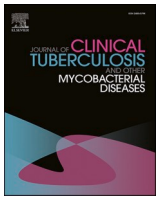
Journal of Clinical Tuberculosis and Other Mycobacterial Diseases 37 (2024) 100493

Contents lists available at ScienceDirect

Journal of Clinical Tuberculosis and Other Mycobacterial Diseases

journal homepage: www.elsevier.com/locate/jctube



Endogenous reactivation cases identified by whole genome sequencing of *Mycobacterium tuberculosis*: Exploration of possible causes in Latvian tuberculosis patients

Anda Viksna [a,b,*], Darja Sadovska [a,c], Vija Riekstina [b,d], Anda Nodieva [e], Ilva Pole [b], Renate Ranka [a,c], Iveta Ozere [a,b]

[a] *Rīga Stradiņš University, Riga, Latvia*
[b] *Riga East Clinical University Hospital, Centre of Tuberculosis and Lung Diseases, Ropaži Municipality, Upeslejas, Latvia*
[c] *Laboratory of Molecular Microbiology, Latvian Biomedical Research and Study Centre, Riga, Latvia*
[d] *Department of Internal Medicine, University of Latvia, Riga, Latvia*
[e] *Sleep Disorder Centre, Riga, Latvia*

ARTICLE INFO

Keywords:
Endogenous reactivation
Tuberculosis recurrence
Tuberculosis reinfection
Whole genome sequencing of *Mycobacterium tuberculosis*
Single nucleotide polymorphism of *Mycobacterium tuberculosis*
Latvia

ABSTRACT

Background: The recurrence of tuberculosis (TB) continues to place a significant burden on patients and TB programs worldwide. Repeated TB episodes can develop either due to endogenous reactivation of previously treated TB or exogenous reinfection with a distinct strain of *Mycobacterium tuberculosis* (Mtb). Determining the precise cause of the recurrent TB episodes and identifying reasons for endogenous reactivation of previously successfully treated patients is crucial for introducing effective TB control measures.

Methods: Here, we aimed to provide a retrospective individual analysis of the clinical data of pulmonary TB patients with assumed endogenous infection reactivation based on WGS results to identify the reasons for reactivation. Patient medical files were reviewed to describe the provoking factors for endogenous reactivation.

Results: In total, 25 patients with assumed endogenous TB reactivation were included in the study group, and 30 patients with one TB episode during the study period were included in the control group. There were no statistically significant differences identified between studied patient groups in patients age ($t_{(53)} = -1.53$, $p = 0.13$), body mass index ($t_{(53)} = 0.82$, $p = 0.42$), area of residency ($\chi^2_{(1;55)} = 0.015$, $p = 0.9$), employment status ($\chi^2_{(1;55)} = 0.076$, $p = 0.78$) and presence of comorbidities ($\chi^2_{(1;55)} = 3.67$, $p = 0.78$). Study group patients had statistically significantly more frequently positive sputum smear microscopy results ($\chi^2_{(1;55)} = 8.72$, $p = 0.0031$), longer time to sputum smear ($t_{(31)} = -2.2$, $p = 0.036$) and sputum culture conversion ($W_{(55)} = 198.5$, $p = 0.0029$). Smoking was statistically significantly ($\chi^2_{(1;55)} = 5.77$, $p = 0.016$) more frequently represented among study group patients. The median treatment duration for drug susceptible TB was 6 months in both in the control group (IQR 6–6) and among study group patients (IQR 6–7.75). The median treatment duration for multidrug-resistant TB was 20 months (IQR 17–23) in the control group and 19 months (IQR 16–19) in the study group patients.

Conclusion: Positive SSM for acid-fast bacteria, delayed time to sputum smear and sputum culture conversion, smoking, and incomplete therapy in the study group patients with multidrug-resistant TB should be considered as potential reasons for reactivation in recurrent TB patient group in our study.

Abbreviations: AFB, acid-fast bacteria; BMI, body mass index; CXR, chest x-ray; DS, drug susceptibility; DS-TB, drug susceptible tuberculosis; HS-resistant, TB isoniazid and streptomycin resistant tuberculosis; MDR-TB, multidrug resistant tuberculosis; Mtb, Mycobacterium tuberculosis; SNV, single nucleotide variant; TB, tuberculosis; tSSC, time to sputum smear conversion; tSCC, time to sputum culture conversion; WGS, whole genome sequencing; WHO, World Health Organization; XDR-TB, extensively drug-resistant tuberculosis.

* Corresponding author at: Rīga Stradiņš University, Riga, Latvia.
E-mail address: anda.viksna@rsu.lv (A. Viksna).

https://doi.org/10.1016/j.jctube.2024.100493

Available online 31 October 2024
2405-5794/© 2024 Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Background

Tuberculosis (TB) remains a global public health threat and is the second leading cause of death due to infection after COVID-19. Worldwide, an estimated 10.6 million people (95 % uncertainty interval 9.9–11.4 million) developed TB and 1.3 million people died of TB in 2022 [1]. Recurrence of TB (or repeated TB episodes) continues to place a significant burden on patients and TB programs worldwide [2]. The rate of TB recurrence varies by country and region [3–6]. Globally, the percentage of estimated incident TB cases that were repeated TB episodes was 6.8 % in 2020 [7].

Recurrent TB episodes can develop either due to endogenous reactivation of previously treated TB or due to exogenous reinfection with a distinct strain of *Mycobacterium tuberculosis* (Mtb). Reactivation rates of 2.7–4.7 % have been reported in countries with a high TB incidence compared with 0.3–1.0 % in countries with a low TB incidence [8,9]. Likewise, higher reinfection rates of 1.5–2.8 % have been found in countries with a high incidence of TB compared with 0.1–0.3 % in countries with a low incidence of TB [6,8,10].

The highest possibility of endogenous infection reactivation is within the first two years after treatment accomplishment, especially for those who interrupted treatment of the first TB episode [11–13]. Risk factors for endogenous reactivation, such as alcohol consumption, comorbidities (diabetes, HIV infection, chronic renal failure), smoking, and increasing age are well documented [6,8,11,14,15].

Identifying the cause of recurrent TB episodes and distinguishing between reinfection and endogenous reactivation is possible using the genotyping of Mtb [13,16,17]. Conventional genotyping methods have been used to distinguish between both conditions since 1990 [13,16–18]. However, these genotyping techniques could not distinguish reinfection cases with the same Mtb genotype [10,16,19]. Whole genome sequencing (WGS) has demonstrated a higher resolution when investigating recurrent TB episodes [16,17,20]. Discrimination of recurrent TB between re-infection and endogenous reactivation has been proposed based on single nucleotide variation (SNV) distance [10,18].

In Latvia, a Baltic state in Northern Europe, recurrent TB episodes from 1999—2007 using conventional genotyping methods were studied by Nodieva *et al.* Authors concluded that non-multidrug-resistant TB patients were at risk for reinfection with multidrug-resistant (MDR) Mtb strains during prolonged hospitalisation in conditions of poor infection control [21]. A recent study conducted by Sadovska *et al.* aimed to determine the causes of recurrent TB episodes using the WGS approach in addition to spoligotyping, and acquired results outlined that endogenous reactivation was the most common possible cause for recurrent TB episodes in the study population [16].

In Latvia, the number of reported TB cases has decreased during the last decades. TB case notification rate was 64.1 per 100 000 population in 2005, and 17.0 per 100 000 population in 2022. A total of 12484 TB cases were registered between 2005 and 2017, including 1705 (13.6 %) recurrent TB cases. Despite the recurrence rate has also decreased from 15.3 % (221 repeated TB cases out of 1443 incident TB cases) in 2005 to 10.9 % (60 repeated TB cases out of 552 incident TB cases) in 2017 [22], it is still above the global percentage of repeated TB episodes in Latvia [7]. Given the relatively high level of TB recurrence in Latvia, it is crucial to identify the provoking factors for repeated TB episodes, particularly for endogenous TB infection reactivation, which can be associated with inappropriate TB case management.

The current study aimed to provide an individual analysis of the clinical data of each patient with assumed endogenous infection reactivation based on Mtb WGS results to identify the reasons for reactivation.

