

Original Research Article

Does Latent Tuberculosis Lead to a Spurious Correlation between BCG and COVID-19 Mortality?

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Abstract

Background: Many factors have been suggested to confound coronavirus disease 2019 (COVID-19) studies, and BCG studies have been criticized for not adjusting for many confounders. We conducted this study to analyze the presumed effectiveness of the Bacillus Calmette–Guérin (BCG) vaccine in decreasing the COVID-19 mortality rate, and to answer the question of whether this is confounded by latent tuberculosis (LTB) prevalence.

Materials and methods: We chose sixty-nine malaria-free countries with different BCG vaccination policies. TB prevalence was considered as a proxy for LTB. The BCG, TB prevalence, and COVID-19 mortality data are publically available. Contingency coefficients (C.C.) and a ROC analysis were used to assess the relationship between TB prevalence and BCG status, and identify cutoff points in each BCG group category. A stem–leaf plot was also used to explore the data’s apparent behavior concerning COVID-19 in relation to the BCG groups.

Results: TB prevalence was significantly associated with BCG status. The BCG vaccination status apparently had a relationship with BCG status.

Conclusions: TB is suggested to have a confounding effect on BCG results, leading to a spurious correlation between BCG and COVID-19 mortality.

Keywords: COVID-19 mortality; latent tuberculosis; SARS-CoV-2; BCG; confounder

High Lights

• Many studies showed a relation between BCG and COVID-19 mortality reduction ,

• Influence of latent TB influence on COVID-19 mortality was also suggested later.

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• Sixty-nine malaria-free countries were selected to look for any association between latent TB prevalence and BCG countries' status, and to look for possible confounded LTB effect on BCG studies.

Introduction

The WHO currently recommends that, in countries with a high tuberculosis (TB) burden, a single dose of the Bacillus Calmette–Guérin (BCG) vaccine should be provided to all infants as soon as possible after birth as part of childhood immunization programs. In countries with low TB incidence rates, the provision of the BCG vaccine may be limited to neonates and infants in recognized high-risk groups, or older children who are negative for TB infection according to the tuberculin skin test (TST).¹ Despite clear evidence and the WHO's recommendations, however, global BCG administration practices appear to be arbitrary.^{2,3} Among 180 countries, 154 reported universal BCG vaccination, 20 reported having had a national BCG policy for everyone in the past, and the remaining six reported selective vaccination for persons in high-risk groups. BCG coverage ranged from 53% to 99%; the coverage was <80% in six high-incidence countries.⁴

Studies on the BCG vaccine's efficacy against TB have been confounded by the cross-reactivity of antigens and absence of measures for excluding latent infection.⁵

Early in the coronavirus disease 2019 (COVID-19) pandemic, Miller *et al.* (2020)⁶ showed that countries with mandated BCG vaccinations had lower COVID-19 morbidity and mortality rates. Furthermore, Sala *et al.*⁷, Berg *et al.*⁸, Dayal *et al.*⁹, Akiyama *et al.*¹⁰, Green *et al.*¹¹, Hegarty *et al.*¹², Shet *et al.*¹³, Ozdemir *et al.*¹⁴, and, more recently, Brooks *et al.*¹⁵

showed significant correlations between BCG and COVID-19 mortality. Early in this pandemic, it was also suggested by a few studies that latent tuberculosis (LTB) could mitigate COVID-19 morbidity and mortality.^{11,16,17,18,19,20,21} Some tested TB prevalence^{16,20,22} as a proxy for LTB, while others considered LTB estimates that might cross-react with BCG and other mycobacteria. Other studies considered positivity in the TST/interferon-gamma release assay (IGRA) as a measure of the potential protective effect of the resident populations' exposure to *Mycobacterium* spp., whether from BCG vaccination or as a result of exposure to environmental mycobacteria.^{17,18,23} Raham TF and Al-Momen H. et al. shed light on the effectiveness of TB prevalence as a proxy for LTB, according to different BCG groups bypassing the BCG effect.^{16,20}

BCG studies are likely to be confounded by LTB, since low-TB countries do not implement vaccination, and countries with high TB prevalence implement BCG programs. About one-quarter of the world's population has LTB,^{24,25} making the latter an important factor to adjust for.

Furthermore, malaria's confounding effect was not adjusted for in most of these studies; this is of special importance because malaria is highly endemic in most high-TB countries.

