicentre study of the early bactericidal activity of anti-tuberculosis drugs. *The Journal of antimicrobial chemotherapy*, *45*(6), 859–870. https://doi.org/10.1093/jac/45.6.859

101. Smythe, W., Khandelwal, A., Merle, C., Rustomjee, R., Gninafon, M., Bocar Lo, M., Sow, O. B., Olliaro, P. L., Lienhardt, C., Horton, J., Smith, P., McIlleron, H., & Simonsson, U. S. (2012). A semimechanistic pharmacokinetic-enzyme turnover model for rifampin autoinduction in adult tuberculosis patients. *Antimicrobial agents and chemotherapy*, *56*(4), 2091–2098. https://doi.org/10.1128/AAC.05792-11

102. Sproston, N. R., & Ashworth, J. J. (2018). Role of C-Reactive Protein at Sites of Inflammation and Infection. *Frontiers in immunology*, *9*, 754. https://doi.org/10.3389/fimmu.2018.00754

103. Strydom, N., Gupta, S. V., Fox, W. S., Via, L. E., Bang, H., Lee, M., Eum, S., Shim, T., Barry, C. E., 3rd, Zimmerman, M., Dartois, V., & Savic, R. M. (2019). Tuberculosis drugs' distribution and emergence of resistance in patient's lung lesions: A mechanistic model and tool for regimen and dose optimization. *PLoS medicine*, *16*(4), e1002773. https://doi.org/10.1371/journal.pmed.1002773

104. Sturkenboom, M.G.G., Van der Lijke, H., Jongedijk, E.M., Kok, W.T., Greijdanus, B., Uges, D.R.A., Alffenaar, J.W. (2015). Quantification of isoniazid, pyrazinamide and ethambutol in serum using liquid chromatography-tandem mass spectrometry. *Journal of Applied Bioanalysis*, *1*(3), 89–98. https://doi.org/10.17145/jab.15.015

105. Sundell, J., Bienvenu, E., Birgersson, S., Äbelö, A., Ashton, M., & Hoffmann, K. J. (2019). Simultaneous quantification of four first line antitubercular drugs and metabolites in human plasma by hydrophilic interaction chromatography and tandem mass spectrometry. *Journal of chromatography. B, Analytical technologies in the biomedical and life sciences*, *1105*, 129–135. https://doi.org/10.1016/j.jchromb.2018.10.027

106. Tostmann, A., Mtabho, C. M., Semvua, H. H., van den Boogaard, J., Kibiki, G. S., Boeree, M. J., & Aarnoutse, R. E. (2013). Pharmacokinetics of first-line tuberculosis drugs in Tanzanian patients. *Antimicrobial agents and chemotherapy*, *57*(7), 3208–3213. https://doi.org/10.1128/AAC.02599-12

107. Ulanova, V., Kivrane, A., Viksna, A., Pahirko, L., Freimane, L., Sadovska, D., Ozere, I., Cirule, A., Sevostjanovs, E., Grinberga, S., Bandere, D., & Ranka, R. (2024). Effect of NAT2, GSTM1 and CYP2E1 genetic polymorphisms on plasma concentration of isoniazid and its metabolites in patients with tuberculosis, and the assessment of exposure-response relationships. *Frontiers in pharmacology*, *15*, 1332752. https://doi.org/10.3389/fphar.2024.1332752

108. Visser, M., Bouter, L. M., McQuillan, G. M., Wener, M. H., & Harris, T. B. (1999). Elevated C-reactive protein levels in overweight and obese adults. *JAMA*, *282*(22), 2131–2135. https://doi.org/10.1001/jama.282.22.2131

109. Walubo, A., Chan, K., Woo, J., Chan, H. S., & Wong, C. L. (1991). The disposition of antituberculous drugs in plasma of elderly patients. II. Isoniazid, rifampicin and pyrazinamide. *Methods and findings in experimental and clinical pharmacology*, *13*(8), 551–556.