2. Materials and methods

The study population of the current retrospective research was patients from the previously conducted study by Sadovska *et al.* with assumed endogenous pulmonary TB reactivation based on Mtb WGS results [16]. The study by Sadovska *et al.* was conducted on TB patients diagnosed with pulmonary TB from 2002 to 2019 in the Centre of Tuberculosis and Lung Diseases, Riga East University Hospital, the nationwide TB diagnostic and treatment centre. In this retrospective research by Sadovska *et al* all available paired Mtb isolates with identical spoligotypes that were acquired from pulmonary TB patients during 2002–2019 were included.

The study group of the current investigation included all paired Mtb isolates with a pairwise distance of $\leq$ 10 SNVs, which were obtained from the patients primary and recurrent TB episodes (selected from a study by Sadovska *et al* as determined previously) [16]. Only those patients who had a complete medical history available were included in the study group. For selected patients the treatment outcome of the first TB episode was defined as "cured" according to the WHO definition [23] before the second isolate acquisition date was considered as evidence of TB recurrence [16]. The control group included all available patients diagnosed with pulmonary TB in 2002–2019, whose Mtb isolates underwent WGS, and who were not diagnosed with recurrent TB episodes and were still alive up to July 2024.

The current study defined assumed endogenous TB infection reactivation as a recurrent pulmonary TB case with similar Mtb strains in both episodes having pairwise distances of $\leq$ 10 SNVs as indicated by WGS [10,25].

Mycobacterial DNA samples were extracted from cultures grown on Löwenstain-Jensen media at the time of diagnosis according to the cetyltrimethyl-ammonium bromide protocol [24]. Spoligotyping, WGS, and bioinformatic data processing are described in detail in the publication [16]. Both pairwise SNV distances and differing SNVs in initial and subsequent Mtb isolates were identified to distinguish between reinfection and reactivation.

Medical records from both TB episodes in the study group patients and only TB episode in the control group patients were reviewed to obtain demographic and clinical data (Tables 1–3). At the time of diagnosis (2002–2019), phenotypic drug-susceptible (DS) tests were used to guide therapy decisions according to the relevant WHO TB treatment guidelines. WGS was applied retrospectively in 2022 [16].

Definitions used for DS /resistance patterns correspond to WHO recommendations that were current at the time of sample collection. In this context, the term "drug-susceptible TB" (DS-TB) refers to tuberculosis that does not show any evidence of drug resistance. Resistance to both isoniazid (H) and streptomycin (S) is defined as isoniazid and streptomycin-resistant TB (HS resistant-TB). Multidrug-resistant TB (MDR-TB) refers to TB that is resistant to both H and rifampicin (R). Extensively-drug resistant tuberculosis (XDR-TB) is MDR-TB, which is also resistant to fluoroquinolones (FQ) and at least one of second-line injectables [26].

The extensity of the disease was evaluated based on radiological abnormality on chest x-ray (CXR) (unilateral/bilateral lung involvement, number of involved lobes, presence of destruction cavities) and radiological abnormality in association with time to sputum smear conversion (tSSC), and time to sputum culture conversion (tSCC).

Treatment outcomes (cured, died, lost to follow up, and treatment failed) were defined according to WHO recommendations that were current at the time of sample collection [27].

2.1. Data analysis

All calculations and statistical analyses were performed using RStudio software (2023.12.0). A level of significance $\alpha = 0.05$ was chosen for all performed tests. The distribution normality of quantitative data was checked using three approaches: constructing a quantile diagram and boxplot and performing the Shapiro-Wilk test. If data were normally distributed, mean value and standard deviation were used as descriptive statistics parameters, the F test was performed to determine if the variances of two populations were equal, and afterwards, a two-sample *t*-

Table 1
Demographic and clinical characteristics of the study group patients with endogenous TB reactivation (N = 25).

Patient ID	Biologic sex	Place of residence	Employment	Comorbidities	Smoking	i/v drug usage	The time between both TB episodes months	Age, years		BMI. kg/m^2		Sputum smears fluorescence microscopy results		tSSC, days		tSCC, days		Bilateral Abnormality on CXR		Presence of cavitation on CXR		Mtb resistance patterns (WGS)		Treatment duration, months		Treatment outcome	
			1st/ 2nd	1st 2nd	1st/ 2nd	1st/ 2nd	1st 2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
P3SIT254.1	F	U	0	0	1	0	72	22	28	18.7	17.3	1	0	59	0	62	0	1	1	1	1	0	0	6	0	cured	died
P4SIT1.3	M	U	0	COPD, CHD	1	0	33	54	57	19.4	21.5	1	1	90	29	90	38	1	1	1	1	0	0	6	9	cured	cured
P6SIT53.1	F	U	0	0	1	0	43	46	50	24.2	23.4	0	1	0	33	28	94	1	1	1	1	0	0	6	8	cured	cured
P8SIT1.4	F	U	1	0	0	0	58	47	52	18.5	19.4	1	1	55	30	55	76	1	0	1	1	0	0	6	6	cured	cured
P9SIT42.1*	F	U	0	0	1	1	126	30	41	21.4	19.4	1	0	19	0	27	77	0	0	1	1	0	0	6	8	cured	cured
P10SIT42.2	M	R	1	DM	1	0	25	41	43	17.4	17.9	1	1	103	62	71	74	1	0	1	1	0	0	6	8	cured	cured
P11SIT1.5*	M	R	1	0	1	0	19	60	61	21.9	20.3	1	0	196	0	196	61	0	1	0	0	0	0	12	8	cured	cured
P14SIT254.3	M	R	0	0	1	0	121	39	49	21.9	19.1	0	1	0	31	55	90	1	1	0	1	0	0	6	7	cured	cured
P15SIT254.4	M	R	0	Alcoholism, VHC	1	0	122	42	52	20.2	21.1	1	0	65	0	65	0	1	0	1	0	0	0	8	1.5	cured	lost to follow-up
P17SIT156.1*	M	R	0	Alcoholism Toxic CMP	1	0	14	48	49	21.9	20.9	1	1	61	109	61	109	1	1	1	1	0	0	6	8	cured	cured
P18SIT47.1*	M	R	1	Alcoholism	1	0	73	23	29	14.0	14.4	1	1	6	0	55	0	1	1	1	1	0	0	12	1	cured	died
P20SIT53.4	M	R	1	0	1	0	20	26	28	19.1	21.6	0	1	0	17	33	17	0	0	1	1	0	0	6	8	cured	cured
P30SIT53.5	M	U	0	0	1	0	74	30	36	20.1	14.2	1	1	44	62	44	117	1	1	0	1	0	0	7	8	cured	cured
P40SIT254.5*	M	R	0	0	1	0	156	39	52	16.3	14.8	1	1	118	0	86	0	1	1	0	1	0	0	11	0	cured	died
P5SIT1117.1	M	U	1	Deafness	1	0	118	60	70	20.0	21.5	1	0	27	0	127	52	1	1	1	1	H R[§] S	H R[§] S	8	10	cured	cured
P23SIT1.8*	M	U	0	Deafness	1	0	26	53	55	18.3	19.2	1	1	95	80	95	80	1	1	1	1	H S Eto PAS	H S Eto PAS	8	12	cured	cured
P31SIT53.6	M	U	1	HIV, Alcoholism	0	0	46	36	39	21.3	22.3	0	0	0	0	98	77	0	1	0	0	H S Eto	H S Eto	10	8	cured	cured
P32SIT53.7	F	U	0	HIV, Alcoholism	1	1	31	34	37	21.6	26.0	1	0	25	0	81	45	1	1	0	0	H S Eto	H S Eto	10	4	cured	lost to follow-up
P33SIT53.8*	M	U	0	HIV, Alcoholism	1	0	17	31	32	16.7	16.4	1	1	95	63	95	63	1	0	0	0	H S Eto	H S Eto	12	8	cured	cured
P39SIT1.15	M	R	1	0	1	0	103	36	44	22.7	20.2	1	0	31	0	33	77	1	0	1	1	H S Eto PAS	H S Eto PAS	8	8	cured	cured
P41SIT1.16	M	R	1	CKD (PD)	1	0	104	59	68	20.3	18.7	1	1	36	32	189	33	0	1	1	1	H R E Z Fq S Km Eto	H R E Z Fq S Km Eto	16	3	cured	died
P22SIT42.4	M	R	0	0	1	1	24	48	50	18.6	18.6	1	0	33	0	61	144	1	1	1	0	H R E Z S Eto	H R E Z S Eto	19	24	cured	cured
P26SIT1.11	M	U	1	0	1	0	83	44	51	18.7	16.1	1	1	114	61	114	0	1	1	1	1	H R E Z S Am Cm	H R E Z Fq[#] S Am	21	13	cured	failed

