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**Estimation of annual risk of *Mycobacterium tuberculosis* infection accounting for tuberculin skin test reversion:
A mathematical modelling approach**

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ABSTRACT:

Background: Over one quarter of the global population is estimated to be infected with *Mycobacterium tuberculosis*. One of the key metrics is the Annual Risk of Infection (ARI), derived from *M. tuberculosis* prevalence data from surveys using the immunoreactive Tuberculin Skin Test (TST). However, the ARI is generally estimated using the dual assumptions of lifelong viable infection as well as persistent immunoreactivity, both of which have been challenged. The study will explore the implications of TST-reversion on ARI estimates. **Methods:** This mathematical modelling study is building upon an existing theoretical exercise by Ian Sutherland, exploring the effect of the varying levels of TST-reversion on the estimate of the ARI. The model was expanded by using empirically estimated age-specific TST-reversion rates, daily timesteps and cubic spline interpolation. Uncertainty was estimated by determining confidence intervals for the TST-reversion proportion. The model was then applied to manually re-estimate ARIs from published TST surveys in Vietnam (Hoa et al.) and South Africa (Wood et al.), by manually fitting observed TST-positivity. Sensitivity analyses included multiple TST-reversion rates and the use of linear interpolation for the ascertainment of TST-positive estimates. **Results:** Constant TST-reversion rates of over 1% had a significant effect on TST-positive prevalence decreasing prevalence by 9%; TST-reversion rates of 10% decreased prevalence by 55% by age 19. When fitted with reversion, the model showed that re-estimated ARIs were 50% to 450% higher than those derived in the TST surveys. Sensitivity analyses also produced similar results. **Conclusions:** Estimation of ARI from TST data without accounting for reversion will consistently underestimate the risk of infection. If we are to understand transmission, we will need to incorporate reversion into our estimates of ARI to facilitate insights into the population at-risk and cost-benefit applications.

ACKNOWLEDGEMENTS:

I would like to thank my supervisor, Rein Houben, for all his contributions to the project. From providing the original idea, to reviewing drafts of this project. Rein welcomed questions and doubts I had, and always provided insightful guidance. He was aware that mathematical modelling was new for me but made this experience highly enjoyable. I am looking forward to working with him again to expand this project or take on new ones.

I am also extremely grateful to Jon Emery and Katherine Horton who were also involved since the beginning providing wholesome feedback. They also provided an immense and invaluable amount of support in R coding. Additional thanks go to Prof. Paul Fine who agreed to meet with us to discuss peculiarities in the base model by Ian Sutherland.

Thanks to Eleonora for the constructive feedback on my grammar and for keeping me writing when I didn't want to write anymore.

I would like to dedicate this to work to my parents who are still in shock that I voluntarily accepted to work on a project involving "something with maths".

BACKGROUND:

TUBERCULOSIS:

Tuberculosis disease (TB) is an airborne infectious disease caused by *Mycobacterium tuberculosis*, which is of considerable global burden as a major cause of morbidity and mortality [1]. Over 10 million new cases of TB and 1.4 million deaths are reported annually, mainly in low- and middle-income countries [2,3]. TB is primarily a disease of the lungs, however, it can manifest throughout the body contributing to various clinical presentations [1,4]. TB has historically been considered distinct from latent infection [5]. Latent TB infection (LTBI) is defined as having a positive immunoreactive test for *M. tuberculosis* infection and no symptoms or microbiologic/pathologic findings of active TB disease [6]. After infection, a small proportion of individuals (5% - 15%) will progress rapidly to TB in the first two years after infection [7,8]; for the rest, it is assumed that infection with the bacterium as LTBI carries a lifelong potential of development to TB disease [4,9].

MYCOBACTERIUM TUBERCULOSIS INFECTION:

It is estimated that one quarter of the global population has LTBI [2,10]. Exposure to *M. tuberculosis* occurs via the respiratory tract through inhalation and inoculation of the bacterium in the alveoli [4]. Other modes of transmission, such as skin inoculation, are possible but extremely rare [4]. The most significant risk factors for infection are close contact and the infectiousness of the source, measured through the density of acid-fast bacilli from a sputum sample [1,4]. *M. tuberculosis* is first met by alveolar macrophages, which act through phagocytosis as part of the innate immune system to stop the infection process [1,11]. If the alveolar macrophages fail to digest *M. tuberculosis*, infection progresses as the bacteria gains entry to the pulmonary parenchyma [11]. A massive recruitment of immune and inflammatory cells occur at the site of entry generating a granuloma [1,11]. Through this host response, viable mycobacteria are walled-off from further spread, however, replication still occurs; this carries the potential of active disease development even decades after infection [1].

The burden of LTBI contributes to the ongoing TB pandemic despite being a non-transmissible state, due to the potential progression to active disease. The estimate of approximately 1.7 billion with LTBI was obtained through reconstruction of TB transmission trends from surveys and World Health Organization (WHO) estimates of TB prevalence in combination with historical projections [10]. However, in this calculation, LTBI is assumed as lifelong. This is an improbable assumption as viable bacteria may be eliminated with the use of medications or through a process of self-clearance in the absence of medications [12].

Furthermore, *M. tuberculosis* infection is indirectly inferred from the presence of TB immunoreactivity, an adaptive immune response to protein components [10,12].

TUBERCULIN SKIN TEST:

Currently, two TB immunoreactivity tests are used to indirectly ascertain infection with *M. tuberculosis*: Tuberculin Skin Test (TST) and Interferon-Gamma Release Assays (IGRAs) [4]. Their main function is to identify individuals with LTBI who may benefit from effective prophylactic treatment that will prevent progression to active disease [1,6]. Although they offer high negative predictive values, both tests are not without their limitations [6]. A positive result cannot distinguish between present or past infection and the positive predictive value for the test is low. [6].

While IGRAs have been developed more recently to overcome some of the limitations associated with the use of tuberculin, TST has been traditionally used to estimate prevalence and transmission trends through population surveys [4,6]. The TST is a low-cost, *in vivo* test, in which antigenic Purified Protein Derivative (PPD) of *M. tuberculosis* is injected intradermally on the forearm to elicit a delayed hypersensitivity reaction [4,6]. TST-positivity occurs approximately 3 to 9 weeks after initial infection [4,13]. The area of injection is examined 48 hours after inoculation and the reaction is quantified by measuring the skin induration diameter in millimetres [6,14]. In order to interpret the reaction as positive, induration measurements must meet certain cut-off values, which depend on the individual's comorbid conditions and geographical location [6,15]. An induration of 10 mm or over is generally considered a TST-positive reaction; nevertheless, false-positive results may arise from exposure to environmental mycobacteria or through Bacillus Calmette-Guérin (BCG) vaccination [4,6,14]. False-negative results may occur in an immunocompromised host with decreased adaptive immune response, such as in individuals infected with the Human Immunodeficiency Virus (HIV) [4]. Furthermore, it is prone to inter- and intra-reader variability and digit bias [6,16]. Regardless of the limitations, TSTs have been a widely used test to assess *M. tuberculosis* burden among diverse populations in the 20th century. For this reason, the study will focus on TST. Nonetheless, how the findings may be affected by the use of IGRA will be discussed.

TST-REVERSION:

There is a conventional assumption that *M. tuberculosis* latent infection means a lifelong presence of viable bacteria from where the individual can develop TB [12,17]. Additionally, this is usually coupled with another assumption that positive immunoreactivity equals persistent *M. tuberculosis* infection [12]. Nevertheless, these assumptions do not

appear to hold. First, studies on prophylactic treatment for LTBI have shown persistent TST-positivity up to nine years after receiving treatment, portraying TST as a poor proxy for viable infection [18]. Of note, a similar phenomenon is seen after treatment for active TB disease [19]. Second, there is substantial evidence on self-clearance of *M. tuberculosis* infection [7,8]; self-clearance is when a proportion of infected individuals eliminate infection in the absence of treatment for TB [17]. Thus, TST-positivity may be a marker of having been infected but should not be used as a marker of persistent, viable *M. tuberculosis* infection since there will be an overestimation of the population at risk of TB disease [12,17].

Regardless of the potential mechanism, TST reactivity is known to change. It wanes over time and there can even be a complete return to negative reactivity [13,20,21]. This phenomenon is called TST-reversion in which a previously TST-positive individual reverts back to a TST-negative reaction [13,20]. Some studies have even observed distinct, age-specific rates of TST-reversion derived from population-wide TST surveys [13,21]. Inferences of TB transmission based on TST-positive prevalence data without consideration of reversion are likely to inaccurately portray the burden, since there is an underestimation of the proportion of individuals who were once infected with *M. tuberculosis* [22]. Furthermore, in consideration of future trends, not accounting for TST-reversions undermines the risk of TB disease as the population at-risk decreases [17]. The challenge is that the prevalence of TST positivity is widely used in estimating TB metrics, including the rate of ongoing transmission in a population, often expressed as the Annual Risk of Infection (ARI).

