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Summary of
Doctoral Thesis

Lauma Freimane

MITOCHONDRIAL AND GENETIC BIOMARKERS IN TUBERCULOSIS: TREATMENT EFFECTS, ADVERSE REACTIONS, AND IMMUNOSENESCENCE

Riga 2026

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For the degree of Doctor of Science in Biology
Subfield of Molecular Biology

Riga 2026

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The Thesis is available at the Library of the University of Latvia, Kalpaka blvd. 4.

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ANNOTATION

Tuberculosis (TB) remains a global health concern, despite advances in drug development, treatment protocols, and diagnostic approaches. TB patients continue to face complications that can compromise their well-being, reduce quality of life, or lead to treatment failure. These challenges highlight the need for novel biomarkers to monitor treatment response and guide clinical decision-making process.

This Thesis investigated blood-based mitochondrial and other DNA-based biomarkers in TB patients, along with potential influencing factors such as patient anthropometric parameters and lifestyle habits, in order to assess the biological impact of TB and the systemic effects of anti-TB chemotherapy.

The results presented in this Thesis suggest that mitochondria play a significant role in TB pathogenesis. TB infection appears to drive immuno-senescence, potentially accelerating the development of active disease. Mitochondrial circulating cell-free DNA exhibited a rapid response to TB treatment, particularly to ethambutol, indicating its potential as a biomarker for treatment monitoring. Moreover, patient lifestyle factors and anthropometric parameters were found to influence the observed biomarkers and should be considered during therapy monitoring. These findings provide a foundation for future research aimed at developing novel TB monitoring tools and host-directed therapies, particularly with respect to the role of mitochondria in TB pathogenesis, which could improve treatment outcomes.

Keywords: tuberculosis, mtDNA copy number, mtDNA variations, telomere length, biomarkers

CONTENTS

ABBREVIATIONS

ADR – adverse drug reactions
AECs – alveolar epithelial cells
AIDS – acquired immunodeficiency syndrome
ATP – adenosine triphosphate
AUC – area under [a receiver-operating characteristic] curve
BMI – body mass index
ccf-DNA – circulating cell-free DNA
CN – copy number
CRP – C-reactive protein
DAMP – damage-associated molecular pattern
ddPCR – droplet digital PCR
DS-TB – drug-sensitive tuberculosis
HIV – human immunodeficiency virus
LC-MS/MS – liquid chromatography-tandem mass spectrometry
MDR-TB – multidrug-resistant tuberculosis
Mtb – Mycobacterium tuberculosis
mtDNA – mitochondrial DNA
nDNA – nuclear DNA
PCR – polymerase chain reaction
rCRS – revised Cambridge Reference Sequence
ROS – reactive oxygen species
rpm – rounds per minute
RR-TB – rifampicin-resistant tuberculosis
TB – tuberculosis
TL – telomere length
WHO – World Health Organization

INTRODUCTION

Tuberculosis (TB) remains one of the major health challenges worldwide and is responsible for millions of deaths each year, especially in low- and middle-income countries (WHO 2024c). While much advancement has been made in developing diagnostic tools and treatment strategies, predicting disease progression and identifying those with a higher chance of experiencing adverse reactions remains a vital gap in the fight against this infectious disease. Biomarkers that provide early insights into disease susceptibility, progression, and treatment response, including the risk of adverse drug reactions, have the potential to improve TB management and patient outcomes significantly.

Regarding biomarkers, mitochondria hold great promise. Although best known for their role in cellular energy production, they have recently been recognized as key players in immune responses and inflammation – processes closely linked to TB pathology – and may therefore serve as host-based biomarkers for treatment monitoring. For example, changes in mitochondrial DNA copy number (mtDNA CN), in combination with variations in telomere length (TL) in circulating blood cells, may be indicative of immunosenescence, which may further influence host susceptibility to infectious diseases such as TB. This raises the question of how DNA-based biomarkers could be used to improve TB monitoring, and what specific aspects of disease progression they may reflect.

Importance of this work: This study explores mtDNA CN, mitochondrial genetic variations, blood cell TL, and individual patient factors as potential biomarkers of the biological impact of TB and the systemic effects of anti-TB chemotherapy. While further research is needed to validate the clinical applicability of the identified biomarkers, this study provides valuable insights into TB pathogenesis and the effects of anti-TB treatment, thereby informing future personalised treatment approaches and improving TB management.

Aims of the study: To investigate blood-based mitochondrial and nuclear DNA biomarkers in TB patients, focusing on their potential role in disease development, treatment response, and adverse anti-TB drug reactions.

Tasks to achieve the aims:

1) Measure and analyse mtDNA CN and TL in blood samples of TB patients and compare them with age- and sex-matched healthy controls;
2) Analyse mtDNA genetic variations in patients with multidrug-resistant TB (MDR-TB) to assess their potential relation to amikacin- and capreomycin-induced adverse events;

3) Measure circulating cell-free mtDNA (ccf-mtDNA) and nuclear DNA (ccf-nDNA) CN in patients with drug-sensitive TB (DS-TB) before and after medication ingestion to determine the short-term systemic effects;
4) Investigate other patient-specific and lifestyle-related factors that may contribute to TB progression and/or observed biomarker values.

This study has been supported by following projects/grants: National Research Program Biomedicine for Public Health (BIOMEDICINE) "Development of Innovative Strategies for the Regulation and Modulation of the Infection Process" (Project No. 7 – Subproject 7.3; Fourth Phase); Rīga Stradiņš University research grants No. 23030103 and No. 6-ZD-22/25/2022; Fundamental and Applied Research Programme funded by the Latvian Council of Science, Project No. lzp2020/1-0050; and the European Union's Recovery and Resilience Mechanism project No.5.2.1.1.i.0/2/24/I/CFLA/001 "Consolidation of the Latvian Institute of Organic Synthesis and the Latvian Biomedical Research and Study Centre".

1. LITERATURE REVIEW

1.1. Introduction to TB

TB is believed to be the deadliest pathogen in human history, responsible for more deaths over time than any other microorganism. Although TB is treatable and somewhat preventable, one in four individuals carries a latent TB infection, which can become active in 5-10% of cases when immunity is weakened (Houben and Dodd 2016; WHO 2024c).

In 2023, an estimated 10.8 million people worldwide developed TB, with 8.2 million being newly diagnosed, and 1.25 million died from this disease, underscoring its persistent global impact (WHO 2024c). Although the overall incidence of TB is decreasing, the prevalence of MDR-TB is on the rise and continues to pose a public health crisis (WHO 2024a). Thus, overall, it was concluded that, despite advancements in TB diagnostics, treatment, and prevention, TB remains a leading cause of death globally, particularly among vulnerable populations such as individuals living with human immunodeficiency virus (HIV), those with chronic illnesses or malnutrition, and people residing in high-burden TB regions (WHO 2024a).