(*continued on next page*)

Table 1 (*continued*)

Patient ID	Biologic sex	Place of residence	Employment	Comorbidities	Smoking	i/v drug usage	The time between both TB episodes months	Age, years	BMI. kg/m^2	Sputum smears fluorescence microscopy results	tSSC, days	tSCC, days	Bilateral Abnormality on CXR	Presence of cavitation on CXR	Mtb resistance patterns (WGS)	Treatment duration, months	Treatment outcome
			1st/ 2nd	1st 2nd	1st/ 2nd	1st/ 2nd	1st 2nd	1st 2nd	1st 2nd	1st 2nd	1st 2nd	1st 2nd	1st 2nd	1st 2nd	1st 2nd	1st 2nd	1st 2nd
P25SIT1.10	M	U	1	0	1	0	46	44 48	18.7 19.1	1 1	127 112	42 112	1 1	1 1	Km Eto / Cm Km Eto	19 15	cured cured
															H R E Z S Am Cm Km Eto / H R E Z Fq[#] S Am Cm Km Eto		
P16SIT1.6	M	R	0	Alcoholism	1	0	55	39 43	20.8 17.0	0 1	0 0	75 0	0 1	0 1	H R E Z S Km Eto PAS / H R E Z S Km Eto PAS	13 0	cured died
								p < 0.001	p = 0.259	p = 0.248	p = 0.011	p = 0.196	p = 0.739	p = 0.414			

Abbreviations: 1st, first TB episode, 2nd, endogenous reactivation; tSSC, time to sputum smear conversion; tSCC, time to sputum culture conversion; F, female; M, male; 1, Yes/positive; 0, No/negative; U, urban; R, rural; COPD, chronic obstructive pulmonary disease; CHD, chronic heart disease; DM, diabetes mellitus; CMP, cardiomyopathy; CKD, chronic kidney disease: PD, peritoneal dialysis; VHC, virus hepatitis C; CXR, chest x-ray; S, streptomycin; H, isoniazid; R, rifampicin; E, ethambutol; Z, pyrazinamide; Am, amikacin; Ofx, ofloxacin; Mfx, moxifloxacin; Lfx, levofloxacin; Cm, capreomycin; Km, kanamycin; PAS, Para-aminosalicylic acid; Thz, thioacetazone; Eto, ethionamide. * Cases of possible reinfection based on WGS and epidemiological data [16]; [#]Cases of resistance development. [§] Phenotypic and genotypic drug susceptibility test difference.

Table 2
Demographic and clinical characteristics of the control group patients (N = 30).

Patient ID	Biologic sex	Place of residence	Employment	Comorbidities	Smoking	i/v drug usage	Age, years	BMI, kg/m^2	Sputum smears fluorescence microscopy results	tSSC, days	tSCC, days	Bilateral Abnormality on CXR	Presence of cavitation on CXR	Mtb resistance patterns (WGS)	Treatment duration, months	Treatment outcome
1	F	U	0	0	1	0	40	19.5	1	55	55	0	1	H R E Z S Am Eto	20	cured
2	M	R	1	0	1	0	31	22.2	1	89	90	1	1	H R E S PAS Eto	20	cured
3	M	U	1	HIV	1	0	40	22.5	0	0	64	0	0	H R E Z S Am PAS Eto	24	cured
4	M	U	0	HIV VHC	1	0	34	22.6	0	0	32	1	0	H R E Z S Fq Eto Km	16	cured
5	M	U	0	0	1	0	28	20.9	0	0	37	0	0	H R Z S Fq Eto Km	14	cured
6	M	U	1	0	1	0	46	17.3	1	47	110	0	1	H R Z S Eto Km	24	cured
7	F	U	1	0	0	0	18	19.1	0	0	44	1	0		6	cured
8	F	R	0	0	0	0	17	22.9	1	18	18	0	1		6	cured
9	M	R	0	0	0	0	21	22.8	0	0	33	0	1		6	cured
10	M	R	0	0	0	0	18	21.8	0	0	16	1	0		6	cured
11	M	R	1	0	1	0	29	24.3	0	0	37	0	1		6	cured
12	M	U	1	0	0	0	43	24.2	0	0	23	0	0		6	cured
13	F	U	1	0	0	0	44	21.5	0	0	14	1	1		6	cured
14	M	U	0	0	1	0	19	18.5	0	0	19	1	0		6	cured
15	M	U	1	0	1	0	22	15.3	0	0	32	0	0		6	cured
16	M	U	0	0	1	0	41	17.0	1	15	54	1	1		6	cured
17	F	R	1	0	0	0	37	18.4	1	8	15	1	0		6	cured
18	M	R	1	0	1	0	44	20.0	0	0	35	1	0		6	cured
19	M	R	0	0	1	0	54	20.8	0	0	63	1	0		6	cured
20	F	R	0	0	0	0	47	17.0	1	23	21	0	1		6	cured
21	F	U	0	0	0	0	19	22.1	0	0	98	1	0		6	cured
22	M	R	0	0	1	0	51	16.8	0	0	60	1	1		6	cured
23	F	R	0	HIV VHC	1	0	27	15.8	1	60	56	1	1		8	cured
24	M	U	0	0	1	0	48	22.7	0	0	95	0	0		6	cured
25	F	R	0	Chr. Pancreatitis	0	0	42	21.8	1	18	40	1	1		6	cured
26	M	R	1	0	1	0	48	22.6	0	0	49	1	0		6	cured
27	M	R	0	0	1	0	25	15.1	1	56	55	0	1		6	cured
28	M	U	0	0	0	0	25	21.5	0	0	48	0	0		6	cured
29	M	R	0	CHD	1	0	66	20.6	0	0	37	1	0		6	cured
30	F	R	0	Alcoholism	0	0	59	20.1	1	6	70	1	0		9	cured

Abbreviations: BMI, body mass index; tSSC, time to sputum smear conversion; tSCC, time to sputum culture conversion; F, female; M, male; 1, Yes/positive; 0, No/negative; U, urban; R, rural; CHD, chronic heart disease; VHC, virus hepatitis C; CXR, chest x-ray; S, streptomycin; H, isoniazid; R, rifampicin; E, ethambutol; Z, pyrazinamide; Am, amikacin; Fq, fluoroquinolone; Cm, capreomycin; Km, kanamycin; PAS, Para-aminosalicylic acid; Eto, ethionamide.

Table 3
Comparison of clinical and demographic characteristics between the study and control group patients.

