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Title: Impact of Reversion of Mycobacterium tuberculosis Immunoreactivity Tests on the Estimated Annual Risk of Infection

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Running Head: Reversion and the Annual Risk of Mycobacterium Tuberculosis Infection

Key words: Tuberculin skin test; Interferon-gamma release assay; Mtb transmission; TST/IGRA surveys

Abbreviations: ARI, annual risk of infection; BCG, Bacillus Calmette-Guérin; IGRA, interferon-gamma release assay; Mtb, Mycobacterium tuberculosis; TB, tuberculosis; TST, tuberculin skin test

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Abstract

A key metric in tuberculosis epidemiology is the annual risk of infection (ARI), which is usually derived from tuberculin skin test (TST) and interferon-gamma release assay (IGRA) prevalence surveys in children. Deriving the ARI assumes that immunoreactivity is persistent over time; however, reversion of immunoreactivity has long been documented. Here we use a deterministic, compartmental model of *Mycobacterium tuberculosis (Mtb)* infection to explore the impact of reversion on ARI estimation using age-specific reversion probabilities for TST and IGRA. Using empirical data of TST reversion (22.2%/year for 0-19yo), the true ARI is 2-5 times higher than estimated from immunoreactivity studies in 8-12 year-olds. Applying empirical reversion probabilities for IGRA (9.9%/year for 12-18yo) showed a 1.5-2-fold underestimation. ARIs are increasingly underestimated in older populations, due to the cumulative impact of reversion on population reactivity over time. Declines in annual risk did not largely affect the results. Ignoring reversion leads to a stark underestimation of the true ARI in populations and our interpretation of *Mtb* transmission intensity. Future surveys should adjust for reversion probabilities and its cumulative effect with increasing age to provide a more accurate reflection of the burden and dynamics of *Mtb* infection.

Introduction

Tuberculosis (TB) remains a major cause of morbidity and mortality worldwide, and it is estimated that one-quarter of the global population is latently infected with *Mycobacterium tuberculosis* (*Mtb*)(1–3). *Mtb* infection is inferred from the presence of a host immune response to *Mtb* protein components with the use of the tuberculin skin test (TST) or interferon-gamma release assay (IGRA)(4,5). While it is known that *Mtb* immunoreactivity does not equate to *Mtb* infection, population surveys of TST positivity have been historically used to derive estimates of

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Mtb infection risk and transmission trends, most conducted in school-aged children (8-12 years)(6). A key metric in TB epidemiology is the annual risk of infection (ARI) which aims to provide a more insightful picture of the risk of *Mtb* transmission (7). It is calculated using *Mtb* immunoreactivity test prevalence data and the mean age of the individuals surveyed (8). In a public health setting, a decrease in the ARI is interpreted as an early indicator of the decline in *Mtb* transmission in a population; on the other hand, an increase could indicate that TB prevention and care measures are insufficient (8).

When calculating ARIs, there is a conventional, usually implicit, assumption that positive *Mtb* immunoreactivity is persistent throughout an individual's lifetime (9). Nevertheless, this assumption does not hold. Tuberculous immunoreactivity can wane over time, and positive TST and IGRAs can revert to negative (reversion)(10–12). Therefore, a major caveat in the ARI is that the phenomenon of reversion is not accounted for in its calculation, thus resulting in a naïve ARI which might differ from the true value. Previous studies have considered the limitations of the current formula in arriving at an accurate estimate and interpretation of the ARI (10,13). In a theoretical study by Sutherland, the effects of TST reversion on the ARI were explored, suggesting a considerable underestimation when annual reversion probabilities exceed 1% (nearly 50% and 67%, when facing a 5% and 10% annual reversion probability, respectively)(13). However, this study only considered low reversion probabilities (0-10%), it did not consider age-specific effects, nor did it link to observed reversion data. While empirically observed reversion probabilities have been documented over a century ago (14), their importance has been largely dismissed. With a few notable exceptions, immunoreactivity surveys do not usually consider reversion when estimating the ARI (10,12). This is an issue as ARI estimates

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without consideration of reversion are likely to underestimate the proportion of individuals once infected with *Mtb* (8).