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The study of risk and confounding factors is very important since previous studies have shown hugely disparate case-fatality rates, in spite of some reporting significant findings. For example, disparities exist among both countries with low LTB incidence or BCG coverage and those with high LTB incidence or BCG coverage.²³ Regarding malaria's influence on COVID-19 mortality, it was suggested by a few preliminary studies to reduce it.^{22,26}

In the context of the conflicting evidence regarding BCG's significant correlations, the growing evidence of a role of LTB and malaria, and a lack of literature defining LTB's confounding effect in malaria-free countries, we suggest that a confounding effect exists, as a background hypothesis for this study. This study is important at this time because several clinical trials are underway to evaluate the efficacy of BCG vaccination for COVID-19.

Materials and Methods

It was not appropriate or possible to involve patients or the public in this work; we used data summated at the general practice level and related, publically published morbidity and mortality statistics.

The main objectives of this study were based on the hypothesis that the decreased mortality rate in BCG studies is related to the influence of LTB rather than the BCG effect, and that BCG studies have been confounded by LTB prevalence. We designed this study to look for an association between TB prevalence (reflected as LTB prevalence) and BCG policy status in the absence of a confounding effect from malaria, through restricting the sample to malaria-free countries. Furthermore, the stem-leaf graphical plot method was proposed to illustrate (apparent) BCG group behavior regarding COVID-19 mortality.

We selected countries that have achieved at least three consecutive years of zero indigenous cases of malaria. The total number of countries was 69, as shown in Appendix A. Countries eligible to apply for WHO certifications of malaria-free statuses were included.²⁷ Countries and territories with populations of less than 1 million were excluded and are listed in Appendix B.

The data for the TB prevalence, BCG, malaria, and COVID-19 mortality are publicly available (references are listed in Appendix B).

The chosen countries were distributed among the BCG category statuses, as shown in Appendix A, and categorized according to three ordinal scales (low: ≤ 15 , moderate: 16–49, and high: ≥ 50), according to the highest-available TB prevalence during 2011–2018. The different classifications of countries according to BCG-vaccine policy status are: just a single current BCG with no previous booster (JSC1-BCG), just a single current BCG with a previous booster (JSC2-BCG), multiple current BCGs (MC-BCG), one previous BCG (JP-BCG), and no previous or current BCG (NP/C-BCG or BCG: 0 or the BCG control group) (Appendix A).

Contingency coefficients (C.C.) and ROC analysis were used to test the relationship between TB prevalence and BCG status, and to identify the cutoff points in each BCG group category. A stem-leaf plot was also used to explore the data's apparent behavior concerning COVID-19 mortality rate for different BCG groups. All the statistical operations were performed using the statistical package SPSS, ver. 22.

Results and Findings

Table 1 shows the distribution of TB prevalence during 2011–2018 in three categorized BCG groups, as well as a comparison that is significant in terms of the contingency coefficient of the reflected relationship between the preceding factors; they had either a random or constrained distribution. The results show a highly significant relationship at $P < 0.01$, indicating that a meaningful constrained distribution is accounted for regarding the studied factors.

Regarding the low-TB-prevalence category the distribution of the NP/C-BCG group was 83.30% (highest), followed by 81.0% of the JP-BCG group, and the lowest was 6.3% of the JSC2-BCG group.

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Regarding the moderate-TB-prevalence category, the distributions were 75.0% of the JSC2-BCG group (highest), and both JP-BCG and MC-BCG were 14.3% (lowest).

Regarding the high-TB-prevalence group, the distributions were 71.4% for MC-BCG and 0.00% for NP/C-BCG (BCG: 0, or the BCG control group).

Table 2 and Figure 1 show estimates of the area of the trade-off between the sensitivity and specificity; sensitivity is plotted against a complementing specificity outcome to examine the trade-off, which is called the ROC curve. The significance level for the testing area was under 50% guideline, with a 95% confidence interval of all the probable combination pairs for the four statuses of the BCG groups under the proposed guideline group (the control) due to NP/C-BCG group status.