	Control group*	Study group*		
Male, n (%)	20 (66.7)	20 (80)		
Female, n (%)	10 (33.3)	5 (20)		
Unemployed, n (%)	19 (63.3)	14 (56)	$\chi^2_{(1;55)} = 0.076$, p = 0.78	Cohen's W = 0.0075, 95 % CI [0.0055, 0.34]
One or more relevant comorbidities, n (%)	6 (20)	12 (48)	$\chi^2_{(1;55)} = 3.67$, p = 0.056	Cohen's W = 0.3, 95 % CI [0.0404, 0.55]
Smokers, n (%)	18 (60)	23 (92)	$\chi^2_{(1;55)} = 5.77$, p = 0.016	Cohen's W = 0.37, 95 % CI [0.14, 0.59]
Intravenous drug users, n (%)	0	3 (12)	p = 0.09	
Residents of rural area, n (%)	16 (53.3)	12 (48)	$\chi^2_{(1;55)} = 0.015$, p = 0.9	Cohen's W = 0.0053, 95 % CI [0.0067,0.34]
Residents of urban area, n (%)	14 (46.7)	13 (52)		
Age in years, mean ± SD	36.1 ± 13.43	41.24 ± 10.99	$t_{(53)} = -1.53$, p = 0.13	Cohen's D = −0.42, 95 % CI [−0.01, 0.11]
BMI, kg/m^2 mean ± SD	20.26 ± 2.66	19.71 ± 2.25	$t_{(53)} = 0.82$, p = 0.42	Cohen's D = 0.22, 95 % CI [−0.34, 0.81]
Positive sputum smear microscopy, n (%)	11 (36.67)	20 (80)	$\chi^2_{(1;55)} = 8.72$, p = 0.0031	Cohen's W = 0.44, 95 % CI [0.18, 0.67]
tSSC, days mean ± SD	35.91 ± 26.85	69.95 ± 46.93	$t_{(31)} = -2.2$, p = 0.036	
tSCC, days median (IQR 25–75)	42 (IQR 32–59)	65 (IQR 55–95)	$W_{(55)} = 198.5$, p = 0.0029	
Bilateral abnormality on CXR, n (%)	17 (56.67)	19 (76)	$\chi^2_{(1;55)} = 1.48$, p = 0.22	Cohen's W = 0.2, 95 % CI [0.015,0.44]
Presence of cavitation on CXR, n (%)	13 (43.33)	17 (68)	$\chi^2_{(1;55)} = 2.43$, p = 0.12	Cohen's W = 0.25, 95 % CI [0.023,0.49]

* The study group included patients with pulmonary TB diagnosed in 2002–2019 with assumed endogenous TB reactivation based on Mtb WGS results [16]; the control group included patients diagnosed with pulmonary TB in 2002–2019 who were not diagnosed with recurrent TB episodes and were still alive up to July 2024.
Abbreviations: tSSC, time to sputum smear conversion; tSCC, time to sputum culture conversion; BMI, body mass index; CXR, chest x-ray.

test was performed. Bayes factor, effect size with 95 % confidence interval (CI) and test power were reported, and, in case of statistically significant differences between groups, a false positive risk (FPR) with the p-equals method was also calculated using public domain https://fpr-calc.ucl.ac.uk. If quantitative data were not normally distributed, median and interquartile range (IQR) were calculated, and the Mann–Whitney–Wilcoxon test was performed for data analysis. The chi-square test of independence and Fisher's exact test were performed on various category data. Bayes factor upper bound, effect size with a 95 % CI, and test power were calculated and reported for all performed non-parametric tests, when possible.

3. Results

In total, 55 patients with pulmonary TB were involved in this study: 25 patients experienced recurrent TB episodes presumably due to endogenous reactivation of TB infection (the study group), as determined previously [16], and 30 patients were diagnosed with active TB only once (the control group).

Altogether 36 Mtb isolate pairs were included in the recurrent TB group in the study by Sadovska *et al.* Among them, the pairwise distance of ≤ 10 SNVs was detected in 30 isolate pairs. Complete medical records of both TB episodes were not available for five of these patients. Consequently, 25 patients with assumed endogenous reactivation of TB were included in the study group for further analysis (Table 1). Demographic and clinical data for both TB episodes in the study group patients are reflected in Table 1, but for control group patients – in Table 2. Descriptive statistics data for both groups are reflected in Table 3.

In the study group, the median time between the first and second TB episodes was 55 months (range 14–156 months; IQR 26–103), while the time lag for 16 % (4/25) patients was 10 years and more. To assess the potential reasons for TB reactivation, frequency of positive sputum smear microscopy (SSM) for acid-fast bacteria (AFB), tSSC, tSCC, disease extensity, treatment duration, age, BMI, place of residency, comorbidities, employment status, and smoking was compared between the primary TB episode in study group and the control group.

3.1. Comparison of the frequency of positive SSM results in the study and control group patients (Tables 1–3)

In the study group, during the initial TB episode, SSM for AFB was positive in 80 % (20/25) of patients, compared to 11 patients (36.67 %) in the control group. The association between the patient groups and SSM results was statistically significant ($\chi^2_{(1;\ 55)} = 8.72$, p = 0.0031). Positive SSM results were distributed with greater observed frequency than expected (standardised residuals > 3) among study group patients and negative SSM results were more common for TB patients in the control group (standardised residuals > 3). Cohen's W value indicated a moderately strong association between these categories (0.44, 95 % CI [0.18, 0.67]). Observations provided no more than 20.32 times bigger support for the existing association between SSM results and patient group (H_A) than its absence (H_0). The test power of 83.16 % highlighted a low probability of not detecting statistically significant differences at this effect size.

3.2. Comparison of the tSSC in the study and control group patients (Tables 1–3)

Comparing tSSC between the patient groups revealed slight data asymmetry and no extreme outliers were observed, and normal data distribution could not be rejected in both the control group ($W_{(11)} = 0.89$, p = 0.16) and the recurrent TB patient group ($W_{(20)} = 0.92$, p = 0.12). The performed F test demonstrated that the variances of the two patient groups were equal ($F_{(2;29)} = 0.33$, 95 % CI [0.12, 1.12], p = 0.074). The mean tSSC was 35.91 ± 26.85 days for patients in the control group and 69.95 ± 46.93 days for recurrent TB patients; the tSSC period was statistically significantly longer among the recurrent TB patients ($t_{(31)} = -2.2$, p = 0.036). Comparing the mean values, the tSSC period was 34.04 days (95 % CI [2.46, 65.62]) longer for the recurrent TB patients during their initial TB episode than for the patients diagnosed with TB once. The observed effect size was large as the mean difference was equal to 0.806 standard deviations (95 % CI [0.26, 1.51]), while the calculated FPR was only 0.17. However, observations provided only 2.02 times greater support for the existing differences in tSSC periods between studied patient groups (H_A) than the presumption that there are none (H_0), and the power of the performed test was only 56.84 %, which highlighted the necessity of the bigger patient cohorts for such analysis.

3.3. Comparison of tSCC in the study and control group patients (Tables 1–3)

Regarding tSCC, slight data asymmetry and multiple outlier values

were present in both groups. Normal data distribution could be rejected in both the control group ($W_{(30)} = 0.92$, $p = 0.035$) and the recurrent TB patient group ($W_{(25)} = 0.86$, $p = 0.0027$). The median tSCC period was 42 days (IQR 32–59) in the control group and 65 days (IQR 55–95) in the recurrent TB patient group. The difference in tSCC periods between the two studied groups was statistically significant ($W_{(55)} = 198.5$, $p = 0.0029$). A moderate positive effect (0.4, 95 % CI [0.16–0.6]) was observed, suggesting that the tSCC period tended to be longer in the recurrent TB patient group. Observations provided no more than 21.64 times greater support for the H_A.

3.4. Comparison of the extensity of the disease in the study and control group patients (Tables 1–4)

No statistically significant association was found between the patient group and bilateral lung abnormalities ($\chi^2_{(1;\ 55)} = 1.48$, $p = 0.22$) and cavitation at the CXR ($\chi^2_{(1;\ 55)} = 2.43$, $p = 0.12$). Cohen's W values indicated a weak association between variables (0.2, 95 % CI [0.015, 0.44] and 0.25, 95 % CI [0.023, 0.49], respectively). Observations provided only 1.1 and 1.45 times bigger support for H_A. The power of the performed tests was only 29.78 % and 35.46 %, respectively, which highlighted a high probability of not detecting statistically significant differences at the existing effect sizes.

3.5. Drug susceptibility patterns of Mtb isolates in the study group patients (Table 1)

In the study group, according to phenotypic DS tests, 56 % (14/25) of patients had DS-TB in both episodes. Simultaneous resistance to H and S was revealed in 24 % (6/25) patients at the first TB episode. Retrospective WGS analysis revealed additional mutations related to Eto and PAS resistance in these HS-resistant Mtb isolates; also, in one phenotypically rifampicin-sensitive isolate, Asp435Tyr in the *rpoB* gene was detected (Table 3). Indeed, according to the literature data, this variant causes low to moderate-level RIF resistance and shows variable minimal inhibitory concentrations (MICs) on different media [16]. Out of the remaining five study group patients, four had MDR-TB and one XDR-TB; retrospective WGS data confirmed this observation.