ANNUAL RISK OF INFECTION:

M. tuberculosis is not a highly infectious pathogen, and its reproduction number varies according to setting [1,23]. Most high-income countries estimate a reproduction number below 1; in contrast, the reproduction number was as high as 4.3 in low- and middle-income countries [23]. There are other reports that an infectious individual might on average infect 3 to 10 people per year, yet the massive LTBI reservoir fuels the yearly ongoing transmission [10,24]. The ARI is a widely used calculation to generate an estimate of the population infected with *M. tuberculosis*, as well as the current transmission intensity [3,13,25]. The ARI is based on the prevalence of the infected reservoir, which has been historically established as the proportion of the population that is TST-positive [22]. Data from TST surveys of school children have been traditionally selected to provide a valid, up-to-date ARI estimate [22].

Unlike prevalence, by considering a temporal component in its calculation, ARI aims to provide a more insightful picture of the risk of transmission of *M. tuberculosis* [25]. The caveat here, however, is the assumption that a TST-reaction remains positive during the

individual's lifetime. As this is unlikely to be true, it is worthwhile examining the impact of TST-reversion, and self-clearance, on estimates of ARI. From a public health perspective, a well-constructed model that considers an important characteristic of the natural history of TB, provides solid estimates that offer accurate insights into the population at-risk.

RATIONALE FOR MODEL:

Mathematical modelling is commonly used in TB research to estimate burden or to understand the consequences of infection. In this project, models will be used to explore the effect of TST-reversion, as a measure of infection clearance, in the estimates of TST-positive prevalence and the ARI. Despite the high morbidity and mortality, TB disease outcome is rare and delayed, which make observational studies often impractical. Lastly, mathematical models facilitate analyses based on various parameters to enable estimates in changing conditions that might not be present during the conduction of real-world studies.

AIMS AND OBJECTIVES:

AIM:

To estimate the annual risk of *Mycobacterium tuberculosis* infection accounting for tuberculin skin test reversion using a mathematical modelling approach.

OBJECTIVES:

- Objective 1: Replicate a model estimating annual TST-positive prevalence accounting for constant TST-reversion rates proposed by Ian Sutherland [20].
- Objective 2: Expand model using empirical data-based age-specific TST-reversion rates from dedicated studies measuring reversion.
- Objective 3: Apply the expanded model to re-estimate the ARI based on published TST surveys of representative populations in Vietnam and a high-incidence region in South Africa.

MATERIALS AND METHODS:

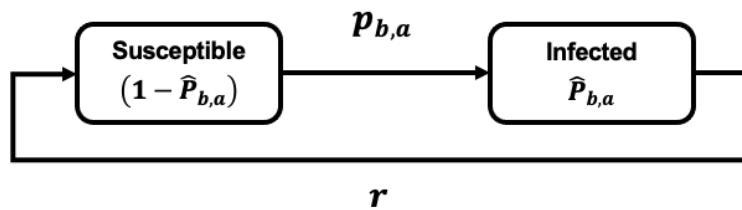
LITERATURE REVIEW:

A review of the private library of the supervisor was performed to find relevant data-based studies measuring TST-reversion according to age group for model expansion. Likewise, the library was searched for population-wide TST surveys to apply the adapted model. A general review of TB literature was conducted to inform the introduction and discussion.

OBJECTIVE 1: REPLICATION OF MODEL WITH CONSTANT TST-REVERSION RATES:

The model is based on a simple deterministic, difference formula on the effect of different levels of TST-reversion upon the estimate of the annual risk of *M. tuberculosis* infection proposed by Ian Sutherland (**Figure 1**) [20].

Figure 1. Model of *M. tuberculosis* infection accounting for TST-reversion.



*Model of *M. tuberculosis* infection accounting for TST-reversion proposed by Ian Sutherland [20]. A description of the model is provided in the main text.*

The proportion of a cohort, born at time b , who are found to be TST positive at age a , is expressed by $\hat{P}_{b,a}$. At birth it is assumed no child is TST positive, therefore $\hat{P}_{b,0} = 0$. The parameter $p_{b,a}$ represents the real infection risk and is calculated with the formula shown below:

$$p_{b,a} = (1 - d_b)^a ARI_{b,0}$$

The formula assumes as an initial parameter of the annual risk of infection at birth, $ARI_{b,0}$, with a subsequent annual decrease in risk based on cohorts from the Netherlands, d_b . Additionally, it includes an annual constant proportion r of individuals with prior TST-positivity that will revert to negativity. The proposed model equation is shown below:

$$\hat{P}_{b,a+1} = \hat{P}_{b,a} + (1 - \hat{P}_{b,a}) p_{b,a} - \hat{P}_{b,a} r$$

The formula has three components that make up the proportion infected in the next year: (1) the proportion of the population infected with *M. tuberculosis* in the current year plus (2) the proportion of non-infected individuals who convert to TST-positive minus (3) the proportion of TST-positive individuals which undergo TST-reversion. The model produces TST-positive proportion estimates successively from birth upwards to 19 years of age, accounting for various annual TST-reversion rates: 0%, 0.1%, 0.5%, 1%, 5%, and 10%. The estimated prevalence at age a was used to calculate the ARI using the formula below [25].

$$ARI_a = 1 - (1 - \hat{P}_{b,a})^{\frac{1}{a}}$$

The ARI estimate for all reversion rates was compared to the annual risk without reversion by computing the relative reduction in ARI. In Sutherland's study the ARI was calculated at age 19, as the degree of underestimation of the ARI was greater in older age groups when compared to younger age groups.

OBJECTIVE 2: MODEL EXPANSION WITH AGE-SPECIFIC TST-REVERSION RATES:

The base model assumed that the TST-reversion rate was constant over ages. However, studies have found distinct reversion rates which are dependent on age and generally, follow a downward trend [13,21]. Therefore, the base model was expanded to account for age-specific TST-reversion parameters from TST surveys conducted in Canada and Malawi [13,21].

The study by Grzybowski et al. was conducted in Victory County, Ontario, Canada where in 1959, a program aimed at the control of TB, carried out five consecutive annual TST surveys among a population of 177,000 individuals of all ages [21]. Chest x-rays were obtained, and chemoprophylaxis was offered to all TST-positive individuals. BCG vaccination was not considered in new-borns or infants and was only recommended for contacts of patients with active TB. Numerators (number of reversions) and denominators (positive reactors retested in one year) used for age group-specific reversion rates were provided; 95% confidence intervals for the given proportion were calculated to account for uncertainty in the TST-reversion rates in the model.

The study by Fine et al. describes a set of over 64,000 TSTs collected in two total population surveys in the Karonga district, in northern Malawi from 1980 to 1989 [13]. TST-

reversion data was available from paired results on 6,991 individuals that participated in both surveys. BCG vaccination was introduced into this population in the mid 1970s, so vaccine coverage was low in older age groups, as was inferred from the presence of a BCG scar. Expected and observed TST-reversion rates in females without a BCG scar were plotted together; expected reversion rates were ascertained by reverse engineering the plot using a web-based plot digitizer [26]. Since the plot only provided point estimates of rates, 95% confidence intervals were not available for these reversion rates. The TST-reversion rates provided by Fine et al. were used for sensitivity analyses [13]. The TST-reversion rates from both studies are displayed on **Table 1**.

Table 1. Age-specific TST-reversion rates.

TST studies	Age group	TST-reversion rate (95% CI)
Grzybowski 1964 [21] Ontario, Canada	0 – 19 years old	22.2% (15.2 – 31.4)
	20 – 39 years old	8.0% (4.9 – 12.6)
	40 – 59 years old	4.8% (3.2 – 6.9)
	60 years old and over	9.0% (6.5 – 12.3)
Fine 1999 [13] Karonga, Malawi	0 – 5 years old	17.9%
	5 – 10 years old	10.2%
	10 – 15 years old	7.5%
	15 – 20 years old	6.1%
	20 – 25 years old	5.3%
	25 – 30 years old	4.8%
	30 – 40 years old	4.1%
	40 years old and over	3.7%

TST-reversion rate estimates also underwent cubic spline interpolation in the middle of every TST-reversion rate age group for smooth plot visualisation. The model was further adapted to calculate by daily timesteps to provide smoother results in contrast to the annual timesteps from the Sutherland model.

There was no available data to incorporate the annual risk percentage decrease parameter in the adapted model. Unlike Sutherland’s theoretical work which built on empirical estimates from a Dutch birth cohort, the model re-estimations (see objective 3) did not provide an estimate for the decrease in ARI.

The model was constructed and run using R v.4.1.0 (2021-05-18) for statistical computing and graphics [27]. Plots were created using the ggplot2 package [28].

MODEL ASSUMPTIONS:

As a simple model, there remain a number of key assumptions, which are listed below. The implication of these on the qualitative and quantitative findings will be addressed in the discussion.

- *Infection with *M. tuberculosis* always develops a TST-positive reaction.*
TST is a marker of TB immunoreactivity, therefore it indirectly tests for *M. tuberculosis* infection and is positive after the first 3 to 9 weeks following infection [4]. However, it is known that not all individuals infected mount an adaptive immune response to TB exposure.
- *No child is TST positive at birth.*
The model assumes that children get exposed to *M. tuberculosis* after birth. Cases of congenital tuberculosis exist, however, these are extremely rare and would not contribute greatly if they were to be considered in the model [29].
- *BCG vaccination does not produce a TST-positive reaction.*
In high-endemic regions, BCG vaccination is administered to neonates and children to prevent the development of disseminated or severe TB disease [4]. BCG vaccination can cross-react with TST producing a false-positive reaction [6,30].
- *BCG vaccination does not confer immunity or affect self-clearance.*
BCG vaccination also impacts TST-positivity by prevention of *M. tuberculosis* infection and through enhancement of self-clearance [31].
- *There is no annual decline in risk of *M. tuberculosis* infection.*
Although considered in Sutherland's base model, the parameter considering an annual decrease in TB burden and transmission was based on epidemiological changes observed in Dutch cohorts of the 20th century. These had only two distinct values: 5% and 13% annual risk decrease [20]. However, recent, generalisable, parametrised data is not available and would likely vary through regions based on their TB control efforts.