The results show that strong and highly significant asymptotic values regarding the area under the curve at $P < 0.01$, concerning the different BCG group categories, in just three groups. There was no significant area under the curve at $P > 0.05$ for the JP-BCG group. This indicates that the TB prevalence rates are good disseminators for the BCG groups: JSC1-BCG, JSC2-BCG, and MC-BCG. The highest TB cutoff point was within MC-BCG, which was 29.50, followed by JSC1-BCG (20.00) and then JSC2-BCG (15.00). The magnitudes of the areas under the curves followed the same ranking and were 0.964, 0.939, and 0.938, respectively, with highly significant asymptotic values and short 95% C.I intervals of 0.872–1.057, 0.848–1.029, and 0.835–1.040, respectively. A non-significant asymptotic value for JP-BCG signifies that TB prevalence does not categorize this group. The cutoff value was the lowest within this table (Table 2, Figure 1).

The stem-leaf graphical plots clearly illustrate the apparent behavior of COVID-19 mortality within the BCG groups (Figure 2); they show that the ranking of mortality rates by BCG group status gives the impression of high mortality within the no-BCG-vaccination groups, and low mortality in countries with BCG vaccination.

Discussion

Many BCG studies showing a significant relationship between BCG and a reduction in COVID-19 mortality and/or morbidity have been criticized for not considering confounding factors, and simply assessing the differences in the incidence/mortality of COVID-19 based on having or not having BCG vaccination policy, as well as sharing the same sources of information with questionable data accuracy.^{25,28,29,30} These studies were, therefore, considered to represent only weak evidence. On the other hand, other studies have found no statistical evidence for an association between BCG vaccination policy and either SARS-CoV-2 morbidity or mortality, as shown by Chimoyi L *et al.*³⁰, Aksu *et al.*³¹, Fukui M *et al.*²⁸, Clément *et al.*³², Hamiel U *et al.*³³, Asahara M³⁴, and Hensel J. *et al.*³⁵. However, these studies also did not define the possible confounding effects of TB or malaria.

A confounding factor may mask an actual association or falsely demonstrate an apparent association between a study's variables where no real association between them exists.³⁶ This confounder may lead to the overestimation of the true association between an exposure and outcome.³⁷ One of limitations of this study is that it did not address whether there is an overestimation of the BCG effect, since it focused on the relationship between TB prevalence and BCG status. Furthermore, it is limited by not measuring BCG vaccination coverage rate, stage of epidemic, socio-economic differences, and differences in practicing of preventive measures to contain the disease, etc.

Comment [U3]: .

The efficacy and effectiveness of BCG vaccination against TB have been found to differ considerably between studies and populations.³⁸

The BCG vaccine has a documented protective effect against TB, meningitis and disseminated TB in children, but it prevents neither primary infection nor, more importantly, the reactivation of a latent pulmonary infection, which is the principal source

of bacillary spread in the community. The impact of BCG vaccination on the transmission of *Mycobacterium* TB is, therefore, limited.³⁹

Despite BCG being effective in 50% of the target population, much controversy surrounds its effect on mild forms of infection, as well as the duration of its effect.²⁵

According to the WHO's recommendations, countries with low TB burdens may limit BCG vaccination to infants in high-risk groups (or TST-negative older children) and adults at high risk for occupational TB exposure and who are TST negative.³⁸ Most people with TB immunoreactivity do not develop active TB upon immunosuppression, suggesting that they have cleared their infections while retaining immunological memory to them.⁴⁰

In most European Union (EU) and Western European countries, the tuberculosis (TB) notification rates are lower than 20 cases per 100,000 population. This rate is decreasing by around 4% yearly in the EU, overall. In 2003, it reached 13.8 per 100,000.^{41,42}

Table 1 shows a highly significant association between TB prevalence and certain BCG groups: countries not implementing BCG vaccinations had low TB prevalence, and vice versa (p value = 0.000). Table 2 and Figure 1 show the ROC analyses indicating that BCG group is significantly associated with corresponding TB prevalence. These results confirm an association between TB prevalence and BCG status.

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The finding of this study that BCG status is highly associated with TB prevalence leads us to conclude that BCG studies can be easily confounded by LTb. The ranking of mortality rates within BCG group statuses shown in a stem-leaf plot (Figure 2) follows the rank of association between TB prevalence and BCG status. This gives the impression of high mortality within groups with no BCG vaccination and low mortalities within countries with BCG vaccination.

This could apply to all BCG studies not adjusting for LTb.