A retrospective WGS analysis showed that, excepting for two cases, the drug resistance profile of Mtb isolates during both episodes of TB was the same. In two cases of endogenous TB reactivation, resistance to FQ was detected in isolates that were still sensitive to FQ when tested using phenotypic methods.

3.6. Comparison of treatment duration in patient subgroups

3.6.1. Treatment duration in patients with DS-TB (Table 1, 2, 4)

Mtb isolates of 24 patients (80 %) in the control group and 14 patients (56 %) in the study group were drug-susceptible. In both patient groups, the asymmetric distribution of treatment duration was observed, and multiple outliers were present. Normal distribution could be rejected in both the control group ($W_{(24)} = 0.32$, $p < 0.001$) and the recurrent TB patient group ($W_{(14)} = 0.64$, $p < 0.001$). The median treatment duration for drug-susceptible TB was 6 months in both the control group (IQR 6–6) and among recurrent TB patients (IQR 6–7.75). The difference in treatment duration for drug-susceptible TB between the two studied groups was statistically significant ($W_{(38)} = 120.5$, $p = 0.035$). A moderate positive effect (0.35, 95 % CI [0.04–0.62]) was observed, suggesting that the treatment duration tended to be longer in the recurrent TB patient group. However, observations provided no more than only 3.11 times greater support for the H_A.

3.6.2. Treatment duration in study group patients with HS-resistant TB (Table 1, 4)

In the study group, for individuals with HS-resistant TB, the mean duration of treatment was 9.33 months (IQR 8–10). HS-resistant TB patients were not represented in the control group.

3.6.3. Treatment duration in patients with MDR-TB and XDR-TB (Table 1, 2, 4 and 5)

Mtb isolates of 5 study group patients (20 %) during first TB episode and 6 patients (20 %) in the control group were MDR-TB and XDR-TB. The median treatment duration for drug-resistant TB was 20 months (IQR 17–23) in the control group and 19 months (IQR 16–19) in the recurrent TB patient group. However, due to the very low patient number in this selection, no additional statistical tests were performed.

3.7. Comparison of age, BMI, place of residency, employment status, comorbidities, and smoking (Tables 1–3)

There were no statistically significant differences between studied patient groups in either patients' age ($t_{(53)} = -1.53$, 95 % CI [−11.87, 1.59], $p = 0.13$) or BMI ($t_{(53)} = 0.82$, 95 % CI [−0.8, 1.9], $p = 0.42$), or area of residency, ($\chi^2_{(1;\ 55)} = 0.015$, $p = 0.9$), or employment status ($\chi^2_{(1;\ 55)} = 0.076$, $p = 0.78$), and presence of comorbidities as well ($\chi^2_{(1;\ 55)} = 3.67$, $p = 0.056$). Nevertheless, the association between the patient group and smoking status was statistically significant ($\chi^2_{(1;\ 55)} = 5.77$, $p = 0.016$), and smokers were present among the recurrent TB-experienced patients with greater frequency than expected (standardised residuals > 2).

Table 4
Duration of treatment of study group patients at first TB episode depending on the extensity of the disease and drug susceptibility/resistance patterns (N = 25).

Radiological extensity of disease at baseline	DS-TB			HS-resistant TB			MDR-TB and XDR-TB		
	Patients, n (%)	Days of tSCC, median (range)	Months of treatment, mean (range)	Patients, n (%)	Days of tSCC, median (range)	Months of treatment, mean (range)	Patients, n (%)	Days of tSCC, median (range)	Months of treatment, mean (range)
Total	14 (56 %)	58 (27–196)	7.43 (6–12)	6 (24 %)	95 (33–127)	9.33 (8–12)	5 (20 %)	75 (42–185)	19 (13–21)
Bilateral abnormality on CXR	11 (44 %)	61 (28–90)	7.27 (6–12)	5 (20 %)	95 (33–127)	9.2 (8–12)	3 (12 %)	61 (42–114)	19 (19–21)
Bilateral abnormality on CXR + cavitary disease at baseline	8 (32 %)	61.5 (28–90)	7.0 (6–12)	3 (12 %)	95 (33–127)	8	3 (12 %)	61 (42–114)	19 (19–21)
Bilateral abnormality on CXR + cavitary disease at baseline + tSCC > 60 days	5 (20 %)	65 (61–90)	6.4 (6–8)	2 (8 %)	111 (95–127)	8	2 (8 %)	87.5 (61–114)	20 (19–21)

Abbreviations: TB, tuberculosis; CXR, chest x-ray; tSCC, time to sputum culture conversion; DS-TB, drug-susceptible TB; HS-resistant TB, isoniazid and streptomycin-resistant TB; MDR-TB and XDR-TB, multidrug-resistant and extensively drug-resistant TB.

Table 5
The treatment regimen for drug-resistant TB patients and HIV-coinfected patients in study group and in control group patients.

Patient ID	HIV coinfection	Antiretroviral treatment	Duration of TB treatment at 1st episode, months	Mtb resistance pattern at 1st TB episode (WGS)	Treatment regimen at 1st TB episode	Mtb resistance pattern at 2nd TB episode (WGS)
Study group						
P5SIT1117.1	0	0	8	H R[§] S	1 HREZ/2KmREZ/ 5 REZ	H R[§]S
P23SIT1.8*	0	0	8	H S Eto PAS	2 REZCmOfxTzdPto/ 6 REZOfxKm/ 2 REZOfx	H S Eto PAS
P31SIT53.6	1	1	10	H S Eto	1 HREZ/ 9 REZ	H S Eto
P32SIT53.7	1	1	10	H S Eto	1 HRESm/3 REKmOfxZ/ 3 REZHKm/1 REZKm/ 2 REZ	H S Eto
P33SIT53.8*	1	1	12	H S Eto	1 REZTzd/3 REZTzdOfx/8 REZOfx	H S Eto
P39SIT1.15	0	0	8	H S Eto PAS	1 HREZKmOfx/ 2 REZOfxKm/ 2 REZOfx /3 REOfx	H S Eto PAS
P41SIT1.16	0	0	16	H R E Z Fq S Km Eto	1 MfxPASPtoTzdCm/ 5 MfxPtoTzdCmAmx/ Clv/ 10 TzdPtoMfxAmx/Clv	H R E Z Fq S Km Eto
P22SIT42.4	0	0	19	H R E Z S Eto	11 KmOfxPtoTzdPAS/ 8 OfxPtoPASTzd	H R E Z S Eto
P26SIT1.11	0	0	21	H R E Z S Am Cm Km Eto	1 CmZOfxCsHRPtoPAS/ 10 CmOfxCsPtoPAS/ 4 OfxCsPtoPAS/ 2 OfxPtoPASHR/ 4 OfxPtoPASCs	H R E Z Fq[#] S Am Cm Km Eto
P25SIT1.10	0	0	19	H R E Z S Am Cm Km Eto	1 HREZ/ 1 CmZOfxCsHRPtoPAS/ 9 CmOfxCsPtoPAS/ 4 OfxCsPtoPAS/ 2 OfxPtoPASHR/ 2 OfxPtoPASCs	H R E Z Fq[#] S Am Cm Km Eto
P16SIT1.6	0	0	13	H R E Z S Km Eto PAS	2 HREZ/ 1 KmEZOfxPtoTzd/ 3 AmEZOfxPtoTzd/ 6 EZOfxPtoTzd/ 3 EOfxEtoTzd	H R E Z S Km Eto PAS
Control group						
1	0	0	20	R S Am H Z E Eto	2 CmLfxPtoTrzEZ/ 5 CmPtoTrzZMfxAmx/Clv/ 6 LfxProTrzBdqLzd/ 7 TrzLfxLzdAmx/Clv	
2	0	0	20	R S H PAS E Eto	1 CmEMfxZTrzPto/ 2 MfxZTrzPto/ 3 MfxZTrzPtoLzdAmx/Clv/ 10 MfxZTrzPtoLzd/ 4 MfxZTrzPto	
3	1	1	24	R S Am H Z PAS E Eto	2 ECmZMfxPtoTrz/ 5 CmMfxPtoTrzAmx/ ClvPAS/ 8 MfxPtoTrzAmx/ClvPAS/ 9 CmMfxPtoTrzAmx/Clv	
4	1	1	16	Fq R S H Eto Z Km E	3 KmMfxTzdPtoZAmx/ClvPAS/ 3 KmTzdPtoZAmx/ClvPASELfx/ 6 AmTzdPtoEBdq/ 4 AmTzdPtoE	
5	0	0	14	Fq R S H Eto Z Km	2 EZPtoTrzCmLfx/ 4 ZPtoTrzCmLfxBdq / 2 ZPtoTrzLfxBdq/ 3 PtoTrzLfxBdqAmx/Clv/ 3 PASTrzLfxBdq	
6	0	0	19	R S H Eto Z Km	1 CmZELfxTrzPto/ 1 ZLfxTrzPtoPASAm/ 4 ZLfxPtoPASAmLzd/ 2 LfxPtoPASAmLzdAmx/ Clv/ 11 LfxPtoPASLzdAmx/Clv	