110. Wang, N., Guo, S., Liu, H., Ding, Y., Yao, R., Liu, Z., Zhu, H., Chen, X., Yang, X., Chen, X., & Lu, Y. (2022). Relevance of gene polymorphisms of NAT2 and NR1I2 to anti-tuberculosis drug-induced hepatotoxicity. *Xenobiotica; the fate of foreign compounds in biological systems*, *52*(5), 520–526. https://doi.org/10.1080/00498254.2022.2092783

111. Wang, X., Zhang, H., Han, Y., Huo, L., Cao, Y., Xu, X., & Ai, L. (2020). Rapid and simultaneous determination of ten anti-tuberculosis drugs in human plasma by UPLC-MS/MS with applications in therapeutic drug monitoring. *Journal of chromatography. B, Analytical technologies in the biomedical and life sciences*, *1152*, 122246. https://doi.org/10.1016/j.jchromb.2020.122246

112. Wang, Y., Xiang, X., Huang, W. W., Sandford, A. J., Wu, S. Q., Zhang, M. M., Wang, M. G., Chen, G., & He, J. Q. (2019). Association of PXR and CAR Polymorphisms and Antituberculosis Drug-Induced Hepatotoxicity. *Scientific reports*, *9*(1), 2217. https://doi.org/10.1038/s41598-018-38452-z

113. Weiner, M., Peloquin, C., Burman, W., Luo, C. C., Engle, M., Prihoda, T. J., Mac Kenzie, W. R., Bliven-Sizemore, E., Johnson, J. L., & Vernon, A. (2010). Effects of tuberculosis, race, and human gene SLCO1B1 polymorphisms on rifampin concentrations. *Antimicrobial agents and chemotherapy*, *54*(10), 4192–4200. https://doi.org/10.1128/AAC.00353-10

114. Weiner, M., Gelfond, J., Johnson-Pais, T. L., Engle, M., Johnson, J. L., Whitworth, W. C., Bliven-Sizemore, E., Nsubuga, P., Dorman, S. E., Savic, R., & Pharmacokinetics/ Pharmacodynamics Group of Tuberculosis Trials Consortium (2021). Decreased plasma rifapentine concentrations associated with AADAC single nucleotide polymorphism in adults with tuberculosis. *The Journal of antimicrobial chemotherapy*, *76*(3), 582–586. https://doi.org/10.1093/jac/dkaa490

115. Wener, M. H., Daum, P. R., & McQuillan, G. M. (2000). The influence of age, sex, and race on the upper reference limit of serum C-reactive protein concentration. *The Journal of rheumatology*, *27*(10), 2351–2359.

116. Werngren, J., Paues, J., Simonsson, U. S. H., & Schön, T. (2018). Distribution of plasma concentrations of first-line anti-TB drugs and individual MICs: a prospective cohort study in a low endemic setting. *The Journal of antimicrobial chemotherapy*, *73*(10), 2838–2845. https://doi.org/ 10.1093/jac/dky268

117. Wilson, D., Moosa, M. S., Cohen, T., Cudahy, P., Aldous, C., & Maartens, G. (2018). Evaluation of Tuberculosis Treatment Response With Serial C-Reactive Protein Measurements. *Open forum infectious diseases*, *5*(11), ofy253. https://doi.org/10.1093/ofid/ofy253

118. World Health Organization. (2021). WHO operational handbook on tuberculosis. Module 2: Screening-Systematic Screening for Tuberculosis Disease. Retrieved from https://iris.who.int/ bitstream/handle/10665/340256/9789240022614-eng.pdf?sequence=1 (accessed 8 January 2025)

119. World Health Organization. (2022a). WHO consolidated guidelines on tuberculosis. Module 4: treatment – drug-susceptible tuberculosis treatment. Retrieved from https://www.who.int/ publications/i/item/9789240048126 (accessed 16 July 2024)

120. World Health Organization. (2022b). WHO operational handbook on tuberculosis. Module 4: Treatment – drug-susceptible tuberculosis treatment. Retrieved from https://www.who.int/ publications/i/item/9789240050761 (accessed 8 January 2025)

121. World Health Organization. (2024). Global tuberculosis report 2024. Retrieved from https://www.who.int/teams/global-tuberculosis-programme/tb-reports/global-tuberculosis-report-2023 (accessed 21 November 2024)