- *All studies have the same definition of TST-conversion and TST-reversion.*
Different cut-off values and incremental changes have been considered for TST-conversion [15]. These criteria have been constantly changing as it was applied in practice and research; **Table 2** shows the definitions for TST-conversion and TST-reversion of the studies used.

Table 2. Definitions of TST-conversion and TST-reversion in studies.

TST studies	TST-conversion	TST-reversion
Grzybowski 1964 [21] Ontario, Canada	Area of induration of 5 mm or more in diameter was considered a positive reaction.	Positive to a negative tuberculin test among positive reactors retested after one year.
Fine 1999 [13] Karonga, Malawi	1980 ATS criterion for conversion: < 10 mm on first test to ≥ 10 mm on second test, with an increase of at least 6 mm.	Defined as the opposite of the conversion definition.

- *Reinfections with M. tuberculosis occur at similar rates as primary infections.*
The model considers population proportions that are shifting between susceptibility and infection. In the model, after TST-reversion occurs the whole susceptible population carries the same risk of infection, regardless of past infection or TST status. Nonetheless, studies have found risk reductions of 41% and 73% when comparing reinfections to primary infections [7,32], although these did not record or consider TST reversion.

OBJECTIVE 3: EXPANDED MODEL APPLICATIONS:

The expanded model was then applied to re-estimate ARI parameters from two population-wide TST surveys: Hoa et al. in Vietnam and Wood et al. in South Africa [33,34]. Firstly, the study by Hoa et al. was a nationwide TST survey among children aged 6 to 14 years carried out in 2006 to 2007, to assess the prevalence of *M. tuberculosis* infection. A total of 21,487 children were tested, of which 82.6% were BCG-vaccinated, as determined by the presence of a BCG scar. No information on HIV infection prevalence was available. The study resulted in an estimated TST-positive prevalence of 16.7%; using this prevalence in a population with a mean age of 10.8 years, an ARI of 1.7% (95% CI: 1.5 – 1.8%) was derived. Secondly, the study by Wood et al. was conducted in high-burden townships in Cape Town, South Africa where 73% of TB cases are estimated to be co-infected with HIV. However, the survey targeted HIV-negative individuals aged 5 to 40 years old. The study derived an ARI of 3.9% (95% CI: 2.2 – 5.7%) from an estimated TST-positive prevalence of 18.1% among 5-

year-olds, an ARI of 3.9% (95% CI: 3.3 – 4.5%) from an estimated prevalence of 32.7% among 10-year-olds, and an ARI of 4.8% (95% CI: 4.1 – 5.5%) from an estimated prevalence of 52.0% among 15-year-olds. Only ARIs for ages 5, 10, and 15, were derived as the sample size was greater in these three age groups. Both TST surveys defined TST-conversion as an induration of ≥ 10 mm [33,34].

Observed TST-prevalence were plotted with the estimates of the model using the original estimate ARI. Then, an ARI parameter that best-fit the observed TST-prevalence data was identified through manual fitting. Lastly, survey-derived ARIs were compared with their corresponding best-fit ARIs.

SENSITIVITY ANALYSES:

As mentioned before, the age-specific TST-reversion rates provided by Fine et al. were used for sensitivity analyses by applying the expanded model to re-estimate the ARI parameters from the two population-wide TST surveys. Additionally, the age-specific TST-reversion rates underwent linear interpolation across age ranges to observe if results were different from those arising from cubic spline interpolation.

ETHICAL CONSIDERATIONS:

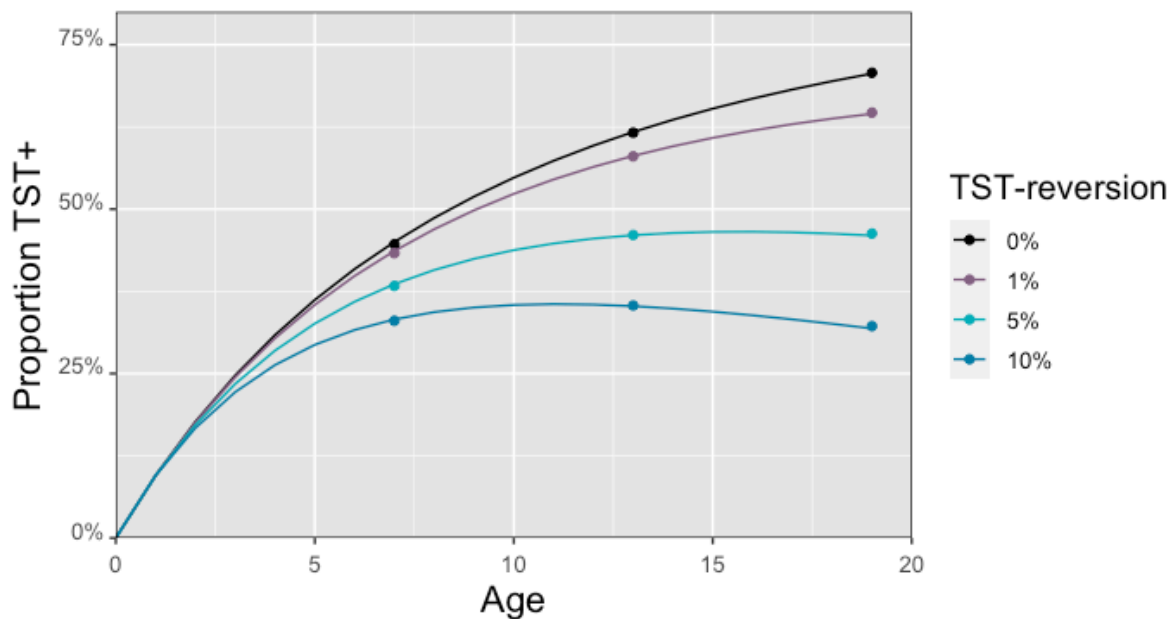
This project did not use any data that required local ethical and/or regulatory approval. Public domain data published as part of prior TST-based population studies or for reference of model parameters for validation were used. The included data did not contain identifiable information of participants. No specific intellectual property rights, copyright or permissions issues apply. This project was assessed by the Research Governance & Integrity Office and deemed as not requiring ethical approval from the MSc Ethics Committee (Ref.: 25969).

RESULTS:

OBJECTIVE 1: REPLICATION OF MODEL WITH CONSTANT TST-REVERSION RATES:

Using the various Dutch birth cohort parameters, the base model was replicated and compared to the tabulations of estimated proportions TST-positive at ages 7, 13 and 19, as well as the annual risk of infections theorised by Ian Sutherland in 1971 [20]. The model was initially tested using the 1912 Dutch birth cohort's baseline parameters: 10% annual risk of infection at birth and 5% annual decline in risk. The model estimated the TST-positive prevalence accounting for the effects of the different rates of reversion. **Figure 2** compares the TST-positive prevalence from the Sutherland model using annual reversion rates of 0%, 1%, 5%, and 10% with the TST-positive prevalence from the model for the same reversion rates at age 7, 13, and 19 years as shown in Sutherland's paper [20]. Similar figures were constructed for all other Dutch birth cohorts in Sutherland's paper (**Appendices 1-5**). **Table 3** shows the calculated and observed TST-positive prevalence values at ages 7, 13, and 19 years with the absolute and relative differences between them.

Figure 2. Comparison of Sutherland's estimates (points) and model estimates (lines) of TST-positive prevalence of 1912 Dutch birth cohort with varying TST-reversion rates.



Base model TST-positive estimates accounting for different levels of TST-reversion. Model parameters based on 1912 Dutch birth cohort: ARI at birth = 10%, annual decrease in risk = 5%. TST-positive prevalence at ages 7, 13, and 19 from theoretical exercise by Ian Sutherland [20].

Table 3. Differences between Sutherland’s estimates and model estimates of TST-positive prevalence of 1912 Dutch birth cohort with varying TST-reversion rates.