* Cases of possible reinfection based on WGS and epidemiological data [16].
§ At the time of diagnosis (year 2004), WGS data were not available. Isolate was phenotypically rifampicin-sensitive, although it harboured Asp435Tyr in the *rpoB* gene. This variant causes low to moderate-level R resistance and shows variable minimal inhibitory concentrations (MICs) on different media [16].
Abbreviations: TB, tuberculosis; HS-resistant TB, isoniazid and streptomycin-resistant TB; MDR-TB, multidrug-resistant TB; XDR-TB, extensively drug-resistant TB; HIV, human immunodeficient virus; Mtb, *Mycobacterium tuberculosis*; WGS, whole genome sequencing; 1, Yes; 0, No; S, streptomycin; H, isoniazid; R, rifampicin; E, ethambutol; Z, pyrazinamide; Ofx, ofloxacin; Mfx, moxifloxacin; Lfx, levofloxacin; Fq, fluoroquinolone; Am, amikacin; Cm, capreomycin; Km, kanamycin; PAS, Para-aminosalicylic acid; Tzd, terizidone; Pto, protionamide; Amx/Clv, amoxicillin/clavulanic acid; Cs, cycloserin. Numbers before drug abbreviation indicate treatment duration in months.
[#] Cases of resistance development.

4. Discussion

Recurrent TB continues to burden TB control worldwide. The reported proportion of recurrence rate varies by country. In Finland, a country with a low TB incidence, only 0.6 % of TB recurrence has been reported [28]. A recurrence proportion of 1.54 % and 1.5 % were identified in Taiwan [3] and Mainland China [29], respectively. In Uganda, a high TB incidence country, 10 % of previously treated patients experienced repeated TB episodes [8]. In Latvia, a low-moderate TB incidence country, a relatively high recurrence rate with bacteriologically confirmed and clinically diagnosed TB was recorded during the study period ranging from 15.3 % in 2005 and 10.9 % in 2017 [22]. Since the year 1996, treatment of drug-sensitive and drug-resistant TB has been provided in line with WHO guidelines in Latvia, including comprehensive, directly observed therapy. More than 85 % of pulmonary TB patients prescribed on treatment had bacteriologically confirmed TB. Successful treatment outcome of primary TB episodes for patients with DS-TB was 71.2 % in 2005 and 87 % in 2015. For patients with MDR-TB and XDR-TB, successful treatment outcome was reached in 70 % of patients in 2005 and 76 % of patients in 2015.

Conventional genotyping methods and WGS of Mtb isolates can help to identify the cause of TB recurrence [16–18]. Episodic WGS of Mtb isolates in Latvia was started in 2020 [30]. The study conducted by Sadovska *et al.* in 2023 so far is the largest local research, including recurrent TB cases when the first and second episodes were caused by Mtb strains with an identical spoligotype pattern from both TB episodes from patients diagnosed with pulmonary TB in 2002–2019. Among 36 studied recurrent TB patients, assumed endogenous reactivation of previous infection was the most common reason for TB recurrence [16]. Other studies that used genotyping of paired Mtb isolates to clarify the reasons for recurrence demonstrated that endogenous reactivation prevailed in some of them [8,31] and reinfection dominated in others [4].

In the present study, we analysed individual patient clinical data for recurrent TB cases assumed as reactivation based on a small genetic distance (≤100 SNV) between Mtb isolates of both TB episodes [16]. Most of the patients with reactivation had advanced disease with bilateral pulmonary involvement (76 %), cavitation (68 %), and sputum smear positivity for AFB (80 %) at the first TB episode (Table 4). Positive

SSM was observed with statistically significantly greater frequency among study group patients. Sputum smear conversion and sputum culture conversion also was statistically significantly longer among recurrent TB patients. Sputum culture conversion for more than 60 days was experienced in 50 % of patients with DS-TB and 82 % of patients with drug resistance (Tables 1–4), being statistically significantly longer for patients with drug resistance. These findings are in line with other studies suggesting that both cavitation and positive culture after two months of therapy increase the risk of endogenous TB reactivation [2,32].

All study group patients with DS-TB at the first episode received at least 6 months and even longer directly observed therapy of first-line TB medicine and were categorised as cured at therapy completion (Table 1). The treatment duration for DS-TB was even statistically significantly longer compared to control group (Tables 2, 3). No patient with DS-TB at the first episode had any drug resistance at reactivation. Based on available data, in the subgroup of patients with DS-TB, we could not identify any other reasons for reactivation except those that are already well described: extensive disease, delayed sputum culture conversion, and smoking [2,11]. The association between the patient group and smoking status was statistically significant. However, other important factors that could influence reactivation were not available for analysis, e.g. Mtb strain virulence, host genetic variability and susceptibility to TB, drug concentration in different pathological tissue, size of Mtb population in TB lesions, *etc*. Although studies show that these factors increase TB recurrence rate [33,34], data are limited regarding TB endogenous reactivation [12].

Six study group patients had phenotypic resistance to H and S at the first TB episode (Table 1). Literature data suggest that isoniazid-resistant TB has a higher potential for relapse, particularly in the absence of a treatment regimen, including FQ [10]. This medication was not included in the treatment regimen of two patients with HS-resistant TB. However, they received an extended treatment regimen that included pyrazinamide throughout all courses of treatment. In four patients, FQ was included in the treatment regimen (Table 5). Three patients with H and S resistance were HIV-coinfected. Some studies have shown that reinfection of TB among HIV patients is more frequent than reactivation [10,12,35].

Five study group patients with MDR-TB and XDR-TB received treatment designed according to WHO recommendations that were current at the time of treatment, but in contrast to DS-TB and HS-resistant TB patients, they did not complete all prescribed treatment regimens (Table 5). The median treatment duration for study group patients with MDR-TB and XDR-TB was 19 months (IQR 16–19), and they were classified as cured after the first TB episode, probably because culture conversion was achieved. The median treatment duration in the control group was 20 months (IQR 17–23) (Table 1, 4, 5). Several studies reported that patients with MDR-TB are at increased risk of TB reactivation [10,11,36] because of long-term treatment and side effects of medication. In our study, incomplete therapy in patients with MDR-TB and XDR-TB should be considered as a potential provoking factor for TB endogenous reactivation.