122. Wu, L., Ye, Z., Liu, H., Guo, H., Lin, J., Zheng, L., Chu, N., & Liu, X. (2020). Rapid and highly sensitive quantification of the anti-tuberculosis agents isoniazid, ethambutol, pyrazinamide, rifampicin and rifabutin in human plasma by UPLC-MS/MS. *Journal of pharmaceutical and biomedical analysis*, *180*, 113076. https://doi.org/10.1016/j.jpba.2019.113076

123. Wyczalkowska-Tomasik, A., Czarkowska-Paczek, B., Zielenkiewicz, M., & Paczek, L. (2016). Inflammatory Markers Change with Age, but do not Fall Beyond Reported Normal Ranges. *Archivum immunologiae et therapiae experimentalis*, *64*(3), 249–254. https://doi.org/10.1007/s000 05-015-0357-7

124. Yang, M., Pan, H., Chen, H., Liu, W., Lu, L., He, X., Yi, H., & Tang, S. (2020). Association between NR1I2 polymorphisms and susceptibility to anti-tuberculosis drug-induced hepatotoxicity in an Eastern Chinese Han population: A case-control study. *Infection, genetics and evolution: journal of molecular epidemiology and evolutionary genetics in infectious diseases*, *83*, 104349. https://doi.org/10.1016/j.meegid.2020.104349

125. Yimer, G., Ueda, N., Habtewold, A., Amogne, W., Suda, A., Riedel, K. D., Burhenne, J., Aderaye, G., Lindquist, L., Makonnen, E., & Aklillu, E. (2011). Pharmacogenetic & pharmacokinetic biomarker for efavirenz based ARV and rifampicin based anti-TB drug induced liver injury in TB-HIV infected patients. *PloS one*, *6*(12), e27810. https://doi.org/10.1371/journal.pone.0027810

126. Zazuli, Z., Barliana, M. I., Mulyani, U. A., Perwitasari, D. A., Ng, H., & Abdulah, R. (2015). Polymorphism of PXR gene associated with the increased risk of drug-induced liver injury in Indonesian pulmonary tuberculosis patients. *Journal of clinical pharmacy and therapeutics*, *40*(6), 680–684. https://doi.org/10.1111/jcpt.12325

127. Zhang, J., Kuehl, P., Green, E. D., Touchman, J. W., Watkins, P. B., Daly, A., Hall, S. D., Maurel, P., Relling, M., Brimer, C., Yasuda, K., Wrighton, S. A., Hancock, M., Kim, R. B., Strom, S., Thummel, K., Russell, C. G., Hudson, J. R., Jr, Schuetz, E. G., & Boguski, M. S. (2001). The human pregnane X receptor: genomic structure and identification and functional characterization of natural allelic variants. *Pharmacogenetics*, *11*(7), 555–572. https://doi.org/10.1097/00008571-200110000-00003

128. Zhang, J., Zhao, Z., Bai, H., Wang, M., Jiao, L., Peng, W., Wu, T., Liu, T., Chen, H., Song, X., Wu, L., Hu, X., Wu, Q., Zhou, J., Song, J., Lyv, M., & Ying, B. (2019). Genetic polymorphisms in PXR and NF-κB1 influence susceptibility to anti-tuberculosis drug-induced liver injury. *PloS one*, *14*(9), e0222033. https://doi.org/10.1371/journal.pone.0222033

129. Zhang, W., He, Y. J., Gan, Z., Fan, L., Li, Q., Wang, A., Liu, Z. Q., Deng, S., Huang, Y. F., Xu, L. Y., & Zhou, H. H. (2007). OATP1B1 polymorphism is a major determinant of serum bilirubin level but not associated with rifampicin-mediated bilirubin elevation. *Clinical and experimental pharmacology & physiology*, *34*(12), 1240–1244. https://doi.org/10.1111/j.1440-1681.2007.04798.x

130. Zheng, X., Bao, Z., Forsman, L. D., Hu, Y., Ren, W., Gao, Y., Li, X., Hoffner, S., Bruchfeld, J., & Alffenaar, J. W. (2021). Drug Exposure and Minimum Inhibitory Concentration Predict Pulmonary Tuberculosis Treatment Response. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America*, *73*(9), e3520–e3528. https://doi.org/10.1093/cid/ciaa1569