In contemporary policy, the ARI remains important and is estimated in TST/IGRA surveys in populations or high-risk settings (15–17). The ARI is also a common parameter in the mathematical modelling of TB, for example, to estimate the global burden of latent *Mtb* infection or to set the intensity of transmission in a population (3,18). Moreover, as novel diagnostic tools that measure *Mtb* immunoreactivity become available and immunoreactivity surveys may be reconsidered in global policy, it is important to consider the level reversion of specific tests so ARI underestimation can be appropriately quantified through current methods. This paper aims to use empirical estimates of reversion for TST and IGRA to quantify the extent of ARI underestimation due to reversion.

Methods

Model overview

We developed a deterministic, compartmental model of *Mtb* infection **(Figure 1)**. It builds on the theoretical study on the effect of constant TST reversion probabilities upon the ARI estimation proposed by Sutherland (13). The proportion of the population found to be immunoreactive at age a is expressed by P_a. The parameter k_a represents the real infection risk, a function of the ARI at birth (ARI_0), with subsequent annual decrease d in risk: $k_a = (1-d)^a \times ARI_0$.

Additionally, the model includes an annual constant proportion r of individuals with positive immunoreactivity that will revert. In order to estimate the proportion infected in the next year, the following formula is used:

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$$P_{(a+1)} = P_a + (1 - P_a) \times k_a - P_a \times r$$

The formula has three components: (1) the proportion of the population infected with *Mtb* in the current year, (2) plus the proportion of non-infected individuals who convert to positive immunoreactivity over the following year, (3) minus the proportion of immunoreactive individuals who revert over the following year. For a fixed initial ARI of 1.5%, *Mtb* immunoreactivity prevalence was calculated in daily timesteps using increasing reversion probabilities from 0% to 50% with 1% increments for 0-80 years. For all ages and *Mtb* immunoreactivity prevalence, the ARI was calculated using the classic formula $ARI_a = 1 - (1 - P_a)^{1/a}$. Then, the base ARI (not accounting for reversion) was compared against each reversion ARI (up to 50% reversion) as a ratio. Since we are considering that reversion is occurring but not accounted for in the calculation of the ARI, this ratio reflects how much the naïve ARI must increase to match the measured prevalence, resulting in the true ARI. The model was constructed and run using R v.4.1.0 (2021-05-18) for statistical computing and graphics (19). Plots were created using the *ggplot2* package (20).

Model assumptions

The key assumption of our model is that *Mtb* infection always leads to *Mtb* immunoreactivity, regardless of different cut-off values and incremental changes considered in conversion criteria (21). Furthermore, the model does not account for reinfections, assuming that reinfections occur at a similar rate as primary infections; this was done for simplicity. Finally, it assumes no child is immunoreactive at birth; therefore, $P_0 = 0$.

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Data sources for ARI estimates

A global ARI estimate was calculated from TST surveys used to re-estimate the global burden of latent TB infection (LTBI) by Houben and Dodd (3). This value was a simple average of ARI estimates from 141 TST surveys collected from Cauthen et al. and a systematic review of the literature (3,8). The resulting global average ARI of 1.5% (95%CI: 1.3-1.7) was used for the primary analysis. For the primary results, k_a was only dependent on ARI_0. The annual decline (2.3%) component of k_a was evaluated further in the sensitivity analyses.

Data sources for Mtb immunoreactivity test reversion

Reversion probabilities — classified per age group — were used to illustrate the degree of ARI underestimation obtained from the model. These were collected from two population-wide TST surveys and one adolescent IGRA survey. The first TST survey (Grzybowski and Allen) was conducted in 1959 on 29,000 individuals of all ages in Victoria County, Ontario, Canada; it consisted of five consecutive annual TST surveys, which considered a positive result as an area of induration of 5mm or more (11). At the time, BCG vaccination was not considered in newborns or infants and was only recommended for contacts of patients with active TB. The study provided numerators (number of reversions) and denominators (positive reactors retested in one year) used for age group-specific reversion probabilities; we calculated 95% confidence intervals for the given proportions to account for uncertainty in the probabilities. The second TST survey (Fine et al.) describes a set of over 64,000 TSTs performed in two total population surveys in the Karonga district, northern Malawi, from 1980 to 1989; TST reversion data was available from paired results from 6,991 individuals that participated in both surveys (10). A

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positive result was considered as an area of induration of 10mm or more. Reversion probabilities in females without a Bacillus Calmette-Guérin (BCG) scar were presented in a plot and were extracted using a web-based plot digitiser (22). Confidence intervals were not available as the absolute numerator and denominator were not provided. On the other hand, in the IGRA survey (Andrews et al.), which was conducted from 2005 to 2007, students aged 12 to 18 years of age were recruited from local schools in Worcester, South Africa (12). The age-specific annual *Mtb* immunoreactivity test reversion probabilities from all studies are displayed in **Table 1**.