The time interval between the first and recurrent TB episodes varies by studies. Some of them had found no significant time difference between the occurrence of reinfection or reactivation after the first TB episode [13,31]. In contrast, others demonstrate that reactivation tends to occur mainly within the first two years after previously treated TB [2,10,12] and the recurrence rate remains high after 2–5 years for patients who completed therapy as well [11]. Our study found that 80 % (16/20) of patients with DS-TB and HS- resistant TB reactivated more than 24 months after treatment completion of the first TB episode, including 45 % (9/20) patients who reactivated five years after treatment completion (Table 1). WGS can distinguish between endogenous reactivation and reinfection caused by the same genotype of Mtb based on the SNV distance; however, this task is much more complicated if the patient is reinfected with the same or closely related Mtb strain [16]. We could not obtain information about the socioeconomic characteristics of patients' lifestyle after completion of therapy for the initial TB episode. However, one can speculate that some proportion of late assumed reactivation was not true reactivation but could be due to reinfection with the same strain of Mtb because patients continue to live in the same environment and keep the same habits and contacts [37]. Thus, pairwise SNV distance analysis needs to be complemented with the delineation of transmission roots within the community and epidemiological data of involved patients. Studies on host demographic and clinical characteristics predisposing to reinfection and knowledge about mycobacterial strain virulence could improve our understanding of reinfection [12,35].

Conditions that affect TB recurrence are complex and diverse. First, a clinical cure does not always indicate a bacteriological cure. In general, a cure is interpreted as "free of disease" after treatment, while its bacteriological base is not strictly defined. Multiple negative cultures at the end of therapy are declared as treatment success, and the patient has been considered as cured. However, in some cases, Mtb replication remains below the detection threshold using smear microscopy and even culture [37]. Especially MDR-TB cases may not be genuinely cured and surviving Mtb replicate and grow to induce disease again [36].

Second, the other potential contributor to recurrence is the size of the bacterial population in TB lesions. The bacillary load in the tuberculous cavity is vast and can reach 10^{11} colony-forming units per gram [38]. The presence and extent of cavitary disease are correlates of poor clinical outcomes and relapse [39]. More than half of our study group patients had cavitary disease visualised on CXR. Considering the low sensitivity of CXR, the number of patients with cavitation is probably higher. Third, the Mtb population consists of distinct bacterial subpopulations, and each of them may be differentially killed by various antimicrobial drugs dependent on phenotypic drug tolerance or anatomical location. In hypoxic microenvironments, persistent mycobacteria have reduced the action of most antituberculosis drugs [38]. Furthermore, antituberculosis drug penetration is not uniform in all types of TB lesions and reaches various concentrations in different pathological tissues. Besides, medicine concentrations in blood can differ from those in TB lesions [40].

Our study had several limitations. The first is a small number of patients for statistical analysis due to the availability of Mtb isolates and WGS data: unfortunately, WGS of Mtb isolates was started in Latvia only recently, and thus it was not carried out as a routine method for the TB patients at the time of diagnosis. Second, we conducted a retrospective study on patients with assumed TB reactivation. Additionally, assumed endogenous reactivation cases analysed in the study represent only a small fraction of all recurrent TB cases in Latvia and probably are not generalisable.

In summary, the study highlighted the complexity of precise cause determination for endogenous reactivation of primary TB disease. Study group patients with DS-TB and HS-resistant TB received treatment regimens according to WHO recommendations which were relevant at that time and were counted as cured. Positive SSM for AFB, longer tSSC and tSCC, and smoking should be considered as potential risk factors for reactivation in these subgroups of patients. Patients with MDR-TB and XDR-TB did not complete prescribed treatment courses, and this potentially could influence on reactivation. Because the common future for all study group patients was an advanced disease, the possibility that clinical cure did not coincide with bacteriological cure and reactivation was caused by multiplication of persistent Mtb should be considered. In some proportion of patients with assumed reactivation based on WGS results, recurrent TB episodes could be caused by reinfection with the same strain of mycobacteria, which caused primary disease.

WGS alone cannot clearly identify the reasons for recurrent TB. A complex approach should be used, including host genetic variability and susceptibility to TB, extensity of the disease, drug concentration in different pathological tissues, Mtb strain virulence, epidemiological links among involved patients and factors predisposing to reinfection.

5. Funding information

This study was funded by State Research program "Public Health", project "New knowledges and approaches to reduce antimicrobial resistance, limit the spread of HIV and expand community vaccination coverage". Nr, VPP-VM-Sabiedribas_Veselība-2023/5-0001.

6. Availability of data and materials

All data generated or analysed during this study are included in this published article.

7. Ethics approval and consent to participate

The study was reviewed and approved by the Ethics Committee of Riga Stradins University Research Ethics Committee (Nr6-1/06/12;28.05.2020); Center for Disease Prevention and Control of Latvia (Nr.6.1-1/2024/2) and the Science Department of Riga East University. Hospital (Nr.AP/08-08/24/60). Consent to participate: Not applicable.