In countries that do not undertake vaccination, confounding occurs simply because of a possible low TB prevalence, giving a false impression that not administering BCG is the cause of high mortality. Another possible confounder is previous TB prevalence, since the immunity generated by TB lasts for a certain period of time. We tried to control for this by considering the highest available TB prevalence during 2011–2018.

For these reasons, BCG studies should be designed properly to avoid bias. Estimation using the TST could also confound the LTB studies since BCG results in a positive TST result. We took the TB prevalence among countries as a proxy reflecting LTB infection to avoid this bias. However, in clinical trials, both TST and IGRA testing seem to be important, since BCG vaccination can cause a positive result for nontuberculous mycobacteria, while IGRA testing does not.⁴³

The low COVID-19 mortality in some countries cannot be explained by either low TB prevalence or malaria-free status, such as in Cyprus, which has not implemented BCG vaccination, and Slovakia, which previously implemented a BCG program. These findings suggest that other factors play roles in decreasing COVID-19 mortality

Conclusions

BCG country status has a highly significant relationship with TB prevalence, which could confound BCG and COVID-19 mortality and morbidity studies. We recommend that TB’s potential confounding effect on BCG results should be considered in ongoing and future studies and trials. The traditional TST might be more appropriate than IGRA testing.

Ethical permission was not necessary for this study, as we analyzed publicly published data and patients were not involved.

Table (1): "Kendall's- τ Correlation Coefficient" mortality rates marker and highest TB prevalence in 2011-2018.

| Marker | Correlation Coeff. and P-value | TB prevalence 2018 |
|---|--------------------------------|--------------------|
| COVID-19 deaths /M in September ,2 ,2020 | Correlation Coefficient | -0.175 * |
| | Sig. (2-tailed) | 0.046 |
| | No. | 69 |
| * Correlation is significant at the .05 level (2-tailed). | | |

Table (2): Distribution of different BCG Status due to COVID-19 deaths /M with comparison's significance.

| BCG Status | No. and % | COVID -19 deaths /M up to August 2, 2020. | | Total | C.S. P-value |
|------------|-----------|---|-----------------|-------|--------------|
| | | < 50 Death / M. | > 50 Death / M. | | |
| | | | | | |

| | | | | | |
|-------------------------------|-------|-------|-------|------|-----------------------------|
| JSC1-BCG | No. | 13 | 6 | 19 | LRT = 9.757 P=0.045 S |
| | % No. | 68.4% | 31.6% | 100% | |
| JSC2-BCG | No. | 7 | 9 | 16 | |
| | % No. | 43.8% | 56.3% | 100% | |
| MC-BCG | No. | 1 | 6 | 7 | |
| | % No. | 14.3% | 68.4% | 100% | |
| JP-BCG | No. | 6 | 15 | 21 | |
| | % No. | 28.6% | 71.4% | 100% | |
| NP/C-BCG (Control) | No. | 2 | 4 | 6 | |
| | % No. | 33.3% | 66.7% | 100% | |
| Total | No. | 29 | 40 | 69 | |
| | % No. | 42.0% | 58.0% | 100% | |

^(*) S: Sig. at P<0.015; testing based on a LRT: likelihood ratio test

Just single current BCG- no previous booster (JSC1-BCG), just single current with previous booster (JSC2-BCG), multiple current BCG (MC-BCG), just previous BCG (JP-BCG), and no previous or current BCG (NP/C-BCG or , BCG : 0 or the BCG controlled group.

Table (3): Descriptive statistics of (COVID -19 deaths /M) and testing of all probable combinations towards countries' BCG status classifications.

| Statistics | | BCG Status | | | | |
|---------------------|----------|----------------------------------|-----|-----|-----|-----|
| | | I | II | III | IV | V |
| 5% Trimmed Mean | | 48 | 89 | 159 | 206 | 310 |
| Median | | 34 | 63 | 119 | 108 | 237 |
| Minimum | | 0 | 5 | 10 | 4 | 17 |
| Maximum | | 182 | 591 | 297 | 622 | 853 |
| Range | | 182 | 586 | 287 | 618 | 836 |
| Interquartile Range | | 63 | 159 | 230 | 370 | 615 |
| Kruskal-Wallis Test | | Chi-Square = 16.482 | | | | |
| P-value | | P=0.022 (S) | | | | |
| Mann-Whitney Test | I X II | Z = - 1.623; P=0.109 ; (NS)/(CE) | | | | |
| | I X III | Z = - 2.342 ; P=0.018; (S) | | | | |
| | I X IV | Z = - 2.804; P=0.004 ; (HS) | | | | |
| | I X V | Z = - 1.814; P=0.069 ; (NS)/(CE) | | | | |
| | II X III | Z = -1.370; P=0.175 ; (NS)/(CE) | | | | |
| | II X IV | Z = -1.364; P=0.175 ; (NS)/(CE) | | | | |