To test the application of the model, ARI estimates from two population-wide TST surveys were used as illustrative examples to calculate the difference between the observed ARI of the studies and the true ARIs of the model. Firstly, the study by Hoa et al. was a nationwide TST survey in Viet Nam among children aged 6 to 14 years carried out from 2006 to 2007; the study resulted in an ARI estimate of 1.7% (95% CI: 1.5 – 1.8%) calculated from a TST-positive prevalence of 16.7% in a population with a mean age of 10.8 years (16). Secondly, the study by Wood et al. was conducted among HIV-negative individuals aged 5 to 40 years old in Cape Town, South Africa (17). 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.

Sensitivity analyses

We performed sensitivity analyses to assess the impact of the parameters on the ARI underestimation output. First, model outputs using the lower and upper bounds of the 95%

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confidence intervals of the baseline ARI (1.3-1.7) were explored. Additionally, considering the heterogeneity in the global TB burden, we also used an initial ARI of 5%, accounting for high-burden settings. Moreover, the component of annual risk decrease was incorporated into parameter k_a. The global annual rate of decline for TB incidence was estimated to be 2.3%, with some regions presenting more notable decreases (2).

Results

Figure 2 shows the degree of ARI underestimation due to ignoring reversion. In the 8-12 years age range, where most TST surveys are conducted, we found that for TST (and in the range of reversion probabilities from Grzybowski and Allen (11,12)), the true ARI is 2-to-5 times higher than estimated under the naïve scenario (i.e. assuming no reversion). With the following age-group reversion probabilities, the ARI underestimation is maintained in the older populations, rising to at least a 5-fold increase of the true ARI after age 60 **(Web Figure 1)**. The lower observed TST reversion probabilities from Fine et al. give a 1.25-to-1.50-fold increase of the true ARI from 3 years of age and a more than a 2-fold increase from age 12 onwards **(Web Figure 2)**. In the case of IGRA, the narrow reversion probabilities lead to a 1.50-to-2-fold increase of the true ARI for ages 12 to 18, within the reversion probabilities from Andrews et al.

Outside of the empirical reversion probabilities, **Figure 2** shows how ARI underestimation grows with increasing levels of annual reversion probabilities as well as with increasing age at which immunoreactivity was tested. Annual reversion probabilities up to 2.5% increase the true ARI by less than 1.25 times. After the first life year, changes in reversion probabilities for a particular age can reach diverse levels of underestimation **(Web Figure 1).**

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Evaluating the impact of reversion on the observed ARI of TST population-wide surveys showed that for the study by Hoa et al., the observed ARI of 1.7% at the mean age of 11, adjusting for reversion (using empirical reversion probabilities from Grzybowski and Allen (11)) showed the true ARI to be twice as that originally observed. Likewise, in the survey by Wood et al., the observed ARI of 3.9% would be increased by a factor of 1.5 at age 5 and by a factor of 2 and more at 10 and 15 years of age (considering the empirical reversion probabilities from Grzybowski and Allen (23)).

Sensitivity analyses

There was no notable difference between the contour maps produced by the lower and upper bounds of the 95% confidence interval of the 1.5% baseline ARI, within the reversion probability ranges from the TST and IGRA surveys **(Web Figure 3 and 4)**. When using a 5% baseline ARI, more discernible true ARI increases were evident at higher reversion probabilities **(Web Figure 5)**. Incorporating the global decline in TB incidence (2.3%) into the model increases the true ARI underestimation, albeit slightly **(Web Figure 6)**.

Discussion

We estimated that the true ARI for *Mtb* immunoreactivity surveys conducted in school-aged children and using empirical data on TST reversion is 2-5 times higher than the baseline value that does not account for reversion. Failing to account for *Mtb* immunoreactivity test reversion in estimating the ARI significantly underestimates the true value and the cumulative effect of reversion can be seen in time. Recent work by Dowdy and Behr explores ARI underestimation due to increasing infection risks in adolescence and early adulthood, resistance to infection, and

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immunoreactivity test reversion, concluding that the latter could underestimate the risk of infection by one-third or more (13). In our study we used empirical data for reversion and explored the impact across age groups in detail, highlighting how reversion is important on its own, but likely differs by age and immunoreactive test. More recent data on reversion, especially of new tools (24), are urgently needed; this is an important concept to explore and consider when interpreting future ARI estimates of recent surveys.