131. Zhou, Z., Wu, X., Wei, Q., Liu, Y., Liu, P., Ma, A., & Zou, F. (2013). Development and validation of a hydrophilic interaction liquid chromatography-tandem mass spectrometry method for the simultaneous determination of five first-line antituberculosis drugs in plasma. *Analytical and bioanalytical chemistry*, *405*(19), 6323–6335. https://doi.org/10.1007/s00216-013-7049-0

132. Zhuang, X., Li, L., Liu, T., Zhang, R., Yang, P., Wang, X., & Dai, L. (2022). Mechanisms of isoniazid and rifampicin-induced liver injury and the effects of natural medicinal ingredients: A review. *Frontiers in pharmacology*, *13*, 1037814. https://doi.org/10.3389/fphar.2022.1037814

133. Ziglam, H. M., Baldwin, D. R., Daniels, I., Andrew, J. M., & Finch, R. G. (2002). Rifampicin concentrations in bronchial mucosa, epithelial lining fluid, alveolar macrophages and serum following a single 600 mg oral dose in patients undergoing fibre-optic bronchoscopy. *The Journal of antimicrobial chemotherapy*, *50*(6), 1011–1015. https://doi.org/10.1093/jac/dkf214

Acknowledgements

After these years dedicated to research, there are many people to whom I owe deep appreciation for their contributions to the success of this PhD journey, each playing a role in one way or another.

First and foremost, I would like to express my sincere gratitude to my scientific supervisor, **Prof. Renāte Ranka**, for fostering and shaping my scientific creativity, guiding my research efforts and, most importantly, providing me with the opportunity to grow as a professional under her leadership.

To my dearest colleagues from the Laboratory of Molecular Biology at the Latvian Biomedical Research and Study Centre – **Darja Sadovska**, **Lauma Feimane**, **Alisa Kazarina**, **Valentīna Čapligina**, **Agne Namiņa**, and **Jānis Ķimsis** – I truly appreciate your time and willingness to share knowledge in my early career steps. I have also been fortunate to work with **Viktorija Ulanova** – she mentored and supported me during the TB PGx project, and over the years, this has evolved into a powerful tandem, bringing countless research ideas to life.

This work would not have been possible without the invaluable contributions of the administrative and clinical personnel, particularly pneumonologist **Anda Vīksna**, at the Centre of Tuberculosis and Lung Diseases (Riga East University Hospital), who demonstrated excellence in patient management and clinical sample collection, as well as provided expertise in interpreting clinical data. Likewise, I extend my gratitude to the National Biobank Genome Database of the Latvian Population for providing specific clinical sample collections required for certain experiments.

Regarding the chemistry aspect of this work, I would like to express special thanks to **Solveiga Grīnberga** from the Mass Spectrometry Group (Laboratory of Physical Organic Chemistry, Latvian Institute of Organic Synthesis) for the opportunity to work under her supervision and to **Eduards Sevostjanovs** for his patience in explaining LC-MS/MS concepts and answering my endless questions during my traineeship in analytical chemistry.

My appreciation also goes to my most recent collaborator, **Leonora Pahirko**, from the Faculty of Science and Technology (University of Latvia), for her assistance in addressing raised scientific questions through statistical analysis.

Many thanks to the Latvian Biomedical Research and Study Centre and the Latvian Institute of Organic Synthesis for state-of-the-art research infrastructure, facilities, and services. Separate thanks to the Genome Centre, the core facility of the Latvian Biomedical Research and Study Centre, for sequencing services.

I am deeply grateful to Rīga Stradiņš University, my *Alma Mater*, for its support through doctoral study grants. Additionally, this work received funding from the Fundamental and

Applied Research project “Tuberculosis treatment: Exploring the perspectives for personalised therapy” (No lzp2020/1-0050), funded by the Latvian Council of Science, and the project “Rīga Stradiņš University internal and Rīga Stradiņš University with Latvian Academy of Sport Education external consolidation” (No 5.2.1.1.i.0/2/24/I/CFLA/005), funded by the European Union’s Recovery and Resilience Mechanism and the Republic of Latvia.

Finally, I would like to thank my **beloved family and friends** for their unlimited patience, deep understanding, and flexibility – whether it was giving me “just one more hour” to finish writing a manuscript chapter before dinner or tolerating my wish to answer “a couple of extremely important” e-mails during vacations. You are my peace, my support, and my inspiration at every step I take.