| | |
|----------|-----------------------------|
| II X V | Z = - 1.290; P=0.203 ; (NS) |
| III X IV | Z = - 0.027; P=1.000 ; (NS) |
| III X V | Z = - 0.714; P=0.534 ; (NS) |
| IV X V | Z = - 0.437; P=0.670 ; (NS) |

HS: Highly Sig. at P<0.01; S: Sig. at P<0.05; NS: Non Sig. at P>0.05. CE: confounding effect if p<0.2

I:(JSC1-BCG :Just single current BCG no previous booster); II:(JSC2-BCG :Just single current with previous booster);III:(MC-BCG :Multiple Current BCG); IV:(JP-BCG :Just Previous BCG); V:(NP/C-BCG(Control) BCG : 0 = No Previous or Current BCG).

Comment [U4]: Arrange this explanation

Table (4): Distribution of highest TB prevalence 2011- 2018 in three categorized responses by different BCG statuses, as well as comparison significant.

| BCG Status | No. and % | Highest TB prevalence recurrently in 2011- 2018 . | | | Total | CS P-value |
|------------|-----------|---|--------|-------|-------|-------------------------------|
| | | Low | Mode. | High | | |
| JSC1-BCG | No. | 2 | 5 | 12 | 19 | C.C. = 0.659 P=0.000 HS |
| | % No. | 10.5% | 26.3% | 63.2% | 100% | |
| JSC2-BCG | No. | 1 | 12 | 3 | 16 | |
| | % No. | 6.3% | 75.0% | 18.8% | 100% | |
| MC-BCG | No. | 1 | 1 | 5 | 7 | |
| | % No. | 14.3% | 14.3% | 71.4% | 100% | |
| JP-BCG | No. | 17 | 3 | 1 | 21 | |
| | % No. | 81.0% | 14.3% | 4.8% | 100% | |
| NP/C-BCG | No. | 5 | 1 | 0 | 6 | |
| | % No. | 83.30% | 16.70% | 0.00% | 100% | |
| Total | No. | 26.0 | 22.0 | 21 | 69 | |
| | % No. | 37.7% | 31.9% | 30.4% | 100% | |

^(*) HS: Highly Sig. at P<0.01, Testing based on a contingency coefficient – C.C. test

Just single current BCG- no previous booster (JSC1-BCG), just single current with previous booster (JSC2-BCG), multiple current BCG (MC-BCG), just previous BCG (JP-BCG), and no previous or current BCG (NP/C-BCG or , BCG : 0 or the BCG controlled group).

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Table (5): ROC analyses with higher available TB prevalence in 2011- 2018 accuracy in discriminating for BCG group status.

| BCG Status | Cutoff Point | Sen. | Spec. | Area | Std. Error | Asymp. Sig. ^(*) | Asymp. 95% C.I. | |
|------------|--------------|-------|-------|-------|------------|----------------------------|-----------------|-------|
| | | | | | | | L.b. | U.b. |
| JSC1-BCG | 20.00 | 0.842 | 1.000 | 0.939 | 0.046 | 0.001 | 0.848 | 1.029 |

| | | | | | | | | |
|----------|-------|-------|-------|-------|-------|-------|-------|-------|
| JSC2-BCG | 15.00 | 0.938 | 0.833 | 0.938 | 0.052 | 0.002 | 0.835 | 1.040 |
| MC-BCG | 29.50 | 0.857 | 1.000 | 0.964 | 0.047 | 0.005 | 0.872 | 1.057 |
| JP-BCG | 19.50 | 0.143 | 1.000 | 0.528 | 0.144 | 0.838 | 0.246 | 0.810 |

(*) HS: Highly Sig. at $P < 0.01$; NS: Non Significant at $P > 0.05$. Just single current BCG- no previous booster (JSC1-BCG), just single current with previous booster (JSC2-BCG), multiple current BCG (MC-BCG), just previous BCG (JP-BCG), and no previous or current BCG (NP/C-BCG or , BCG : 0 or the BCG controlled group).