The original work by Sutherland concluded that reversion probabilities above 1% would significantly impact ARI estimates (24,25). However, as we have seen, empirical data for TST/IGRA reversions in populations have shown that the probabilities strongly exceed 1% per year and vary by age (26,27), although still poorly quantified and understood for new tests. Reversions may result from a myriad of different factors including self-clearance of *Mtb* infection, cross-reactivity with BCG vaccination or non-tuberculous mycobacteria (in the case of TST), and false-negative reactions due to impaired immune response. It is worth noting the difference in ARI underestimation depending on the tool used might not be related to the actual tool but likely depends on the TB incidence in those settings at the time of the surveys, since the likelihood of reversion might be influenced by reinfection. Thus, lower reversion probabilities could be seen in settings with a higher risk of reinfection (25,26). While data on reversion from novel diagnostic methods are non-existent at present, our work highlights why it is crucial to acquire such data and how they may impact ARI estimates. Nonetheless, regardless of how – and to what extent – reversions occur, our findings focus more on the implications of the underestimation and interpretation of the resulting ARI.

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Another essential issue of the interpretation of the ARI is its reliance on the host immune response to *Mtb*, which is an indirect ascertainment of *Mtb* infection. Because of the limitations of *Mtb* immunoreactivity tests, the interpretation of a positive test result as a marker of true infection, i.e., harbouring viable *Mtb* bacilli and being at risk of TB disease, is unclear. While our findings call for conscientious interpretations of the ARI given the reversion phenomenon, TB prevention and care may benefit from an improved biomarker for detecting *Mtb* infection that will enable a more direct estimation of the true ARI. Luckily, some biomarkers are already being explored (27,28); some providing the additional benefit of identifying individuals at higher risk of progression to active TB disease (28).

ARI estimates are key to understanding time trends in TB burden and dynamics and are important to inform subsequent policy. Given the substantial impact of reversion on ARI estimates, this naturally occurring phenomenon should be recognised in ARI calculations or, at the minimum, its interpretations (29). Our exploratory analysis of the TST prevalence surveys by Hoa et al. and Wood et al. illustrates how the true ARI can be at least two times higher than the naïve ARI (3). We may apply our understanding of the impact of ARI estimation to other existing surveys, such as India's recent nationally representative survey, as reversion would mean a true transmission risk of 2-5 times as high (30). Caution in the interpretation of the majority of published ARIs to date is essential, including global estimates of individuals recently or remotely infected with *Mtb* (30).

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Limitations

The reversion probabilities used to highlight the degree of underestimation may differ by TB incidence in the setting, the immunoreactivity test and the cut-off used. For the latter, issues arise from the use of reversion probabilities from the report of Grzybowski and Allen because of the instability of the test, mainly the variability around the 5mm single cut-off point (10). This issue is exacerbated by inter-reader variability and digit bias often encountered when using TSTs (31). In turn, the reversion probabilities from Fine et al. are more convincing as they adhere to the ATS/CDC definitions which address this variability (12,31). Despite this, we opted to base our TST results based on the reversion probabilities by Grzybowski and Allen since they provide a range of uncertainty in their estimates (32). Similarly, IGRA reversions are also overemphasized in the so-called uncertainty zone (0.2-0.7 IU/ml) around the default cut-off value, where they are as high as 52%, declining as the value increases (12,32) By presenting a wide range of reversion probabilities (up to 50%), we provide a contour map that serves as a guide that could be used to explore the ARI underestimation as seen by other empirical probabilities. While our simple model with two binary outcomes enables a clear analysis of the impact of reversion, it excluded other phenomena which could also play a role in *Mtb* immunoreactivity and, subsequently, the ARI. The model assumed that the risk of *Mtb* immunoreactivity was the same for primary infections and reinfections, and while it is not possible to determine if there would also be a reduced risk of immunoreactivity conversion (33), studies have shown a risk reduction in the progression of TB disease in previously 'infected' individuals, i.e. with positive *Mtb* immunoreactivity (34). Hypothetically, if we assumed that a risk reduction would be observed among individuals who had converted before, then, for the estimates accounting for reversion, the *Mtb* immunoreactivity prevalences — and their corresponding ARIs — would have been lower than those obtained in the primary analysis, thus resulting in a higher ratio and higher