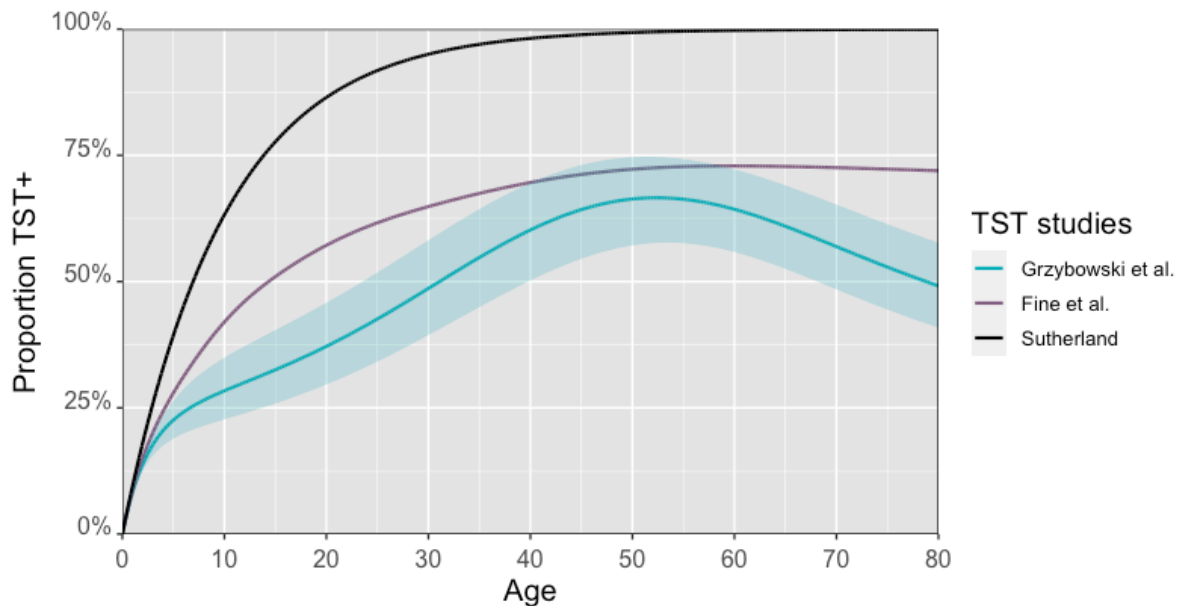
Age	TST-positive prevalence	TST-reversion rates			
		0%	1%	5%	10%
7	Model	45.0%	43.6%	38.6%	33.2%
	Sutherland	44.7%	43.3%	38.3%	33.0%
	Relative difference	0.8%	0.8%	0.7%	0.6%
13	Model	61.7%	58.1%	46.0%	35.3%
	Sutherland	61.6%	58.0%	46.1%	35.4%
	Relative difference	0.2%	0.1%	-0.1%	-0.3%
19	Model	70.6%	64.5%	46.0%	31.9%
	Sutherland	70.7%	64.7%	46.3%	32.2%
	Relative difference	-0.2%	-0.3%	0.7%	-1.1%

The replication of the model showed the effect that the varying levels of TST-reversion have on the predicted TST-positive prevalence as previously theorised by Sutherland. The plot shows decreasing levels of TST-positivity as the TST-reversion rate is increased. When comparing TST-positive prevalence at age 19 across TST-reversion rates with no reversion, rates of 1%, 5%, and 10% estimate a prevalence of with a relative decrease of 9%, 35%, and 55%, respectively.

OBJECTIVE 2: MODEL EXPANSION WITH AGE-SPECIFIC TST-REVERSION RATES:

The adapted model was trialled with age-specific reversion rates from the population-wide TST-studies by Grzybowski et al. and Fine et al [13,21]. Following the parameters of the 1912 Dutch birth cohort, an ARI at birth of 10% was used without any rate of annual decline of risk, as explained in the assumptions of the adapted model. **Figure 3** compares the estimated TST-prevalence accounting for the two sets of age-specific TST-reversion rates.

Figure 3. Estimated TST-positive prevalence per age accounting for age-specific TST-reversion rates.



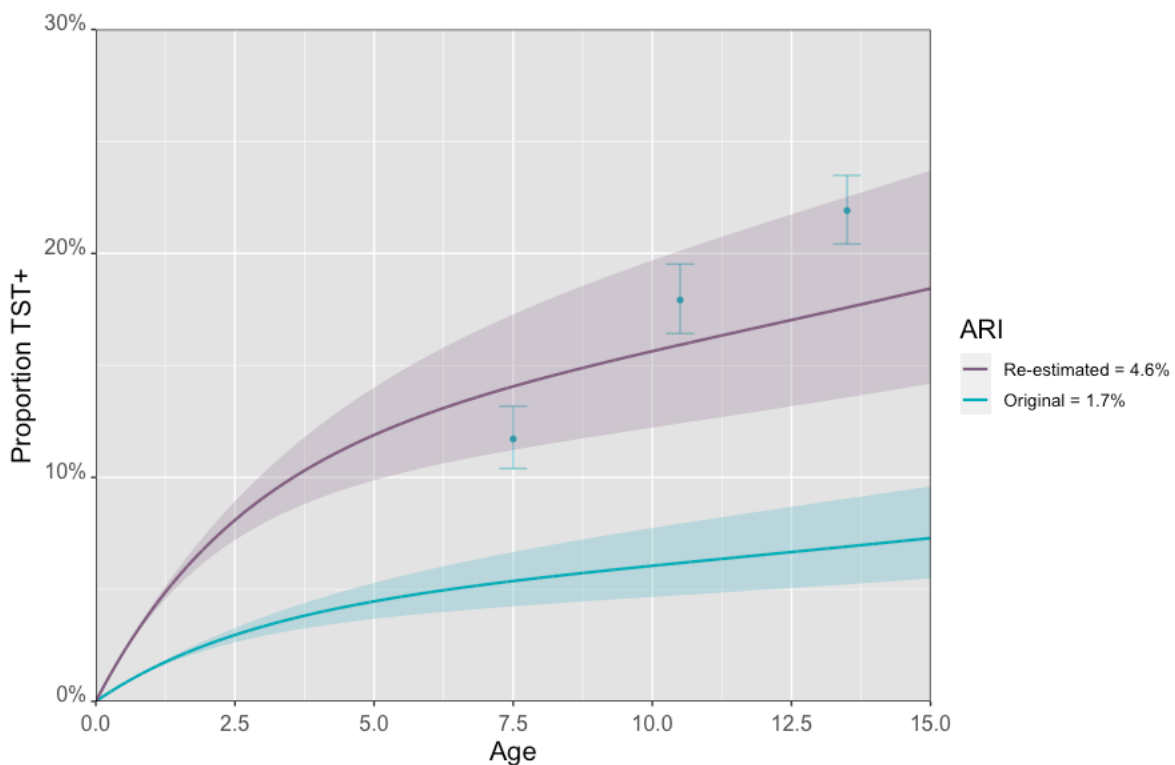
Expanded model TST-positive estimates accounting age-specific TST-reversion rates from Grzybowski et al. and Fine et al. [13,21]. For reference, theorised TST-positive estimates without accounting for reversion from Sutherland [20]. Model parameters: ARI at birth = 10%.

TST-positive prevalence is inversely correlated with the TST-reversion rates of each age group. The calculated TST-positive prevalence using the age-specific TST-reversion rates from Fine et al. display a smooth logarithmic growth as the TST-reversion rates follow a downward trend from 17.9% at the 0- to 5-year-old age group to 3.7% at the 40-year-old and over age group. In this scenario, a maximum TST-positive prevalence of 72.9% is reached at age 60 before it begins a slight decrease to 71.9% at age 80. On the other hand, the calculated TST-positive prevalence using the age-specific TST-reversion rates from Grzybowski et al. follows a different pattern with a high reversion rate of 22.2% (95% CI: 15.2 – 31.4%) in the 0- to 19-year-old age group that declines to 4.8% (95% CI: 3.2 – 6.9%) in the 40- to 59-year-old age group, which later increases to 9% (95% CI: 6.5 – 12.3%). A maximum TST-positive prevalence of 66.6% (95% CI: 57.7 – 74.7%) is reached at age 52. Due to the absence of confidence intervals in the TST-reversion rates from Fine et al., it is not possible to determine if there are significant differences in the TST-positive prevalence estimates when comparing it with the estimates using the TST-reversion rates from Grzybowski et al. Both lines generally overlap between the ages of 40 to 60 years old, despite the lack of confidence intervals in the estimates from Fine et al.

OBJECTIVE 3: RE-ESTIMATION OF ARI FROM DATA OF TST SURVEYS:

The age-specific TST-reversion rates were used to re-estimate the original ARIs from data-based population-wide TST surveys. As described in the methods, the TST survey by Hoa et al. derived an ARI of 1.7%. First, the TST-positive prevalence accounting for the TST-reversion rates of Grzybowski et al. was plotted, and the model was applied to varying levels of ARI to manually ascertain the best-fit to the observed data. An ARI of 4.8% was deemed best-fit for the model that accounted for TST-reversion. **Figure 4** shows the model estimates based on the ARI of 1.7% and 4.8% alongside the measured TST-positive prevalence for ages 7.5, 10.5, and 13.5 years. **Table 4** shows the observed and estimated TST-positive prevalence for ages 7.5, 10.5, and 13.5 years.

Figure 4. TST-positive prevalence per age accounting for age-specific TST-reversion rates based on original estimate and best-fit ARI parameters.



Expanded model TST-positive estimates accounting age-specific TST-reversion rates from Grzybowski et al. [21]. Re-estimation of ARI parameter observed by Hoa et al.[33]. Observed TST-positive prevalence at age 7.5, 10.5, and 13.5.