1.1.1. *Mtb* pathogenicity mechanisms

The main causative agent of TB is *Mycobacterium tuberculosis (Mtb)*, which is a member of the *Mtb* complex discovered by Robert Koch in 1882. *Mtb* is an airborne, intracellular microorganism transmitted via aerosols released when individuals with active TB cough, sneeze, or talk. Inhaling as few as one to five *Mtb* bacteria can be enough to establish an infection (Tellier et al. 2019). After entering the respiratory tract, the innate immune system activates the body's first line of defence through the epithelial barrier: alveolar epithelial cells (AECs) and alveolar macrophages – which are also the primary targets of *Mtb* (Nunes-Alves et al. 2014).

Infected alveolar macrophages (or *Mtb* bacteria directly) migrate into the lung's interstitial tissue, where the bacteria infect a variety of monocyte-derived and tissue-resident macrophages, dendritic cells and neutrophils (Chandra, Grigsby, and Philips 2022). If the innate immune response is effective, *Mtb* is completely eliminated, preventing disease onset (Chandra et al. 2022; Nunes-Alves et al. 2014).

If the first line of defence fails to eliminate *Mtb,* adaptive immune responses are activated. Dendritic cells or inflammatory monocytes migrate to the pulmonary

lymph nodes, where antigen-specific T cells are primed (Chandra et al. 2022). This triggers the recruitment of immune cells to the lung parenchyma, leading to the formation of granulomas (Pai et al. 2016). Granulomas consist of an amorphous mass of immune cells surrounding a necrotic core of *Mtb*-infected alveolar macrophages, designed to contain the bacterial spread (Ramakrishnan 2012). Although granulomas form to limit the spread of bacteria, they can also provide a protective environment for bacterial populations, enabling them to evade detection and clearance by the host immune system. This allows the infection to persist in a clinically latent state while still permitting bacterial replication (Luies and du Preez 2020).

Latent TB is characterized by a persistent immune response to *Mtb* antigens without clinical manifestations of active TB but with an increased risk of developing the disease (Narasimhan et al. 2013). This latent state can persist for decades, reactivating under conditions favourable to transmission or when the bacterial load becomes excessive. This can cause the granuloma to fail, allowing bacteria to enter the bloodstream or re-enter the respiratory tract for release (Anes et al. 2023; Lin et al. 2014; Pai et al. 2016).

Mtb employs a variety of strategies to survive within host cells and manipulate immune responses, effectively evading or delaying them. These include inducing apoptosis in infected macrophages and necrosis in AECs, arresting phagosome maturation in infected cells, and impairing dendritic cell maturation to delay T cell priming; additionally, *Mtb* can reprogram immune cell metabolism and disrupt mitochondrial function, leading to T cell exhaustion and a weakened adaptive immune response (Ankley, Thomas, and Olive 2020; Chandra et al. 2022; Danelishvili et al. 2003; Russell et al. 2019; Vázquez et al. 2017).

1.2. TB treatment and adverse drug reactions

The TB treatment regimen has significantly improved in recent years, becoming shorter and more optimised for better outcomes. DS-TB is now treated exclusively with oral medications. Standard DS-TB treatment consists of an intensive phase of 2 months, followed by a continuation phase of 2–4 months with 4–5 different antibiotics. Since 2021, the 6-month regimen remains the gold standard, and it is suitable for all patients regardless of age, disease severity, or HIV status (WHO 2022a). The 6-month regimen consists of 2 months of daily first-line TB medicines – isoniazid, rifampicin, pyrazinamide, and ethambutol – followed by 4 months of isoniazid and rifampicin.

MDR-TB is defined as TB caused by *Mtb* strains with resistance to at least two first-line anti-TB drugs, rifampicin and isoniazid (WHO 2024b). The global pooled prevalence of MDR-TB is estimated to be around 11.6%, with the highest rates observed in Eastern European countries, Russia, Central Asia, and parts of China (Salari et al. 2023).

Treatment of MDR-TB is significantly more challenging than DS-TB. Before 2016, MDR-TB treatment typically took 18-24 months and included several injectable agents, which were often associated with severe adverse events. Beginning in 2022, WHO guidelines promoted the use of shorter, all-oral regimens rather than longer regimens, and in 2025 they advanced to recommending all-oral regimens as the global standard (WHO 2025). Treatment regimens for MDR-TB can now be categorized into 6-month, 9-month, or longer regimens. Baseline drug susceptibility testing will confirm eligibility for different regimen options (WHO 2022b).

The 6-month regimen consists of 4 anti-TB drugs: bedaquiline, pretomanid, linezolid, and moxifloxacin (WHO 2022b). The 9-month regimen is suitable for patients who are ineligible for the shorter 6-month regimen. It consists of a 4-month intensive phase (bedaquiline, levofloxacin or moxifloxacin, ethionamide, ethambutol, high-dose isoniazid, pyrazinamide, and clofazimine), followed by a continuation phase for the remaining 5 months (levofloxacin or moxifloxacin, clofazimine, ethambutol, and pyrazinamide). The longer MDR-TB regimen (18-20 months) is tailored for patients with additional drug resistance or other complicating factors. The choice of regimen is influenced by clinical factors (e.g., disease severity, hepatic or renal failure, uncontrolled diabetes mellitus, or HIV status), contextual factors (e.g., prevalence of HIV or isoniazid resistance), and access factors (e.g., availability and cost of TB drugs) (WHO 2022a). The duration of therapy may be adjusted based on the patient's response to treatment (WHO 2022b).

1.2.1. Adverse drug reactions in TB treatment

Adverse drug reactions (ADRs) can affect different organ systems and typically range from mild (Grade 1) to potentially lethal (Grade 4). The severity of ADRs can be classified using the *Division of AIDS* table for grading the severity of adult and paediatric adverse events:

- **Grade 1 (Mild):** symptoms cause no or minimal interference with usual social and functional activities;
- **Grade 2 (Moderate):** symptoms cause more than minimal interference with usual social and functional activities;
- **Grade 3 (Severe):** symptoms cause an inability to perform usual social and functional activities;
- **Grade 4 (Potentially life-threatening):** symptoms cause an inability to perform basic self-care functions or require medical or surgical intervention to prevent permanent impairment or persistence;
- **Grade 5 (Death):** the adverse event results in death (NIH 2017).

Based on WHO data, around 19.3% of DS-TB patients develop Grade 3 or higher ADRs (WHO 2022a). However, the data are controversial and vary across different geographical regions. A 2022 study conducted in Brazil by Flávia

et al. reported that 78.8% of patients experienced at least one ADR. The most common ADRs were hyperuricaemia (27.8%), dyspeptic syndrome (22.9%), and dermatological syndrome (17.1%) (Sant´Anna et al. 2022). Nevertheless, 88.7% of DS-TB patients who developed ADRs experienced mild or moderate damage (Sant´Anna et al. 2022).