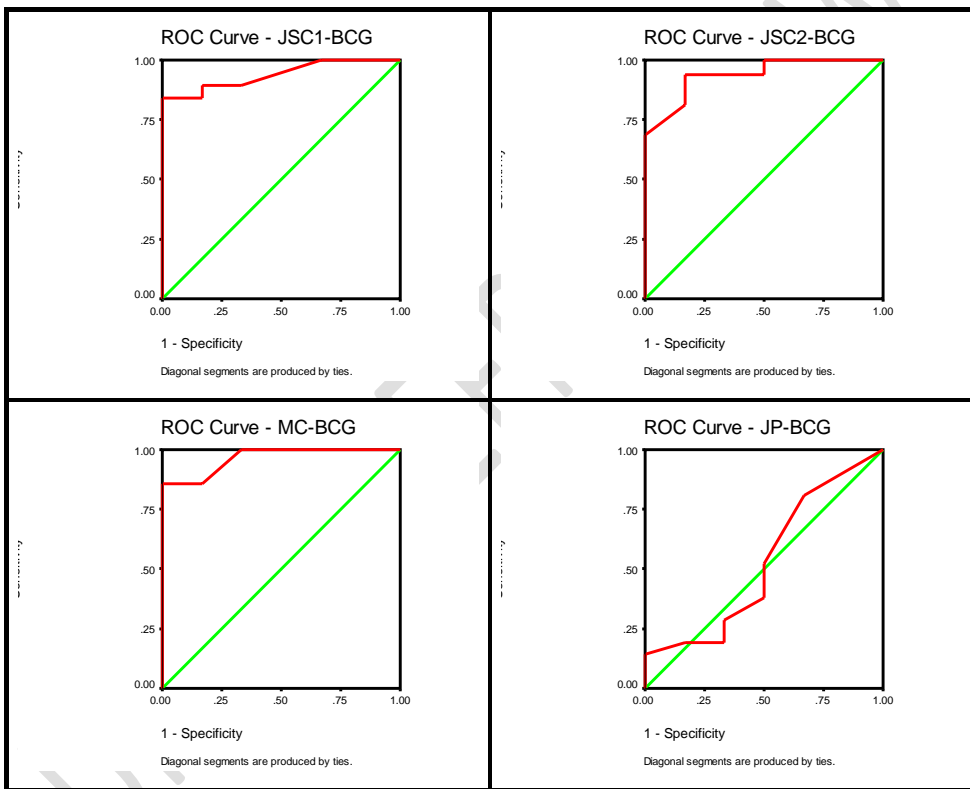


Figure (1): ROC Curve plots for studied TB prevalent rates in relation with BCG different categorized groups.