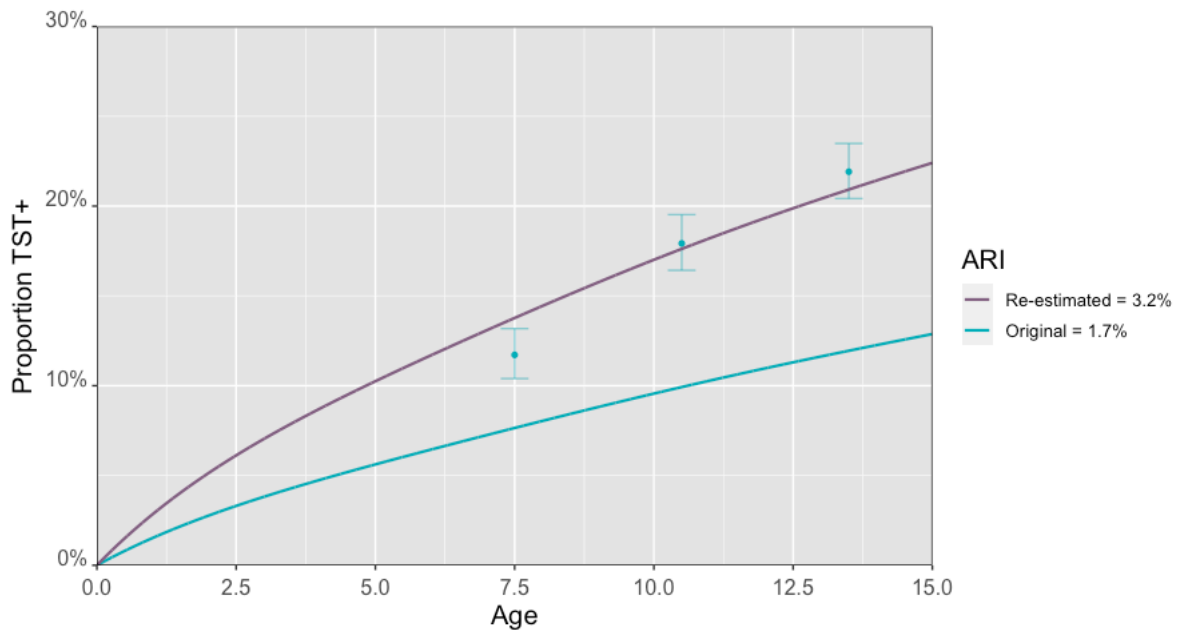
Table 4. TST-positive prevalence per age accounting for age-specific TST-reversion rates based on original estimate and best-fit ARI parameters.

Age	ARI = 1.7% (95% CI) (Original estimate)	ARI = 4.8% (95% CI) (Model best fit)	Observed prevalence (95% CI)
7.5	5.4% (4.2 – 6.7%)	14.1% (11.2 – 17.3%)	11.7% (10.4 – 13.2%)
10.5	6.2% (4.7 – 7.9%)	15.9% (12.4 – 20.1%)	17.9% (16.4 – 19.5%)
13.5	6.9% (5.2 – 9.1%)	17.6% (13.6 – 22.5%)	21.9% (20.4 – 23.5%)

Expanded model TST-positive estimates accounting age-specific TST-reversion rates from Grzybowski et al. [21]. Re-estimation of ARI parameter observed by Hoa et al.[33].

Then, as part of the sensitivity analyses, the TST-positive prevalence accounting for the TST-reversion rates of Fine et al. was plotted and the model was applied to varying levels of ARI to ascertain the best-fit of the observed data. An ARI of 3.2% was deemed best-fit for the model that accounted for TST-reversion. **Figure 5** demonstrates the model estimates based on the ARI of 1.7% and 3.2% alongside the measured TST-positive prevalence for ages 7.5, 10.5, and 13.5 years. **Table 5** shows the observed and estimated TST-positive prevalence for ages 7.5, 10.5, and 13.5 years.

Figure 5. TST-positive prevalence per age accounting for age-specific TST-reversion rates based on original estimate and best-fit ARI parameters.



Expanded model TST-positive estimates accounting age-specific TST-reversion rates from Fine et al. [13]. Re-estimation of ARI parameter observed by Hoa et al.[33]. Observed TST-positive prevalence at age 7.5, 10.5, and 13.5.

Table 5. TST-positive prevalence per age accounting for age-specific TST-reversion rates based on original estimate and best-fit ARI parameters.

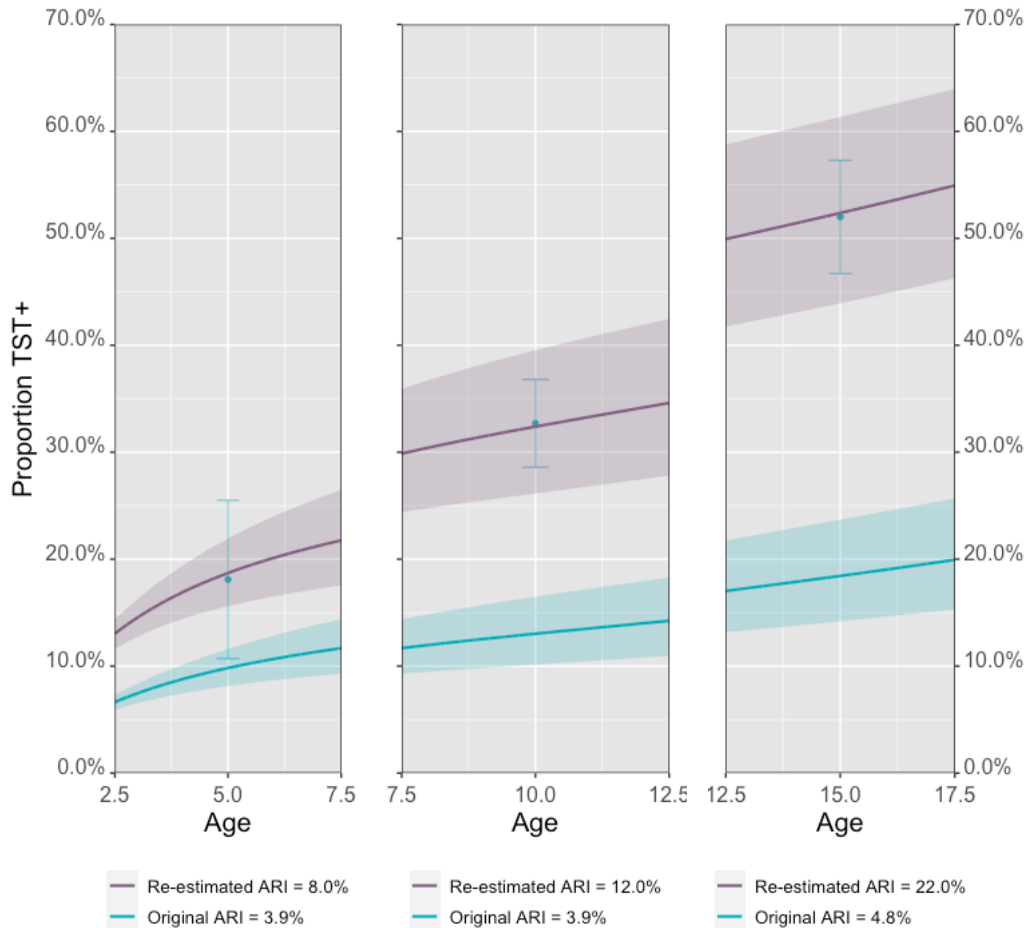
Age	ARI = 1.7% (Original estimate)	Best-fit ARI = 3.2% (Model best fit)	Observed prevalence (95% CI)
7.5	7.6%	13.8%	11.7% (10.4 – 13.2%)
10.5	9.9%	17.6%	17.9% (16.4 – 19.5%)
13.5	11.9%	20.9%	21.9% (20.4 – 23.5%)

Expanded model TST-positive estimates accounting age-specific TST-reversion rates from Fine et al. [13]. Re-estimation of ARI parameter observed by Hoa et al.[33].

As described in the methods, the TST survey by Wood et al. derived an ARI of 3.9% among 5- and 10-year-olds, and an ARI of 4.8% among 15-year-olds. First, the TST-positive prevalence accounting for the TST-reversion rates of Grzybowski et al. was plotted and the model was applied to varying levels of ARI to ascertain the best-fit of the observed data for each age group separately. An ARI of 8%, 12%, and 22% were deemed best-fit for the TST-positive prevalence at age 5, 10, and 15, respectively. **Figure 6** demonstrates the model calculations based on the estimated and best-fit ARI alongside the measured TST-positive

prevalence for ages 5, 10, and 15 years. **Table 6** shows the observed and estimated TST-positive prevalence for ages 5, 10, and 15 years.

Figure 6. TST-positive prevalence per age accounting for age-specific TST-reversion rates based on original estimate and best-fit ARI parameters.



Expanded model TST-positive estimates accounting age-specific TST-reversion rates from Grzybowski et al. [21]. Re-estimation of ARI parameter per age observed by Wood et al.[34]. Observed TST-positive prevalence at age 5, 10, and 15.

Table 6. TST-positive prevalence per age accounting for age-specific TST-reversion rates based on original estimate and best-fit ARI parameters.