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degree of underestimation. Another phenomenon that could affect the estimated ARI is individuals who are resistant to *Mtb* infection (i.e. repeatedly negative *Mtb* immunoreactivity tests in individuals who have had close contact with pulmonary TB patients, such as household contacts, miners, etc.)(35–37). Including an *Mtb* resistance parameter would affect the naïve and true ARI in similar ways, therefore, it is expected that it would not alter the true to naïve ARI ratios observed in our primary results. Finally, BCG vaccination and non-tuberculous mycobacteria exposure are known to cause false TST positives (10), which may contribute to a degree of overestimation when using reactivity to assess ARI. However, their contribution to reactivity and whether and how they may modify infection risks and reversion probability is unknown, so we did not include them in the model.

Conclusions

Not accounting for reversion leads to a stark underestimation of the true ARI in populations, which changes our understanding and interpretation of *Mtb* transmission intensity. Considering our findings, interpretations of ARI estimates should be handled prudently. Categorisation by ARI levels and mathematical models of TB disease relying on ARI as a parameter would need to be amended. Reversion probabilities specific to a region, test, and even age are needed to increase the interpretation of ARIs from future cross-sectional surveys. Adjusting for reversion probabilities and its cumulative effect with increasing age will provide a more accurate reflection of the burden and dynamics of *Mtb* infection.

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Table 1. Age-specific annual *Mtb* immunoreactivity test reversion probabilities.

First Author, Year (Reference No.) and Age Group, Years	Setting (Date Range)	Annual Reversion Probability, %	95% CI
TST surveys			
Grzybowski, 1964 (11)	Ontario, Canada (1958-1962)		
0-19		22.2	15.2, 31.4
20-39		8.0	4.9, 12.6
40-59		4.8	3.2, 6.9
≥ 60		9.0	6.5, 12.3
Fine, 1999 (10)	Karonga, Malawi (1980-1989)		
0-4		17.9	
5-9		10.2	
10-14		7.5	
15-19		6.1	
20-24		5.3	
25-29		4.8	
30-39		4.1	
≥ 40		3.7	
IGRA surveys			
Andrews, 2015 (12)	Worcester, South Africa (2005-2007)		
12-18		9.9	8.8, 11.1

CI, confidence interval; TST, tuberculin skin test; IGRA, interferon-gamma release assay.

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Figure 1. Model of immunoreactivity accounting for reversion.

Mtb: Mycobacterium tuberculosis; P_a: Proportion of the population found to be Mtb immunoreactive at age a; k_a: Real infection risk, which is a function of the annual risk of infection (ARI) at birth (ARI_0), with subsequent annual decrease d in risk; r: annual constant proportion of individuals with positive immunoreactivity that will revert.

Figure 2. Contour map of annual risk of infection (ARI) underestimation by varying annual reversion probabilities.

The ratio between true (varying reversion levels) and naïve annual risk of infection (ARI) (no reversion) represents true ARI increase. Baseline parameters: 1.5% ARI at birth and no decline in annual risk. Tuberculin skin test (TST) reversion probabilities from Grzybowski and Allen (represented by the red line; dotted red lines represent 95%CI) and (interferon-gamma release assay) IGRA reversion probabilities from Andrews et al. (represented by the yellow line; dotted yellow lines represent 95%CI) (11). White dotted lines represent the age range of populations where most TST surveys are conducted. The study by Grzybowski and Allen was conducted in Ontario, Canada (1958-1962) and the study by Andrew et al. was conducted in Worcester, South Africa (2005-2007).