Compared to DS-TB, MDR-TB treatment requires a longer duration, a higher pill burden, increased dosages, and includes medications with a greater risk of toxicity. Additionally, patients may experience severe and irreversible ADRs, leading to poorer treatment outcomes, with approximately 15% of MDR/RR-TB patients dying from the disease. Severe ADRs may necessitate changes in TB therapy, further compromising treatment efficacy. While shorter TB treatment regimens are associated with a lower incidence of ADRs, implementing a 6-month treatment protocol is not always feasible (WHO 2022b).

Based on a recent meta-analysis of MDR-TB treatment in 2020, only 61.3–69.7% of patients achieved successful therapy (Lan et al. 2020). Additionally, approximately 24% of patients experienced at least one ADR severe enough to require permanent drug discontinuation. For example, injectable drugs, amikacin and kanamycin, were most frequently discontinued due to ototoxicity (87% and 75%, respectively), while nephrotoxicity accounted for 51% of capreomycin-associated ADRs leading to drug discontinuation (Lan et al. 2020).

1.3. Significance of DNA-based biomarkers in TB

Existing diagnostic tools, such as *Mtb* antigen tests (tuberculin skin test, interferon-gamma (IFN-γ) release assay, or T-SPOT assay), sputum smear microscopy, chest radiographs, and the Xpert MTB/RIF automated molecular test, play a crucial role in TB diagnostics, management of active disease, treatment monitoring, and outcome prediction (Heyckendorf et al. 2022; Huang et al. 2022; Nogueira et al. 2022). However, these methods have limitations, including low or variable sensitivity, time consumption, and reduced efficacy in paediatric patients (Huang et al. 2022). Additionally, traditional approaches may be less effective in detecting extrapulmonary, smear-negative, or latent TB infections (Huang et al. 2022; Jain et al. 2024). Emerging biomarkers hold promise for improving diagnostic sensitivity, specificity, and accessibility, potentially addressing these existing gaps.

Considering that biomarker research is actively focused on increasing specificity and sensitivity while keeping the procedure as non-invasive as possible, DNA-based biomarkers are emerging, both whole blood-based and circulating cell-free, known as liquid biopsy (Li et al. 2024). DNA-based biomarkers hold several advantages over conventional methods: high sensitivity and specificity, a fast approach with low concentration input, which can be used in latent infection diagnostics, and several methods that can be integrated with

portable devices, facilitating use in resource-limited settings (Heyckendorf et al. 2022; Nathavitharana et al. 2022; Wang et al. 2022; Yayan et al. 2024).

However, no single test can address all clinical questions. Therefore, patients' treatment response, disease progression, and outcomes should be assessed using a combination of diagnostic approaches, including both traditional routine tests and innovative methods. Currently, there is no consensus on host-specific DNA biomarkers for diagnostics or treatment response, highlighting an urgent need for their development (Shaik, Pillay, and Jeena 2024).

Among the promising DNA-based biomarkers, those derived from blood will be discussed in greater detail, highlighting their potential to enhance TB diagnosis, track disease progression, and demonstrating the molecular and physiological basis of this Thesis.

1.3.1. DNA-based biomarkers from whole blood: the diagnostic potential of mtDNA and telomere length

As established in the previous chapter, DNA-based biomarkers hold great potential and are currently the focus of active research. However, most studies focus on the pathogen's DNA rather than the host's. Among host DNA biomarkers, mtDNA analysis shows great promise for infection monitoring.

Mitochondria are multifunctional organelles that possess their own DNA and are responsible not only for ATP (adenosine triphosphate) production and oxidative phosphorylation (OXPHOS) but also for modulating cellular REDOX balance, reactive oxygen species (ROS) production, pH, and Ca^{2+} concentrations and their homeostasis. Additionally, mitochondria regulate the cell cycle, initiate apoptosis, and participate in various signalling pathways and epigenomic regulation through Krebs cycle intermediates (Wallace 2015; Wallace and Fan 2010; Weinberg, Sena, and Chandel 2015). Apart from the functions already mentioned, mitochondria are further implicated in the regulation of the innate immune response through various mechanisms.

Mitochondria are an excellent source of damage-associated molecular patterns (DAMPs) due to the relative hypomethylation of mtDNA, its structural diversity, and its susceptibility to oxidative damage. DAMPs derived from mtDNA subsequently activate innate immune response sensors, stimulate cytokine secretion, and contribute to the pathogenesis of various inflammatory diseases (West and Shadel 2017). *Mtb* infection has been shown to directly increase mitochondrial stress and ROS production, triggering the release of oxidized mtDNA into the cytosol, where it acts as a DAMP, mediating innate antimicrobial immunity and local inflammation (West and Shadel 2017).

The mtDNA CN in circulating blood cells may serve as an indicator of mitochondrial dysfunction, which is also induced by chronic *Mtb* infection and antigen presentation (Malik and Czajka 2013; Russell et al. 2019). Although mtDNA CN is largely associated with age-related diseases, cardiovascular

health, cancer, and other chronic conditions, studies have also reported its usefulness in predicting predisposition to infectious diseases, disease severity, and the progression of adverse events (Castellani et al. 2020; Sun et al. 2019; Udomsinprasert et al. 2022; Wang et al. 2012).

Systemic oxidative stress and mitochondrial dysfunction are also associated with T cell dysfunction and inflammaging – a condition characterized by the accumulation of senescent immune cells, which further increases systemic inflammation and weakens the immune response (Escrig-Larena, Delgado-Pulido, and Mittelbrunn 2023). Several biomarkers can describe inflammaging, including DNA-based markers, such as mtDNA CN, and also leukocyte TL (Lin and Epel 2022). In response to oxidative stress, telomeres shorten, threatening overall DNA stability. Shorter TL has also been associated with higher mortality rates from various age-related pathologies (Dolcini et al. 2020). TL is already reported as predictive of TB treatment outcomes, as recent studies have shown that patients with longer TL at the time of diagnosis have better treatment outcomes (Katoto et al. 2021).