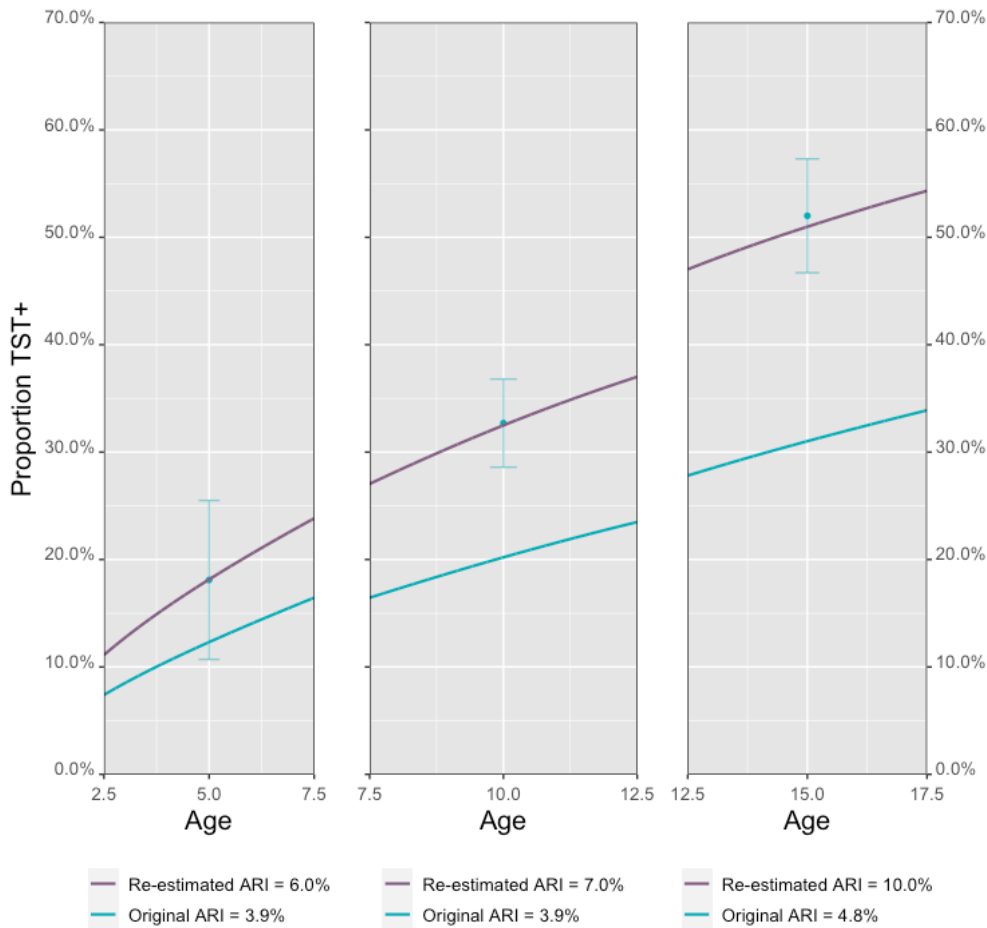
Age	ARI (95% CI) (Original estimate)	Best-fit ARI (95% CI) (Model best-fit)	Observed prevalence (95% CI)
5	9.8% (8.1 – 11.6%)	18.7% (15.6 – 21.9%)	18.1% (10.7 – 25.5%)
10	13.0% (10.1 – 16.5%)	32.4% (26.1 – 39.5%)	32.7% (28.6 – 36.8%)
15	18.4% (14.2 – 23.7%)	52.4% (43.9 – 61.4%)	52.0% (46.7 – 57.3%)

Expanded model TST-positive estimates accounting age-specific TST-reversion rates from Grzybowski et al. [21]. Re-estimation of ARI parameter per age observed by Wood et al.[34]. Original

estimated ARIs are 3.9%, 3.9%, and 4.8% at age 5, 10, and 15, respectively. Best-fit re-estimated ARIs are 8%, 12%, and 22% at age 5, 10, and 15, respectively.

Then, as part of the sensitivity analyses, the TST-positive prevalence accounting for the TST-reversion rates of Fine et al. was plotted and the model was applied to varying levels of ARI to ascertain the best-fit of the observed data for each age group separately. An ARI of 6%, 7%, and 10% were deemed best-fit for the TST-positive prevalence at age 5, 10, and 15, respectively. **Figure 7** shows the model calculations based on the estimated and best-fit ARI alongside the measured TST-positive prevalence for ages 5, 10, and 15 years. **Table 7** shows the observed and estimated TST-positive prevalence for ages 5, 10, and 15 years.

Figure 7. TST-positive prevalence per age accounting for age-specific TST-reversion rates based on original estimate and best-fit ARI parameters.



Expanded model TST-positive estimates accounting age-specific TST-reversion rates from Fine et al. [13]. Re-estimation of ARI parameter per age observed by Wood et al.[34]. Observed TST-positive prevalence at age 5, 10, and 15.

Table 7. TST-positive prevalence per age accounting for age-specific TST-reversion rates based on original estimate and best-fit ARI parameters.

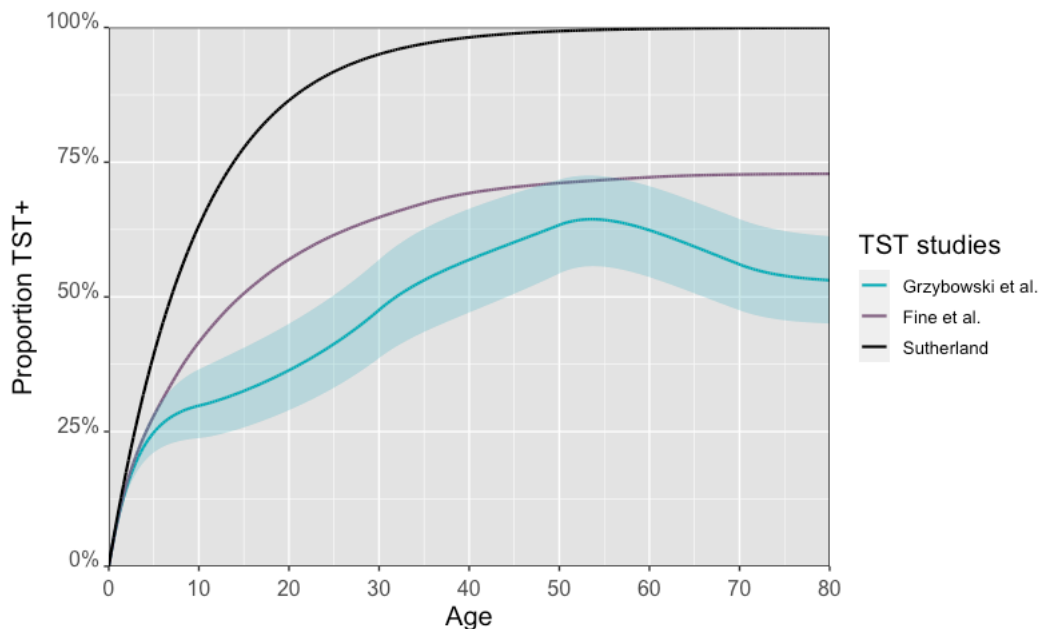
Age	ARI (Original estimate)	Best-fit ARI (Model best-fit)	Observed prevalence (95% CI)
5	12.3%	18.1%	18.1% (10.7 – 25.5%)
10	20.2%	32.5%	32.7% (28.6 – 36.8%)
15	31.0%	51.0%	52.0% (46.7 – 57.3%)

Expanded model TST-positive estimates accounting age-specific TST-reversion rates from Fine et al. [13]. Re-estimation of ARI parameter per age observed by Wood et al.[34]. Original estimated ARIs are 3.9%, 3.9%, and 4.8% at age 5, 10, and 15, respectively. Best-fit re-estimated ARIs are 6%, 7%, and 10% at age 5, 10, and 15, respectively.

FURTHER SENSITIVITY ANALYSES:

Expanded model estimates underwent linear interpolation to observe if results were different from those arising from cubic spline interpolation. Following the parameters of the 1912 Dutch birth cohort, an ARI at birth of 10% was used without any rate of annual decline of risk. **Figure 8** plots similar to **Figure 3**, where cubic spline interpolation is used. No major difference can be observed between the two figures.

Figure 8. Estimated TST-positive prevalence per age accounting for age-specific TST-reversion rates using linear interpolation.



Expanded model TST-positive estimates accounting age-specific TST-reversion rates from Grzybowski et al. and Fine et al. [13,21]. For reference, theorised TST-positive estimates without accounting for reversion from Sutherland [20]. Model parameters: ARI at birth = 10%.

The expanded model by means of linear interpolation was employed to re-estimate the original estimate ARI from the TST surveys by Hoa et al. in Vietnam [33]. The TST-positive prevalence accounting for the TST-reversion rates of Grzybowski et al. was plotted, and the model was applied to varying levels of ARI to ascertain the best-fit of the observed data. An ARI of 4.6% was deemed best-fit for the model that accounted for TST-reversion; a slightly lower ARI from the 4.8% ARI estimated using cubic spline interpolation. **Table 8** shows the observed and estimated TST-positive prevalence for ages 7.5, 10.5, and 13.5 years, which have been somewhat altered due to the linear interpolation and re-estimation of best-fit ARI.

Table 8. TST-positive prevalence per age accounting for age-specific TST-reversion rates based on original estimate and best-fit ARI parameters using linear interpolation.