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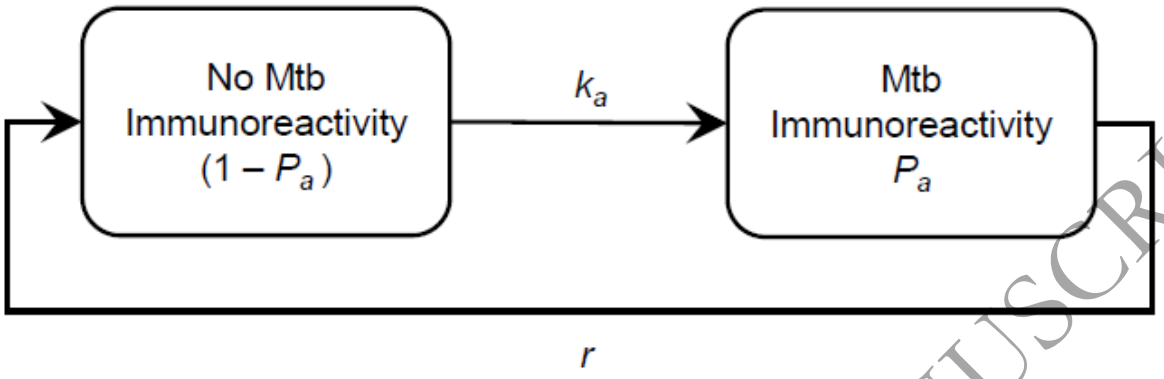

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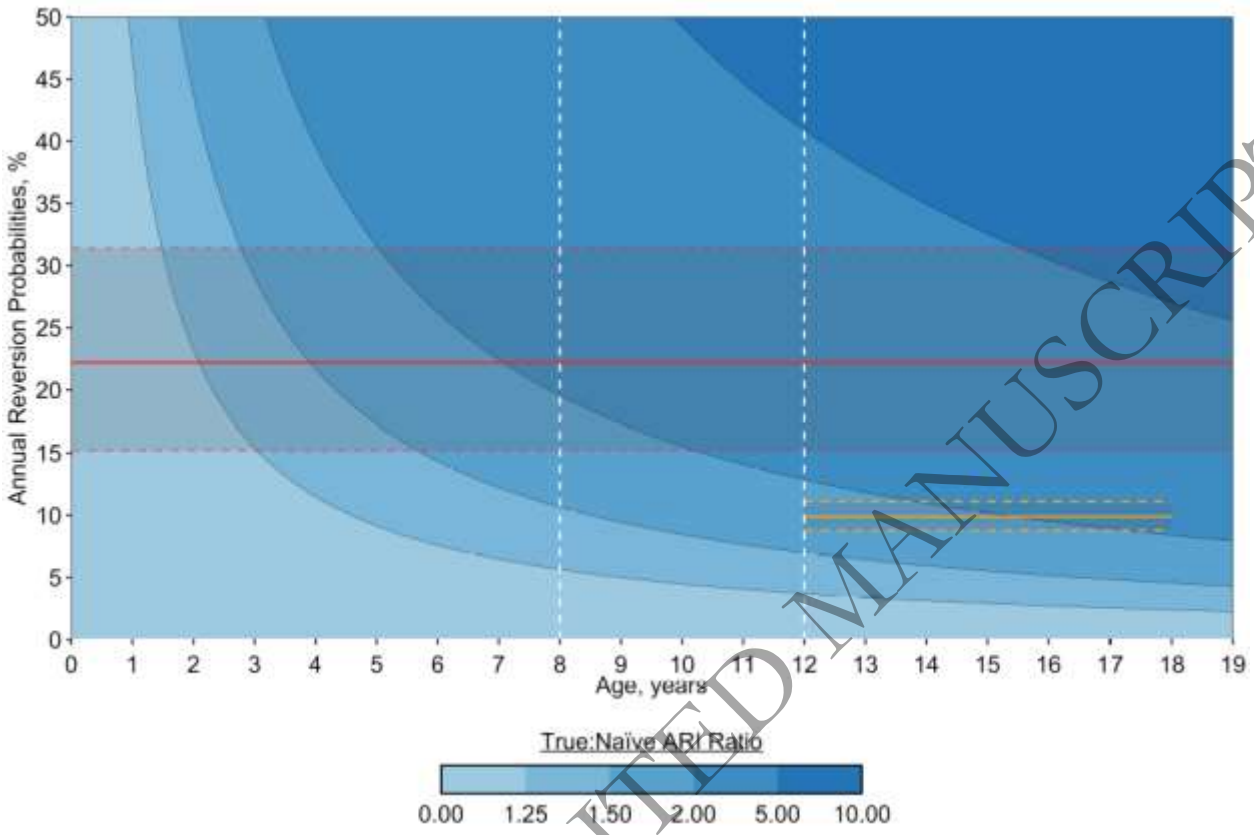

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