Further, approximately 93% of the mtDNA sequence is coding and contains evolutionarily conserved, commonly inherited variants that indicate affiliation with a specific mitochondrial haplogroup (Chinnery and Hudson 2013). Since mtDNA essentially lacks introns, it is more prone to somatic mutations with functional consequences in response to oxidative stress and other environmental factors (Ferreira and Rodriguez 2024). Both inherited and acquired mtDNA variants can impact mitochondrial activity, respiratory chain function, and overall mitochondrial functionality (Heidari et al. 2022; Sanchez-Contreras and Kennedy 2022; Schaefer et al. 2022; Vázquez-Coto et al. 2022). This suggests that the presence of common haplogroups or somatic mtDNA variants may influence inflammatory pathology (West and Shadel 2017). At present, the impact of mtDNA variations on immune response modulation remains unclear and requires further investigation. In the case of TB, several mtDNA variant involvement has been confirmed in relation to adverse events induced by aminoglycosides, second-line injectable drugs (Gao, Chen, and Guan 2017; Jones et al. 2021).

1.3.2. Cell-free DNA-based biomarkers from blood plasma

Liquid biopsy has gained increasing attention over the past decade, showing great promise in early diagnostics due to its low material requirements and broad range of potential applications (Adhit et al. 2023; Caputo et al. 2023). Liquid biopsy targeting human ccf-DNA is already used in various diagnostic contexts, including prenatal care, cancer detection, and age-related conditions (Zimmer et al. 2022). Additionally, it can assist in identifying DNA derived from infectious agents (Fernández-Carballo et al. 2019; Szilágyi et al. 2020).

It is believed that in healthy individuals, ccf-nDNA in blood plasma primarily originates from lymphoid and myeloid cell apoptosis, whereas ccf-mtDNA mainly derives from circulating cell-free mitochondria (Roch et al. 2021; Snyder et al. 2016). While ccf-nDNA is more stable than ccf-mtDNA, its levels can fluctuate depending on an individual's sex and age (Meddeb et al. 2019). In contrast, ccf-mtDNA concentration in plasma can rise rapidly, responding quickly to both psychological and physiological stress (Trumpff et al. 2019). As ccf-mtDNA is secreted and recognised as a DAMP, it has been proposed to serve as a functional link between mitochondrial damage and systemic inflammation (Cossarizza et al. 2011; West et al. 2015).

Variations in ccf-DNA levels have been extensively studied in patients with infectious diseases, physical trauma, critical illnesses, and cardiovascular conditions, highlighting its potential for facilitating early diagnostics across diverse medical scenarios (Jackson Chornenki et al. 2019; Peng et al. 2024; Trulson et al. 2023; Vajpeyee et al. 2020; Zhang et al. 2024). Additionally, ccf-DNA serves as a versatile biomarker for guiding treatment, monitoring therapeutic responses, identifying microbial resistance, and detecting minimal residual disease, making it particularly valuable in the management of chronic infections (Nyaruaba et al. 2019; Rahat et al. 2020).

In TB research, the detection of circulating bacterial DNA in the plasma of pulmonary TB patients has been shown to be a versatile biomarker for diagnostic purposes, while ccf-mtDNA CN has recently been identified as a potential biomarker for differentiating latent and active TB infection (Nyaruaba et al. 2019; Pan et al. 2022). Similar findings have also been demonstrated in COVID-19 patients, where ccf-mtDNA levels were used to distinguish asymptomatic and symptomatic individuals from controls (Shoraka et al. 2023). These findings highlight the significant potential of liquid biopsies as a promising tool in routine diagnostics, particularly in the context of TB, where they could enhance early detection and personalised treatment strategies.

2. MATERIALS AND METHODS

2.1. Study design and sample collection

The aim of this work was to investigate blood-based mt- and nDNA biomarkers in TB patients, with a focus on their potential roles in disease development, treatment response, and adverse reactions to anti-TB drugs, while also exploring the complex interactions between immunosenescence, mitochondrial function, and lifestyle factors in patients with DS- or MDR-TB. At the outset of this study, one of the perspective future goals was the potential development or identification of candidate biomarkers that could be used in clinical practice as tools for monitoring disease progression or assessing TB severity. For this reason, the molecular methods employed were intentionally kept simple to facilitate their potential integration into clinical practice, if needed (Table 1).

The first two publications, from 2021 and 2023, describe retrospective studies, with a primary focus on MDR-TB patients. The patient cohort was collected in the period from 2014 to 2017. DNA samples were obtained from the Genome Database of the Latvian Population according to the protocol described by Rovite et al. (Rovite et al. 2018).

The third publication is a cross-sectional study investigating rapid changes in ccf-DNA levels in the blood plasma of patients with DS-TB. A different TB patient cohort was used for several reasons: (1) patients with DS-TB receive fewer therapeutic drugs, resulting in fewer metabolite interactions; (2) fresh plasma samples could be collected, which was crucial for accurate metabolite analysis; and (3) these patients were newly diagnosed, with no severe comorbidities or relevant disease history, reducing potential confounding factors. Samples were collected at the Centre of Tuberculosis and Lung Diseases, and DNA extraction was carried out in Latvian Biomedical Research and Study Centre.

Table 1. **Summary of participant characteristics and materials in publications underlying this Thesis.**

	Publication I (Freimane et al. 2021)	**Publication II (Freimane et al. 2023)**	**Publication III (Freimane et al. 2025)**
Analysis type	Retrospective	Retrospective	Cross-sectional
Sample source	Genome Database of the Latvian population (Rovite et al. 2018)	Genome Database of the Latvian population (Rovite et al. 2018)	Patients admitted to the Centre of Tuberculosis and Lung Diseases, Riga East Clinical University Hospital
Subjects	$n_{MDR\text{-}TB} = 51$ $n_{controls} = 57$	n = 47	n = 25
Health status	MDR-TB patients and sex and age matched healthy controls	MDR-TB patients	DS-TB patients
Biological material used	DNA from peripheral blood	DNA from peripheral blood	DNA from peripheral blood plasma
Main molecular methods	Real-time PCR	Full mtDNA genome sequencing using next-generation sequencing (NGS)	1) droplet digital PCR (ddPCR) 2) LC-MS/MS
Aim	To investigate whether MDR-TB is associated with alterations in aging biomarkers in peripheral blood mononuclear cells	To analyse the occurrence of ADRs and evaluate the role of multiple patient-, disease-, and therapy-related factors	To investigate fluctuations in ccf-mtDNA and ccf-nDNA copy number in DS-TB patients' plasma after anti-TB drug ingestion

The inclusion criteria for all three studies were as follows: age over 18 years old; signed informed consent; a diagnosis of MDR-TB or DS-TB; the availability of clinical data. The exclusion criteria were applied only in the third publication: pregnancy, and co-infection with other microbial or viral agents (e.g., hepatitis C/B virus, HIV).

Information about patients' lifestyle choices, anthropometric data, and other potential influencing factors was obtained in collaboration with the Centre of Tuberculosis and Lung Diseases at Riga East University Hospital and the national biobank of the Genome Database of the Latvian Population. The study was conducted in accordance with the Helsinki Declaration and approved by the Central Medical Ethics Committee of Latvia (Approval No. 01-29.1/1 and No. 01-29.1.2/1736), 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). Informed consent was obtained from all participants.