Age	ARI = 1.7% (95% CI) (Original estimate)	Best-fit ARI = 4.8% (95% CI)	Observed prevalence (95% CI)
7.5	5.9% (4.7 – 7.2%)	14.9% (11.9 – 17.9%)	11.7% (10.4 – 13.2%)
10.5	6.5% (4.9 – 8.4%)	16.1% (12.5 – 20.4%)	17.9% (16.4 – 19.5%)
13.5	7.1% (5.3 – 9.3%)	17.2% (13.2 – 22.2%)	21.9% (20.4 – 23.5%)

Expanded model TST-positive estimates accounting age-specific TST-reversion rates from Grzybowski et al. [21]. Re-estimation of ARI parameter observed by Hoa et al.[33].

DISCUSSION:

The TST prevalence estimates of the model accounting for age-specific TST-reversion rates were consistently lower than those calculated from prevalence data in TST surveys, when using the calculated ARI. The model showed that higher ARIs were needed to fit the prevalence data observed in the studies. Best-fit ARIs were 1.54 to 4.58 times higher than those estimated in TST surveys. Similar results were found in the sensitivity analyses. Accounting for TST-reversion rates, instead of the historical paradigm of lifelong TST-positivity, shows that individuals are at higher risks of *M. tuberculosis* infection.

Therefore, adequate recognition of TST-reversion rates will greatly impact TST-positivity estimations. As shown by Ian Sutherland, constant TST-reversion rates of above 1% lead to a high degree of underestimation (from 50% to 70%) of the ARI, when comparing to estimates not accounting for reversion [20]. Empirical data for TST-reversions in populations have shown that the rates are much higher than 1%, and vary by age [13,21].

The high rate TST-reversion has substantial consequences for key metrics in TB, including the derived ARI [13]. TST-positivity reflects immunoreactivity to either past or present infection, therefore, the reported burden of LTBI will be overestimated if no reversion is observed (thus, higher ARI), even by eliminating the threat of viable mycobacteria through effective treatment, prophylaxis, or self-clearance [35,36]. Apart from test stability, there is wide variability in antigen preparations, dosages, and methods of administration of TST in epidemiological surveys; nevertheless, these are not expected to introduce large differences in the results [22]. Additionally, surveys often use different cut-off values for TST-positivity that could lead to an overestimation of TST-reversions if less stringent criteria for positivity are used. Despite this, similar conclusions might be reached as any substantial TST-reversion rate (above 1%) would have a notable effect on the TST-positive estimates.

One reason for the previous underestimation of ARI may have been due to successful exposure and initial infection which then rapidly self-cleared without leaving trace of *M. tuberculosis* immunoreactivity in the host [11]. Some of these 'infected' individuals may not ever have been at risk of disease. However, one hypothesis is that inoculums of *M. tuberculosis* can induce immunoreactivity without a successful infection in the lower respiratory tract [37]. In this scenario, immunisation occurs in the mucosa of the upper airways [37]. Reversion happens for IGRA as well, but this remains poorly quantified and understood [36,38–40]. Negative IGRA results should be interpreted carefully as some studies suggest that the rate of IGRA-reversion exceeds that of TST [40]. This furthers the issue of current

unreliable tests to determine viable *M. tuberculosis* infection and provide control measures to prevent further spread of TB.

Given all peculiarities and limitations of TSTs, the interpretation of TST-positivity as a marker of real infection, of harbouring viable *M. tuberculosis*, and of being at-risk of TB disease is unclear. TB control warrants an improved biomarker for detection of LTBI; luckily, some are already being explored [41,42]. However, at present, if the interpretation of TST remains as is, without considering self-clearance, then the number of people who have become infected by *M. tuberculosis* will always be miscalculated. This in turn carries implications in the risk of progression to disease and actual number at-risk.

MODEL LIMITATIONS:

As with most mathematical models, several assumptions were put into the model to simplify a complex reality; some of which pose important limitations to the results obtained. One limitation is that the model assumed that the risk of *M. tuberculosis* infection was the same for primary infection and reinfections. Previous work by Sutherland has shown a lower risk of developing tuberculosis within 5 years in males aged 15-69 years in those with a distant primary infection who had a recent reinfection (1.91% per year) when compared to those with a primary infection (5.06% per year) [43]. For females, the results were similar with an annual risk of 1.10% and 5.85% following reinfection and primary infection, respectively. Other studies have found risk reductions of 41% and 73% when comparing reinfections to primary infections [7,32]. A recent meta-analysis has estimated the risk reduction to be even greater at 79% [9]. Of note, these studies looked at the risk of disease following reinfection and derived that the increased risk is related to the naivety of the host in primary *M. tuberculosis* infections. Consideration of the risk reinfections in the model would most likely lead to lower estimated TST-prevalence which would heighten with increasing levels of TST-reversion. Adding a measure of protection from reinfection, would result in a lower ARI than estimated. Nonetheless, it would not be considerable enough to arrive at different conclusions as the ARI would still be more than originally estimated.

Another limitation with the model is that the effects of BCG vaccination have not been considered. BCG vaccination is a major component of worldwide TB control, with over 90% of new-borns being vaccinated annually [44]. This wide coverage compromises the usefulness of TST as a measure of *M. tuberculosis* infection [22]. BCG vaccination can cause TST false-positive reactions as protein components of *M. bovis* cross-react with PPD [4]. The likelihood of this cross-reactivity decreases with age, but lingers in some individuals [6,30]. Age of

vaccination also influences tuberculin cross-reactivity, with delayed (after 1 year of age) BCG vaccination leading to a higher prevalence of TST-positive individuals, compared to those who did not receive BCG vaccination [6,31]. Similarly, false positives can be seen in surveys vulnerable to TST boosting in which retesting the same individuals acts as sufficient exposure to elicit an immunoreactive response [22]. Considering this effect on TST data, the model would have produced higher TST-positive estimates, thus, higher ARIs, resulting in more pronounced differences when compared to the original estimates. Restraining the use of this measure in the model provides a more conservative estimate of ARI.

On the other hand, BCG vaccination may be indirectly targeting and enriching innate immunity, facilitating the role of early clearance [31]. Results from a randomised controlled trial evaluating a novel vaccine candidate and BCG revaccination have found reduced TB immunoreactivity conversion among those receiving BCG, compared to those receiving placebo [45]. A meta-analysis of observational studies further concluded a protective efficacy of 19% against *M. tuberculosis* infection following exposure among BCG-vaccinated children compared to unvaccinated children [46]. With over 150 countries currently recommending universal BCG vaccination, this conferred protection, although small, has a massive impact in the control of TB [44]. Sutherland's model was built based on Dutch birth cohorts where BCG vaccination was not recommended or enforced, allowing for the true risk of infection for that population to be estimated without accounting for the effects of the vaccine [20,25]. Both studies, which provided TST-reversion rates, recommended BCG vaccination and might have contributed to the high TST-reversion rates evidenced in the younger age groups [13,21]. It is possible that due to the low cut-off score of 5 mm in the TST survey by Grzybowski et al, a large subset of the TST-reversions observed in the 0- to 19-year-old age group were due to the stability of the TST [21]. The model is not considering vaccination coverage nor its protective effects; while that might overestimate the TST prevalence estimates, the relative difference when comparing models accounting for TST-reversion would be maintained.

On the other hand, false-negative reactions can occur in individuals with compromised immunity as with HIV infection, prolonged corticosteroid use or protein malnutrition [47–50]. The TST survey by Wood et al. was challenged by the high co-infection rates of TB and HIV; by just considering HIV-negative individuals as eligible to participate, the TST-results would not bear the potential of increased false-negatives [34]. However, the risk ascertained must be interpreted in terms of the setting considering a population with a higher risk of *M. tuberculosis* infection and exposure due to the co-infection [51]. Moreover, infection can be reverted hastily through the phenomenon of early clearance, which is the eradication of *M.*

tuberculosis before the development of an adaptive immune response [52]. Here, physical barriers or the innate immune response (particularly alveolar macrophages) plays a vital role in preventing a successful infection [31,52]. Hypothesised stages of response to *M. tuberculosis* infection suggests that half of those exposed will clear infection via innate immune response [11]. If considered in the model, all these factors underestimate the risk of *M. tuberculosis* infection as a proportion of infections would not be properly recorded by TSTs.

Lastly, the annual decline in the risk of infection was withdrawn from the base model. Studies have demonstrated a steady decrease in risk globally [22,23]. At present, countries are working towards the goals of the WHO “End TB Strategy” and declines are being seen in reported incidence rates as better diagnostic tools and efficacious treatments are available [53]. Considering risk decline in the model would decrease TST-positivity prevalence and the differences between the original and the re-estimated ARI would be further emphasised.

RECOMMENDATIONS:

In conclusion, calculation of ARI without accounting for TST-reversion will consistently underestimate the risk of infection. Age-specific TST-reversion rates illustrate how this phenomenon decreases as age progresses, although the underestimation remains non-trivial. While there are many limitations associated with the use of TST, reversion has generally not been considered despite its enormous effect upon ARI calculations. In order to make progress towards the goals of the WHO “End TB Strategy”, estimates must consider TST-reversion to provide accurate estimates and facilitate insights into the population at-risk and cost-benefit applications.