CRediT authorship contribution statement

Anda Viksna: Investigation. **Darja Sadovska:** Investigation, Data curation, Conceptualization. **Vija Riekstina:** Investigation. **Anda Nodieva:** Investigation. **Ilva Pole:** Investigation. **Renate Ranka:** Writing – review & editing, Visualization, Supervision, Conceptualization. **Iveta Ozere:** Writing – review & editing, Writing – original draft, Supervision, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] Global Tuberculosis Report 2023. Geneva: World Health Organization 2023.
[2] Romanowski K, Balshaw RF, Benedetti A, Campbell JR, Menzies D, Ahmad Khan F, et al. Predicting tuberculosis relapse in patients treated with the standard 6-month regimen: an individual patient data meta-analysis. Thorax 2019;74(3):291–7. https://doi.org/10.1136/thoraxjnl-2017-211120.
[3] Lee C-S, Ho C-H, Liao K-M, Wu Y-C, Shu C-C. The incidence of tuberculosis recurrence: Impacts of treatment duration of and adherence to standard anti-tuberculous therapy. J Infect Public Health 2023;16(11):1778–83. https://doi.org/10.1016/j.jiph.2023.09.005.
[4] Afshar B, Carless J, Roche A, Balasegaram S, Anderson C. Surveillance of tuberculosis (TB) cases attributable to relapse or reinfection in London, 2002–2015. PLoS One 2019;14(2):e0211972.
[5] Cohen DB, Davies G, Malwafu W, Mangochi H, Joekes E, Greenwood S, et al. Poor outcomes in recurrent tuberculosis: More than just drug resistance? PLoS One 2019;14(5):e0215855.
[6] Gadoev J, Asadov D, Harries AD, Parpieva N, Tayler-Smith K, Isaakidis P, et al. Recurrent tuberculosis and associated factors: a five - year countrywide study in Uzbekistan. PLoS One 2017;12(5):e0176473.
[7] Global Tuberculosis Report 2020. Geneva: World Health Organization 2020.
[8] Luzze H, Johnson DF, Dickman K, Mayanja-Kizza H, Okwera A, Eisenach K, et al. Relapse more common than reinfection in recurrent tuberculosis 1–2 years post treatment in urban Uganda. Int J Tuberc Lung Dis 2013;17(3):361–7. https://doi.org/10.5588/ijtld.11.0692.
[9] Marx FM, Dunbar R, Enarson DA, Williams BG, Warren RM, Van Der Spuy GD, et al. The temporal dynamics of relapse and reinfection tuberculosis after successful treatment: a retrospective cohort study. Clin Infect Dis 2014;58(12):1676–83. https://doi.org/10.1093/cid/ciu186.
[10] Guerra-Assunção JA, Houben RMGJ, Crampin AC, Mzembe T, Mallard K, Coll F, et al. Recurrence due to Relapse or Reinfection WithMycobacterium tuberculosis: A Whole-Genome Sequencing Approach in a Large, Population-Based Cohort With a High HIV Infection Prevalence and Active Follow-up. J Infect Dis 2015;211(7):1154–63. https://doi.org/10.1093/infdis/jiu574.
[11] Erkens C, Tekeli B, Van Soolingen D, Schimmel H, Verver S. Recurrent tuberculosis in the Netherlands – a 24-year follow-up study. Eurosurveillance 2022;27(12). https://doi.org/10.2807/1560-7917.es.2022.27.12.2100183. 1993 to 2016.
[12] Qiu B, Wu Z, Tao B, Li Z, Song H, Tian D, et al. Risk factors for types of recurrent tuberculosis (reactivation versus reinfection): A global systematic review and meta-analysis. Int J Infect Dis 2022;116:14–20. https://doi.org/10.1016/j.ijid.2021.12.344.
[13] Shao Y, Song H, Li G, Li Y, Li Y, Zhu L, et al. Relapse or Re-Infection, the Situation of Recurrent Tuberculosis in Eastern China. Front Cell Infect Microbiol 2021;11:638990. https://doi.org/10.3389/fcimb.2021.638990.
[14] Weiangkham D, Umnuaypornlert A, Saokaew S, Prommongkol S, Ponmark J. Effect of alcohol consumption on relapse outcomes among tuberculosis patients: A systematic review and meta-analysis. Front Public Health 2022;10:962809. https://doi.org/10.3389/fpubh.2022.962809.
[15] Córdoba C, Buriticá PA, Pacheco R, Mancilla A, Valderrama-Aguirre A, Bergonzoli G. Risk factors associated with pulmonary tuberculosis relapses in Cali, Colombia. Biomédica 2020;40(Supl 1):102–12. https://doi.org/10.7705/biomedica.5061.
[16] Sadovska D, Nodieva A, Pole I, Ķimsis J, Vīksna A, Ozere I, et al. Advantages of analysing both pairwise SNV-distance and differing SNVs between Mycobacterium tuberculosis isolates for recurrent tuberculosis cause determination. Microb Genom 2023;9(3). https://doi.org/10.1099/mgen.0.000956.
[17] Shanmugam S, Bachmann NL, Martinez E, Menon R, Narendran G, Narayanan S, et al. Whole genome sequencing based differentiation between re-infection and relapse in Indian patients with tuberculosis recurrence, with and without HIV co-infection. Int J Infect Dis 2021;113(Suppl 1):S43–7. https://doi.org/10.1016/j.ijid.2021.03.020.
[18] Parvaresh L, Crighton T, Martinez E, Bustamante A, Chen S, Sintchenko V. Recurrence of tuberculosis in a low-incidence setting: a retrospective cross-sectional study augmented by whole genome sequencing. BMC Infect Dis 2018;18(1). https://doi.org/10.1186/s12879-018-3164-z.
[19] Uys P, Brand H, Warren R, Van Der Spuy G, Hoal EG, Van Helden PD. The Risk of Tuberculosis Reinfection Soon after Cure of a First Disease Episode Is Extremely High in a Hyperendemic Community. PLoS One 2015;10(12):e0144487.
[20] Du J, Li Q, Liu M, Wang Y, Xue Z, Huo F, et al. Distinguishing Relapse From Reinfection With Whole-Genome Sequencing in Recurrent Pulmonary Tuberculosis: A Retrospective Cohort Study in Beijing, China. Front Microbiol 2021;12. https://doi.org/10.3389/fmicb.2021.754352.
[21] Nodieva A, Skenders G, et al. Who are at risk for tuberculosis recurrence? Eur Respir J 2011;38(Suppl 55):p4377.
[22] SPKC [Internet]. Available from: https://www.spkc.gov.lv/lv/tuberkuloze-1.
[23] World Health Organization Operational handbook on tuberculosis. Module 4: Treatment. Drug resistant tuberculosis treatment. 2022. update.; Geneva: World Health Organization 2022.
[24] Van Soolingen D, Hermans PW, De Haas PE, Soll DR, Van Embden JD. Occurrence and stability of insertion sequences in Mycobacterium tuberculosis complex strains: evaluation of an insertion sequence-dependent DNA polymorphism as a tool in the epidemiology of tuberculosis. J Clin Microbiol 1991;29(11):2578–86. https://doi.org/10.1128/jcm.29.11.2578-2586.1991.
[25] Bryant JM, Harris SR, Parkhill J, Dawson R, Diacon AH, van Helden P, et al. Whole-genome sequencing to establish relapse or re-infection with Mycobacterium tuberculosis: a retrospective observational study. Lancet Respir Med 2013;1(10):786–92. https://doi.org/10.1016/s2213-2600(13)70231-5.
[26] World Health Organization consolidated guidelines in tuberculosis. Module 4: Treatment Drug-resistant tuberculosis treatment. Geneva: World Health Organization; 2020.
[27] Companion handbook to the WHO guidelines for the programmatic management of drug-resistant tuberculosis; Geneva: World Health Organization 2014.
[28] Korhonen V, Soini H, Vasankari T, Ollgren J, Smit PW, Ruutu P. Recurrent tuberculosis in Finland 1995–2013: a clinical and epidemiological cohort study. BMC Infect Dis 2017;17(1). https://doi.org/10.1186/s12879-017-2818-6.
[29] Jiang H, Yin J, Liu F, Yao Y, Cai C, Xu J, et al. Epidemiology of recurrent pulmonary tuberculosis by bacteriological features of 100 million residents in China. BMC Infect Dis 2022;22(1). https://doi.org/10.1186/s12879-022-07622-w.
[30] Aleinikova D, Pole I, Kimsis J, Skangale A, Bobrikova O, Kazelnika R, et al. Application of whole-genome sequencing in a case study of renal tuberculosis in a child. BMC Infect Dis 2020;20(1). https://doi.org/10.1186/s12879-020-4832-3.
[31] He W, Tan Y, Song Z, Liu B, Wang Y, He P, et al. Endogenous relapse and exogenous reinfection in recurrent pulmonary tuberculosis: a retrospective study revealed by whole genome sequencing. Front Microbiol 2023;14. https://doi.org/10.3389/fmicb.2023.1115295.
[32] Chen M-Y, Lo Y-C, Chen W-C, Wang K-F, Chan P-C. Recurrence after Successful Treatment of Multidrug-Resistant Tuberculosis in Taiwan. PLoS One 2017;12(1):e0170980.
[33] Mirsaeidi M, Sadikot RT. Patients at high risk of tuberculosis recurrence. Int J Mycobacteriol 2018;7(1). https://doi.org/10.4103/ijmy.ijmy_164_17.
[34] van Coller A, Glanzmann B, Cornelissen H, Möller M, Kinnear C, Esser M, et al. Phenotypic and immune functional profiling of patients with suspected Mendelian Susceptibility to Mycobacterial Disease in South Africa. BMC Immunol 2021;22(1):62. https://doi.org/10.1186/s12865-021-00452-6.
[35] Sonnenberg P, Murray J, Glynn JR, Shearer S, Kambashi B, Godfrey-Faussett P. HIV-1 and recurrence, relapse, and reinfection of tuberculosis after cure: a cohort study in South African mineworkers. Lancet 2001;358(9294):1687–93. https://doi.org/10.1016/S0140-6736(01)06712-5.

[36] Shen X, Yang C, Wu J, Lin S, Gao X, Wu Z, et al. Recurrent tuberculosis in an urban area in China: Relapse or exogenous reinfection? Tuberculosis 2017;103:97–104. https://doi.org/10.1016/j.tube.2017.01.007.

[37] Pérez-Lago L, Monteserin J, Paul R, Maus SR, Yokobori N, Herranz M, et al. Recurrences of multidrug-resistant tuberculosis: strains involved, within-host diversity, and fine-tuned allocation of reinfections. Transbound Emerg Dis 2022;69 (2):327–36. https://doi.org/10.1111/tbed.13982.

[38] Evangelopoulos D, Fonseca JDD, Waddell SJ. Understanding anti-tuberculosis drug efficacy: rethinking bacterial populations and how we model them. Int J Infect Dis 2015;32:76–80. https://doi.org/10.1016/j.ijid.2014.11.028.

[39] Urbanowski ME, Ordonez AA, Ruiz-Bedoya CA, Jain SK, Bishai WR. Cavitary tuberculosis: the gateway of disease transmission. Lancet Infect Dis 2020;20(6): e117–28. https://doi.org/10.1016/S1473-3099(20)30148-1.

[40] Strydom N, Gupta SV, Fox WS, Via LE, Bang H, Lee M, et al. Tuberculosis drugs' distribution and emergence of resistance in patient's lung lesions: a mechanistic model and tool for regimen and dose optimization. PLoS Med 2019;16(4): e1002773.