2.2. Molecular methods

2.2.1. Publication I – real-time PCR

In this study the relative TL and mtDNA CN quantification were performed using real-time PCR. mtDNA CN was measured using a multiplex reaction with Maxima Probe/ROX qPCR Master Mix (2X) reagents (Thermo Scientific, USA), targeting the mitochondrial minor arc (minArc) as the mitochondrial template. The *GAPDH* gene (OMIM: 138400) was used for normalisation, as previously described (Phillips, Sprouse, and Roby 2014; Zole and Ranka 2019).

For TL quantification Maxima SYBR green qPCR Master Mix (2X) (Thermo Scientific, USA) was used, with the *HBB* (β-globin) gene (OMIM: 141900) serving as a control single-copy gene for normalization, as described elsewhere (Kim et al. 2013). The real-time PCR conditions for telomere amplification were 95 °C for 10 minutes, followed by 40 cycles of 95 °C for 10 seconds and 58 °C for 1 minute. Each sample was measured in triplicate, and relative quantification calculations were performed using the 2-ΔΔCT method with Pfaffl's modification (Livak and Schmittgen 2001; Pfaffl 2004).

2.2.2. Publication II – full mtDNA genome sequencing

Full mtDNA genome sequencing was performed using IonTorrent technology and the Personal Genome Machine (Thermo Fisher Scientific, USA). First, the entire mitochondrial genome was amplified in two separate PCR reactions, producing fragments A and B, each over 8 kb in length, as previously described (Fendt et al. 2009).

A and B amplicons were pooled and fragmented by sonication into 200–250 bp-long fragments. DNA fragment libraries were prepared using the Ion Plus Fragment Library Kit (Thermo Fisher Scientifc, Carlsbad, CA, USA) and the Ion XpressTM Barcode Adapters Kits (Thermo Fisher Scientifc, Carlsbad, CA, USA).

The sequencing data were uploaded to the Galaxy web platform, where the public server at *usegalaxy.org* was used to analyse the data (Afgan et al. 2018). BAM data sets were converted into the FASTQ format using the *Convert, Merge, Randomize* tool (Barnett et al. 2011). FASTQ sequences were filtered by quality (the cut-off value = 20; percent of bases in a sequence that must have quality equal to or higher than the cut-off value = 90). The tool *Trim Galore!* was used to remove adapter sequences from the data file (Krueger et al. 2023). *Bowtie 2* was used to map the trimmed reads to the reference genome using standard revised Cambridge Reference Sequence (rCRS, NC_012920.1, *Homo sapiens* mitochondrion complete genome) (Langmead and Salzberg 2012). Aligned reads were analysed with the *FreeBayes tool* for haploid genomes using a population model as shown in Nekrutenko & Ostrovsky, 2021 (Nekrutenko and Ostrovsky 2021).

Single-nucleotide variants were defined as positions with a nucleotide different from the rCRS; indels were not analysed. To exclude sequencing errors, each sample was also examined manually using the Integrative Genomics Viewer (IGV) tool (Robinson et al. 2011). VCF files were uploaded to the HaploGrep 2.0 classification tool to determine a detailed mtDNA haplogroup (Weissensteiner et al. 2016). Mutation analyses were conducted using the HmtVar and MITOMAP databases (Preste et al. 2019; Ruiz-Pesini et al. 2006).

2.2.3. Publication III – DNA isolation and ddPCR

Blood samples were collected by medical personnel using vacutainers with EDTA (BD, Plymouth, UK) at three time points: in the morning on an empty stomach before drug intake (referred to hereafter as the 0 h time point), and at 2 h and 6 h after drug intake. Blood samples were immediately centrifuged at 4,000 rpm (3488×g) for 15 minutes at 4 °C, and blood plasma aliquots were immediately frozen and stored at –70 °C until further processing.

Plasma ccf-DNA was extracted from 200 µL of thawed plasma aliquots using QIAamp DNA Blood mini kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. DNA was eluted in 100 µL elution buffer and stored at –20 °C until further analysis.

For absolute quantification of ccf-mtDNA and ccf-nDNA in blood plasma samples, **ddPCR** was employed to target *MT-ND1* (OMIM: 516000) and *B2m* (OMIM: 109700) gene fragments, respectively. According to the manufacturer`s

recommendations, the measurements were conducted in a 20 µL ddPCR reaction mix containing 10 µL of 2x ddPCR supermix for probes (Bio-Rad Laboratories, Dubai, UAE), 1 µL of each primer (final concentration 900 nM), 1 µL probe (final concentration 250 nM), 2 µL of plasma DNA sample, and nuclease-free water. Primers and probes were purchased from Metabion, Germany, and have been described elsewere (Ye et al. 2017; Belmonte et al. 2016). To amplify *MT-ND1*, the plasma DNA was diluted with nuclease-free water at a ratio of 1:10.

Droplets were generated in accordance with the manufacturer's recommendations using the droplet generator machine (Bio-Rad Laboratories, Hercules, USA). Subsequently, droplets from the cartridge were transferred onto a 96-well ddPCR plate. The PCR reaction involved an initial incubation at 95 °C for 10 minutes, followed by 40 cycles of 94 °C for 30 seconds and 60 °C for 60 econds. The reaction was concluded with a final hold at 98 °C for 10 minutes. The droplets were then read using a QX200 droplet reader machine (Bio-Rad Laboratories, Hercules, USA) and analysed using QuantSoft™ Analysis Pro (version 1.0.596). The absolute CN of ccf-mtDNA and -nDNA per µL of fresh plasma was calculated, factoring in the dilution.

2.2.4. Determination of pharmacokinetic parameters of anti-TB drugs with LC-MS/MS

A validated LC-MS/MS method was employed to determine concentrations of isoniazid, rifampicin, pyrazinamide and ethambutol in blood plasma samples as described previously (Kivrane et al. 2021). The area under the time-concentration curve from 0 to 6 h ($AUC_{0\text{-}6\ h}$) was calculated using the linear trapezoidal rule based on data obtained in the three time points: pre-dose (0 h) and 2 and 6 h post-dose, which are usually the most informative for therapeutic drug monitoring (Peloquin 2002).

2.3. Bioinformatics and statistical analyses

We explored various patient-, lifestyle-, and disease-related factors in each study. The similarities and differences in the analysed contributing factors of each publication are listed in Table 2. These factors were chosen to normalize the data and to identify potential biomarker-influencing variables that should be considered in future studies or during data interpretation.