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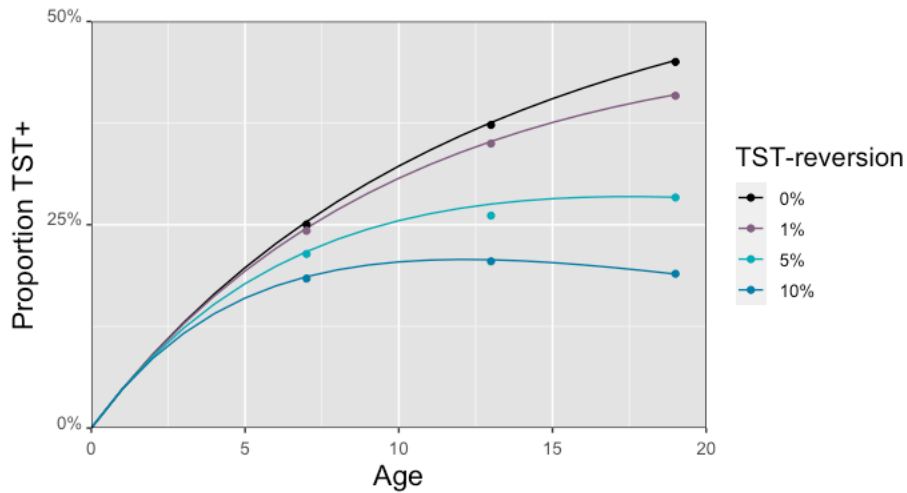
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APPENDICES:

APPENDIX 1:

Comparison of expected and observed TST-positive prevalence of 1925 Dutch birth cohort with varying TST-reversion rates.

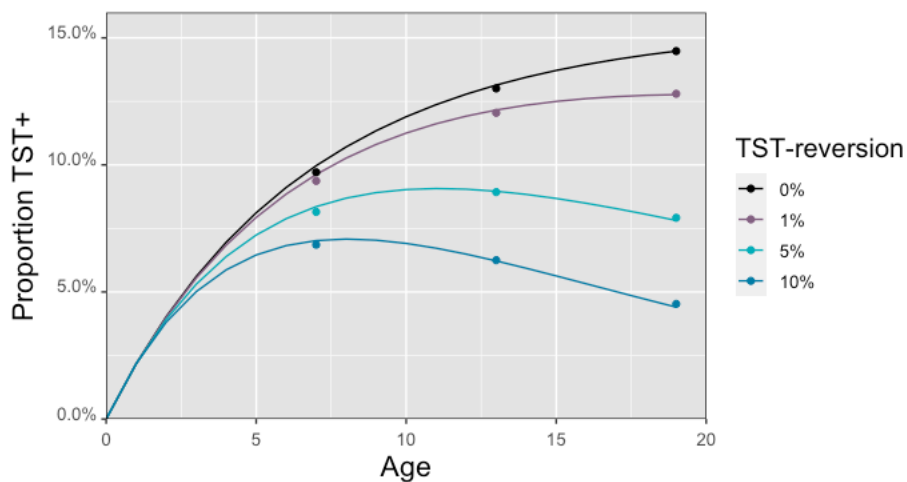


Base model TST-positive estimates accounting for different levels of TST-reversion. Model parameters based on 1925 Dutch birth cohort: ARI at birth = 5%, annual decrease in risk = 5%.

Observed TST-positive prevalence at ages 7, 13, and 19.

APPENDIX 2:

Comparison of expected and observed TST-positive prevalence of 1938 Dutch birth cohort with varying TST-reversion rates.

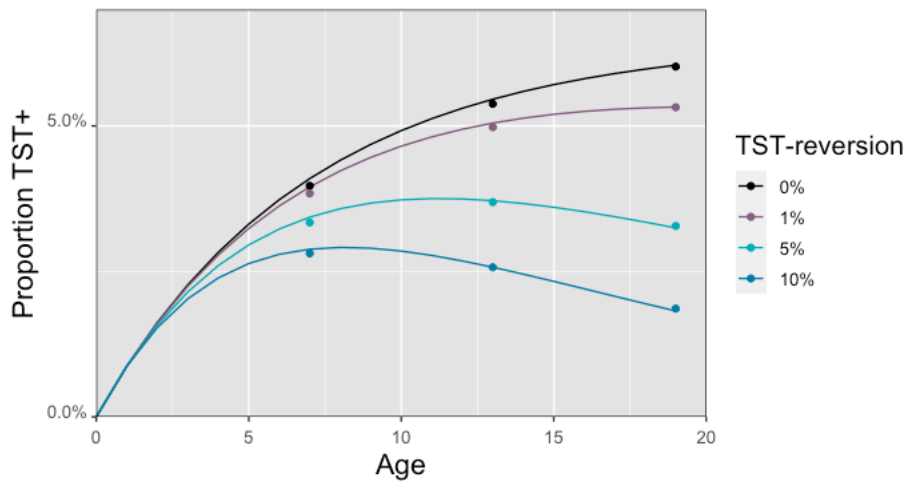


Base model TST-positive estimates accounting for different levels of TST-reversion. Model parameters based on 1938 Dutch birth cohort: ARI at birth = 2.5%, annual decrease in risk = 13%.

Observed TST-positive prevalence at ages 7, 13, and 19.

APPENDIX 3:

Comparison of expected and observed TST-positive prevalence of 1945 Dutch birth cohort with varying TST-reversion rates.

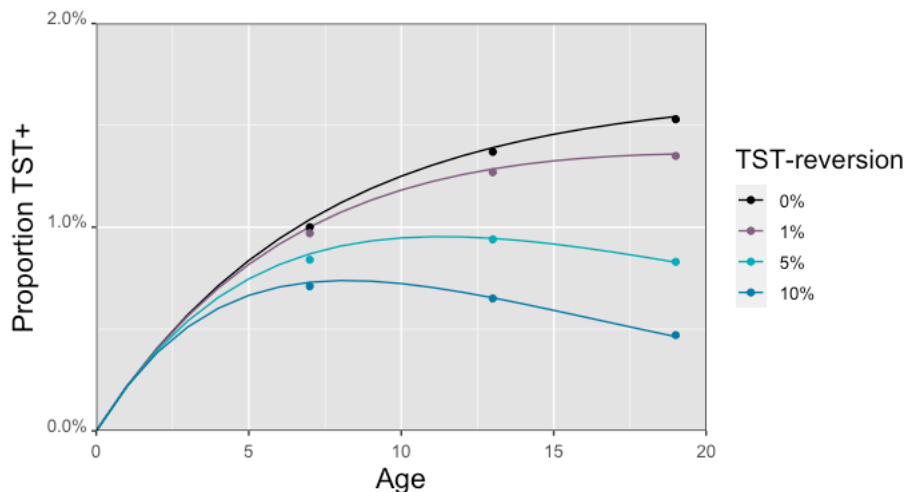


Base model TST-positive estimates accounting for different levels of TST-reversion. Model parameters based on 1945 Dutch birth cohort: ARI at birth = 1%, annual decrease in risk = 13%.

Observed TST-positive prevalence at ages 7, 13, and 19.

APPENDIX 4:

Comparison of expected and observed TST-positive prevalence of 1955 Dutch birth cohort with varying TST-reversion rates.

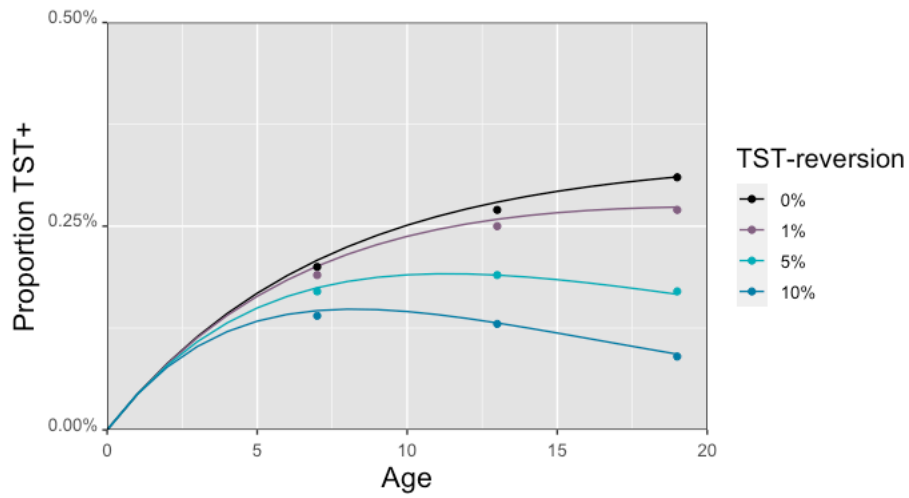


Base model TST-positive estimates accounting for different levels of TST-reversion. Model parameters based on 1955 Dutch birth cohort: ARI at birth = 0.25%, annual decrease in risk = 13%.

Observed TST-positive prevalence at ages 7, 13, and 19.

APPENDIX 5:

Comparison of expected and observed TST-positive prevalence of 1967 Dutch birth cohort with varying TST-reversion rates.



Base model TST-positive estimates accounting for different levels of TST-reversion. Model parameters based on 1967 Dutch birth cohort: ARI at birth = 0.05%, annual decrease in risk = 13%.

Observed TST-positive prevalence at ages 7, 13, and 19.