Table 2. **Factors analysed and statistical tests used in the publications presented in this Thesis.**

Factors analysed			
Common in all publications	**Applicable to Publication I**	**Applicable to Publication II**	**Applicable to Publication III**
▸ Sex ▸ Age ▸ Tobacco smoking experience ▸ BMI ▸ Alcohol consumption ▸ Comorbidities	▸ MDR-TB status ▸ TB incident case ▸ Disease location	▸ Type of injectable agent administered during therapy ▸ Number of injectable doses received ▸ Simultaneous antiretroviral therapy ▸ TB incident case ▸ mtDNA variations	▸ C-reactive protein (CRP) levels ▸ Use of additional drugs ▸ Hypertension drug use ▸ Presence of cavitations ▸ Drug-induced liver injury ▸ $AUC_{0-6\,h}$ of the anti-TB drugs used
Statistical tests used			
▸ ANOVA ▸ ANCOVA	▸ t-test ▸ Pearson's correlation	▸ Chi-square test ▸ Fisher's exact test ▸ Logistic regression	▸ t-test ▸ Linear regression ▸ Wilcoxon signed-rank test ▸ Mann-Whitney U-test ▸ Spearman's correlation ▸ Shapiro-Wilk test

Statistical tests were selected based on the type of data, its conformity to a normal distribution, and the specific research question being addressed. The statistical tests used are also reported in Table 2.

A significance level of $\alpha = 0.05$ was applied. Statistical analyses were conducted using GraphPad Prism version 5.01 for Windows, R Statistical Software (v4.1.2), and XLSTAT (Adinsoft 2021; GraphPad Software 2007; R Core Team 2021).

3. RESULTS AND DISCUSSION

3.1. MDR-TB patients demonstrate immunosenescence traits

In our cohort, MDR-TB patients showed significantly shorter telomeres ($P < 0.0001$) and higher mtDNA CN ($P = 0.0068$) compared to healthy controls, and this difference was not associated with age. Furthermore, ANCOVA analysis indicated that the most significant factor for both TL shortening and mtDNA CN increase was the infection with *Mtb* ($P = 0.002$ and $P = 0.019$, respectively). mtDNA CN was also influenced by the patient's sex ($P < 0.001$), where females showed no significant change in mtDNA CN compared to controls, although they were underrepresented in this cohort. Other analysed factors, such as BMI, smoking, and the type of TB infection (extrapulmonary), may have varying effects on mtDNA CN and TL dynamics; however, infection with *Mtb* remains the most influential factor.

This raises the question of whether *Mtb* infection induces immunosenescence or if individuals with pre-existing immunosenescence are more susceptible to infection. Theoretically, both scenarios could be true. Telomere shortening is associated with increased inflammatory processes and may serve as a biomarker of liver injury caused by excessive ROS production induced by anti-TB drugs (Ruiz et al. 2021; Udomsinprasert et al. 2020). In infectious environments, immune cells with shortened telomeres are associated with severe lymphopenia and increased mortality (Ruiz et al. 2021). Telomere shortening contributes to progressive atrophy and functional decline in highly proliferative organs such as the intestines and hematopoietic system, while also causing significant impairment in relatively quiescent organs like the liver and heart (Sahin et al. 2011). Furthermore, telomere shortening and dysfunction are linked to impaired mitochondrial biogenesis and function, reduced gluconeogenesis, and increased ROS production, further exacerbating telomere damage (Sahin et al. 2011).

This suggests that when *Mtb* infection increases mitochondrial ROS production to amplify the inflammatory response, it also contributes to telomere damage. In turn, telomere shortening further accelerates mitochondrial dysfunction and mtDNA release, ultimately providing *Mtb* with a survival advantage. This creates a self-perpetuating cycle of infection progression and immune senescence, where each process reinforces the other (Ellzey, Patrick, and Watson 2023; Qian et al. 2019).

3.2. Assessment of amikacin- and capreomycin-related ADRs in patients with MDR-TB

In our second study, 47 patients with MDR-TB received injectable anti-TB drugs: amikacin and/or capreomycin. More than half of these patients experienced aminoglycoside-associated ADRs, including ototoxicity and/or nephrotoxicity (n = 26/47, 55.3%). Ototoxicity was more frequently observed in patients receiving amikacin injections (logistic regression analysis, P = 0.049). This adverse effect is primarily driven by mitochondrial translation impairment, followed by oxidative stress-induced mitochondrial dysfunction, ultimately leading to cell death (Shulman et al. 2014). Like previous researchers, we propose that these adverse events are primarily caused by the biochemical properties of aminoglycosides, in combination with other predisposing factors such as mitochondrial functionality, genetic predisposition, and increased aminoglycoside accumulation in cells particularly sensitive to them – specifically, inner ear hair cells and renal proximal tubular epithelial cells (McDermott et al. 2022; Rivetti et al. 2023; Vuda and Kamath 2016). Ototoxicity events were not associated with the doses received or treatment duration per se, which is in accordance with the currently accepted opinion in the scientific literature. This emphasizes the role of patient variability and the potential impact of genetic background.

However, nephrotoxicity was more likely associated with pre-existing renal impairment and was more common in older patients (not significant in logistic regression, but significant in Fisher's exact test: P = 0.017). Since renal function declines with age, it is unsurprising that nephrotoxicity was more frequently observed in older patients (Noronha et al. 2022); however, this finding was not confirmed by multifactorial logistic regression analysis.

3.3. Exploring the role of mitochondrial genetic factors in amikacin- and capreomycin-related ADRs

Full mitochondrial genome sequencing did not reveal specific ADR-associated variants, and no significant differences in adverse event occurrence are observed for any specific variants, mitochondrial genes, or haplogroups.

Our findings suggest that mitochondrial genetic variability is too extensive to draw definitive conclusions regarding specific variants associated with aminoglycoside-related ADRs. Although 34% (n=16) of patients treated with aminoglycosides in our study developed ototoxicity, none of the known risk variants were detected. However, we identified several novel variants in patients experiencing aminoglycoside-related ADRs. Due to the low frequency and heterogeneity of these variants across patients, it is difficult to determine whether they are truly associated with the observed phenotype. Notably, one variant,

m.961T>A, was found in a patient with ototoxicity. This variant is located in the *MT-RNR1* gene, a known hotspot for ototoxicity-associated mutations. In the gnomAD v4.1.0 population database, m.961T>A has been reported only four times, with an estimated frequency of 0.007%. Previous population studies have identified this variant only twice – once in our prior analysis of mtDNA variants in the ethnic Latvian population and once in an individual from Russia, as reported by Dzhemilova and colleagues (Dzhemilova et al. 2009; Igumnova et al. 2018).

Although we did not identify any known variants associated with aminoglycoside-induced ototoxicity in MDR-TB patients, almost all individuals carried additional variants that were not linked to their confirmed haplogroup. It is possible that the mtDNA haplogroup could suppress a potentially pathogenic or phenotype-modifying variant by altering its expression range (La Morgia et al. 2020; Vila-Sanjurjo, Smith, and Elson 2021). Alternatively, the phenotype might be balanced by the cell itself, silencing the modifying variant (Münch and Harper 2016; Richter-Dennerlein et al. 2016).

3.4. Fluctuations in ccf-mtDNA and -nDNA CN in blood plasma after anti-TB drug intake in patients with DS-TB

After anti-TB drug administration, the average levels of ccf-mtDNA CN in blood plasma showed rapid fluctuations, with a 1.5-fold increase at the 2-hour time point, followed by a 1.3-fold decrease after 6 hours. We also calculated the ccf-mtDNA$_{\Delta 2\,h/0\,h}$ ratio, which represents relative changes within the first two hours post-drug administration. This ratio was patient-specific and influenced by age, ethambutol AUC$_{0\text{-}6h}$, and BMI. The rapid response of ccf-mtDNA to physiological stressors suggests its potential utility for analysing various influencing factors and monitoring treatments with significant physiological impact. For example, ccf-mtDNA levels have already been reported to distinguish latent TB infection from active disease, reinforcing the idea that they reflect disease dynamics (Pan et al. 2022). This finding also supports the hypothesis that baseline variability in ccf-mtDNA may indicate infection severity or impaired innate immune responses, but further studies are needed to confirm this.

In contrast, ccf-nDNA CN levels in blood plasma remained relatively stable during the 6-hour period following drug administration, supporting their use as an internal control for ccf-mtDNA monitoring, especially in cases where extreme ccf-mtDNA CN values were observed. We also found that patients with pulmonary cavitations had higher ccf-nDNA values, although the difference was not statistically significant ($p = 0.055$). The limited sample size may have reduced the statistical power to detect this association.

In conclusion, with respect to DNA-based biomarkers for assessing predisposition to TB, whole blood or leukocyte mtDNA CN and TL appear to be

more suitable markers due to their more stable concentrations. In contrast, ccf-DNA markers may be better suited for monitoring rapid treatment responses.

3.5. Additional factors influencing TB-related biomarkers

In all the publications presented in this Thesis, the author has analysed various factors influencing TB-related and patient-related properties to assess their potential interactions and applicability in TB monitoring.

Patient sex emerged as a significant factor only in mtDNA CN from whole blood in MDR-TB patients but not for TL, with males demonstrating a relatively higher mtDNA CN than females. Overall, women did not exhibit significant changes in the senescence biomarkers analysed compared to matched healthy controls. Several possible explanations for this finding exist. First, there is the underrepresentation of women in our sample cohort (n=12; 23.5%). Another potential factor is differences in white blood cell counts or oestrogen levels, as oestrogens regulate mitochondrial bioenergetics (Klinge 2020; Shim et al. 2020).

In our first study, we did not observe an **age**-related influence on mtDNA CN and TL, despite these associations being well established in population studies (Zole and Ranka 2018). We attributed this to the specific age range of our patients, the absence of children and young adolescents, and the impact of the disease itself. However, in our most recent study, age emerged as a significant factor in ccf-mtDNA fluctuations following drug consumption in multivariate analysis, though it was not statistically significant in univariate analysis. This suggests that age may act as a modifying factor.

Age is naturally associated with chronic diseases, systemic wear and tear, cumulative allostatic load, and increased ccf-DNA CN (Memiah et al. 2021; Tessier et al. 2023; Wang 2022). Aging also affects drug pharmacokinetics, influencing absorption in the gastrointestinal tract, phase I metabolism in the liver, and excretion due to age-related renal impairment (Reis da Silva 2024). However, since this association was observed only for ccf-mtDNA CN and not nDNA, the author proposes that it is more likely linked to inflammation and therapy response, which may be more complex in older individuals (Negin, Abimbola, and Marais 2015).

Body composition, represented as **BMI**, played a role in two of the studies presented in this Thesis. First, in MDR-TB patients, a normal BMI was associated with longer telomeres in whole blood samples. This aligns with broader research suggesting that obesity is linked to shorter telomeres due to cumulative oxidative stress and chronic inflammation (Warmelink et al. 2011). However, no impact was observed on mtDNA CN.

Second, in DS-TB patients, those with higher BMI were more likely to exhibit negative ccf-mtDNA dynamics ($\Delta_{2\ h/0\ h}$) after drug ingestion, whereas underweight patients showed a greater relative increase in ccf-mtDNA CN.

These findings suggest that BMI can influence the analysed biomarkers, though likely in distinct ways, as they represent different biological parameters and responses. This underscores the complex impact of body composition on disease pathogenesis and treatment outcomes.

Changes in body composition due to aging and chronic illnesses like TB often manifest as muscle loss and an increased fat tissue proportion. These alterations can affect therapeutic drug performance, as muscle mass plays a crucial role in drug metabolism, distribution, and elimination (Chung 2014; Reis da Silva 2024). Additionally, TB-related weight loss itself can be a risk factor for hepatic injury (Warmelink et al. 2011). Conversely, inadequate nutrition and low body weight are well-known risk factors for TB (Choi et al. 2021).

Our findings indicate that **ethambutol** strongly influences ccf-mtDNA dynamics, likely due to its chemical properties, which impact mitochondrial function by disrupting the electron transport chain (Sarkar and Ganguly 2016). This disruption can lead to excessive ROS generation and decreased ATP levels, both of which can trigger ccf-mtDNA release (Reddam, McLarnan, and Kupsco 2022; Zhao et al. 2021). In other studies, presented in this Thesis, the medications used or their pharmacokinetic parameters were not analysed as separate factors due to the highly variable drug regimens in MDR-TB patients and the small sample size, which was insufficient for achieving statistical power.

We believe that due to the limited sample size – resulting from strict exclusion criteria and the relatively small number of new patients each year in the Latvian population – some potential associations may have been missed. Certain patterns may only be observable in isolated models. For example, non-smokers had slightly longer telomeres than patients with a history of smoking, and patients with pulmonary TB had longer telomeres than those with extrapulmonary TB (including those with both pulmonary and extrapulmonary TB); however, these differences were not statistically significant. Additionally, MDR-TB patients generally had a significantly higher proportion of individuals with a history of smoking, increased alcohol consumption, and lower body weight compared to healthy age- and sex-matched controls. Similarly, the association between CRP and ccf-nDNA was significant only in univariate analysis. However, CRP is a well-known marker of inflammation activity and severity, particularly in TB, where it can play a protective role against bacterial infection (Sproston and Ashworth 2018).

These findings emphasize the complex interplay between systemic inflammation, lifestyle factors, and molecular markers of disease. Many of the factors discussed, such as smoking, lower BMI, and alcohol consumption, have been previously reported (Min et al. 2023; Narasimhan et al. 2013; Siddalingaiah et al. 2023). However, inconsistencies with other studies may arise due to the small sample size and the wide diversity of analysed factors.

The fact that each of the studies presented in this Thesis explored different aspects and identified distinct sets of potential influencing factors highlights the diverse pathophysiological mechanisms underlying variations in whole blood mtDNA CN, TL, and plasma ccf-DNA. This underscores the multifunctional nature of these biomarkers and their potential applications in different contexts.

3.6. Limitations of the study

The results of this study were interpreted with a critical perspective, acknowledging several limitations. One of the primary constraints was the small sample size, which, combined with the high sensitivity of the applied methods to errors, may have introduced variability in the results. External factors such as blood or plasma sample processing, storage, transportation, overall sample quality, and operator errors could have also influenced the findings.

Complete exclusion of platelet contamination in plasma was not possible, as some degree of activation is inevitable during blood centrifugation (Söderström et al. 2016). Additionally, ccf-mtDNA levels are known to decrease in certain conditions, such as neurodegenerative diseases, where they may serve as predictive biomarkers (Lowes et al. 2020). Moreover, mtDNA CN varies significantly between different cell types (Filograna et al. 2021), a factor that was not accounted for in this study. Even if this had been considered, reference values for mtDNA CN, ccf-mtDNA CN, or other DNA-based markers in healthy individuals and various tissues or bodily fluids are still not well established. This lack of standardised reference ranges presents challenges for implementing these biomarkers, particularly in smaller hospital settings with limited sample sizes. Additionally, the applicability of these biomarkers in paediatric patients remains unclear.

CONCLUSIONS

1. Patients with MDR-TB exhibit an immunosenescent phenotype compared with healthy controls.
2. Ototoxicity in MDR-TB patients could not be explained by known mitochondrial variations associated with aminoglycoside-induced ADRs, suggesting potential inter-population genetic variability and the involvement of additional, yet unidentified, factors.
3. Nephrotoxicity observed in MDR-TB patients receiving amikacin or capreomycin injections was more likely associated with pre-existing renal impairment than with genetic predisposition or lifestyle factors.
4. Ccf-mtDNA, but not ccf-nDNA, demonstrated a measurable response to anti-TB treatment in patients with DS-TB, particularly to ethambutol administration. This may reflect individual pharmacokinetic variations in drug metabolism and clearance.
5. Lifestyle-related factors were observed to significantly influence the analysed DNA-based biomarker values.

THESIS

1. The immunosenescent phenotype observed in patients with MDR-TB, characterised by altered mtDNA CN and shortened TL, may result from both active disease development and chronic antigen presentation, suggesting a multifactorial origin.
2. ADR associated with injectable agents – amikacin and capreomycin – appear to be more influenced by the specific drug used and the patient's pre-existing health conditions.
3. Ccf-DNA demonstrated a potential as a biomarker for TB therapy monitoring, as ccf-mtDNA levels respond rapidly to drug administration, whereas ccf-nDNA may serve as a useful internal control to validate observed changes.

PUBLICATIONS

I. **Freimane L**, Barkāne L, Igumnova V, Kivrāne A, Zole E, Ranka R. Telomere length and mitochondrial DNA copy number in multidrug-resistant tuberculosis. *Tuberculosis*. 2021;131:102144. doi: 10.1016/j.tube.2021.102144. PMID: 34781086.

II. **Freimane** L, Barkāne L, Kivrāne A, Sadovska D, Ulanova V, Ranka R. Assessment of Amikacin- and Capreomycin-Related Adverse Drug Reactions in Patients with Multidrug-Resistant Tuberculosis and Exploring the Role of Genetic Factors. *J Pers Med*. 2023;13(4):599. doi:10.3390/jpm13040599. PMID: 37108985.

III. **Freimane L**, Kivrāne A, Ulanova V, Vīksna A, Sevostjanovs E, Grīnberga S, Cīrule A, Krams A, Ranka R. Fluctuations in circulating cell-free mitochondrial and nuclear DNA copy numbers in blood plasma after anti-tuberculosis drug intake in patients with drug-susceptible tuberculosis. *Tuberculosis*. 2025;151:102611. doi: 10.1016/j.tube.2025.102611. PMID: 39862444.

APPROBATION OF RESEARCH

1. **Freimane L**, Zole E, Barkāne L, Ranka R. MDR-TB impacts patient's leukocyte telomere length and mitochondrial DNA copy number. 27.03.2020. *RSU International Student Conference 2020,* Riga, Latvia. Oral presentation.
2. **Freimane L**, Zole E, Barkāne L, Ranka R. Insights on senescence and therapy-related adverse effects in tuberculosis patients. *2020. International Scientific Conference on Medicine 2020,* Riga, Latvia. Abstract book.
3. **Freimane L**, Igumnova V, Sadovska D, Kivrāne A, Ķimsis J, Barkāne L, Ranka R. Mitochondrial DNA variations and K haplogroup frequencies differs between TB patients and healthy controls. 25.03.2022. *80th International Scientific Conference of the University of Latvia 2022,* Riga, Latvia. Oral presentation.
4. **Freimane L**, Barkāne L, Ulanova V, Kivrāne A, Sadovska D, Ranka R. Multirezistentās tuberkulozes injicējamo medikamentu izraisīto blakusparādību ietekmējošie riska faktori. 10.02.2023. *Latvijas Universitātes 81. starptautiskā zinātniskā konference 2023*, Riga, Latvia. Oral presentation.
5. **Freimane L**, Vīksna A, Ulanova V, Kivrāne A, Sadovska D, Ranka R. Droplet digital PCR – a fast clinical method for *M. tuberculosis* infection progression monitoring in patients with tuberculosis – pilot study phase I. 25.–28.06.2023. *The 43rd Annual Meeting of the European Society of Mycobacteriology*, Tirana, Albania. Abstract book.
6. **Freimane L**, Kivrāne A, Ulanova V, Sadovska D, Ranka R. Cirkulējošās šūnu brīvās mitohondriālās DNS fragmentu koncentrācija kā biomarķieris pacientiem ar tuberkulozi. 16.02.2024. *Apvienotā LaBS un LU konference*, Riga, Latvia. Oral presentation.
7. **Freimane L**, Ulanova V, Kivrāne A, Sadovska D, Vīksna A, Ranka R. Within-day variation of cell-free mitochondrial and nuclear DNA in blood plasma of patients with tuberculosis: associations with drug exposure and patient-related factors. 29.06.–03.07.2024. *48th FEBS Congress,* Milan, Italy. Abstract book.

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