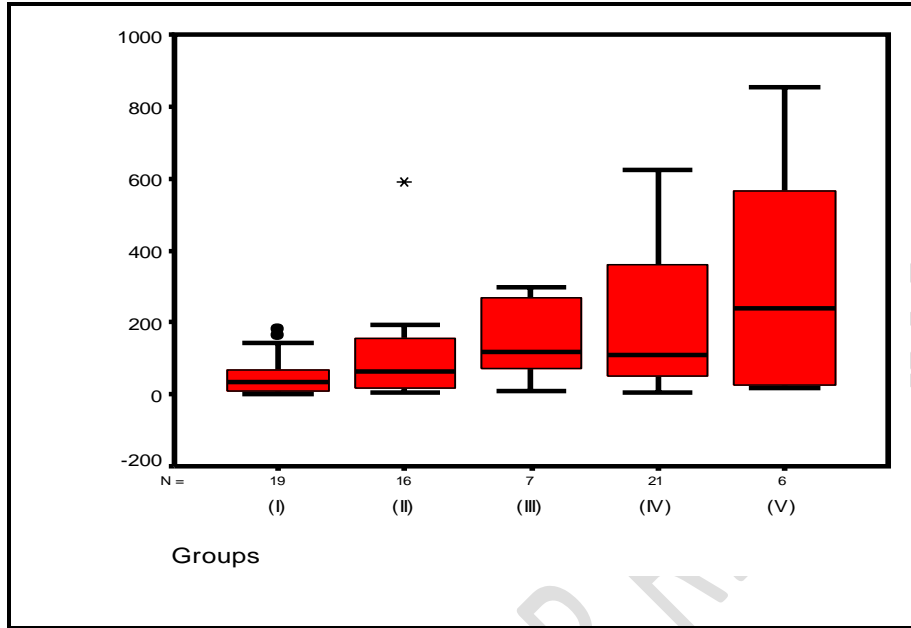


Figure (2): Stem-Leaf plots of (COVID-19 deaths /M up to August 2, 2020) due to effects of studied marker (BCG status). I:(JSC1-BCG :Just single current BCG no previous booster); II:(JSC2-BCG :Just single current with previous booster);III:(MC-BCG :Multiple Current BCG); IV:(JP-BCG :Just Previous BCG); V:(NP/C-BCG(Control) BCG : 0 = No Previous or Current BCG).

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References

- ¹ Global tuberculosis report 2017. Geneva: World Health Organization; 2017. Licence: CC BY-NC-SA 3.0 IGO.p103
- ² Zwerling A, Behr MA, Verma A, Brewer TF, Menzies D, Pai M. The BCG World Atlas: a database of global BCG vaccination policies and practices. PLoS Med. 2011;8:e1001012. [DOIExternal LinkPubMedExternal Link](#)
- ³ Pooransingh S, Sakhamuri S. Need for BCG Vaccination to Prevent TB in High-Incidence Countries and Populations. Emerg Infect Dis. 2020;26(3):624-625. <https://dx.doi.org/10.3201/eid2603.191232>
- ⁴ World Health Organization. Global tuberculosis report 2018 [cited 2019 Aug 4]. <https://apps.who.int/iris/handle/10665/274453>

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- ⁵ Updated guidelines for using Interferon Gamma Release Assays to detect Mycobacterium tuberculosis infection - United States, 2010.
- ⁶ Miller A, Reandelar MJ, Fasciglione K, Roumenova V, Li Y, Otazu GH. Correlation between universal BCG vaccination policy and reduced morbidity and mortality for COVID-19: an epidemiological study. MedRxiv. 2020.
- ⁷ Sala G, Miyakawa T. Association of BCG vaccination policy with prevalence and mortality of COVID-19. MedRxiv. 2020.
- ⁸ . Berg MK, Yu Q, Salvador CE, Melani I, Kitayama S. Mandated Bacillus Calmette-Guérin (BCG) vaccination predicts flattened curves for the spread of COVID-19. Medrxiv. 2020; 6(32):eabc1463.
- ⁹ Dayal D, Gupta S. Connecting BCG vaccination and COVID-19: additional data. MedRxiv. 2020.
- ¹⁰ Akiyama Y, Ishida T. Relationship between COVID-19 death toll doubling time and national BCG vaccination policy. MedRxiv. 2020
- ¹¹ Green CM, Fanucchi S, Fok ET, Moorlag SJ, Dominguez-Andres J, Negishi Y, *et al.* COVID-19: A model correlating BCG vaccination to protection from mortality implicates trained immunity. MedRxiv. 2020.
- ¹² Hegarty PK, Sfakianos JP, Giannarini G, DiNardo AR, Kamat AM. COVID-19 and Bacillus Calmette-Guérin: What is the link?. European Urology Oncology. Eur Urol Oncol. 2020;3(3):259-61.
- ¹³ Shet A, Ray D, Malavige N, Santosham M, Bar-Zeev N. Differential COVID-19-attributable mortality and BCG vaccine use in countries. MedRxiv. 2020.
- ¹⁴ . Ozdemir C, Kucuksezer UC, Tamay ZU. Is BCG vaccination effecting the spread and severity of COVID-19?. Allergy. 2020;75(7):1824-1827
- ¹⁵ Brooks NA, Puri A, Garg S, Nag S, Corbo J, Turabi AE, Kaka N, Zimmel RW, Hegarty PK, Kamat AM. The association of Coronavirus Disease-19 mortality and prior bacille Calmette-Guerin vaccination: a robust ecological analysis using unsupervised machine learning. Sci Rep. 2021 Jan 12;11(1):774. doi: 10.1038/s41598-020-80787-z. PMID: 33436946; PMCID: PMC7804196.
- ¹⁶ TF,Raham. TB Prevalence Influence on Covid-19 Mortality. medRxiv 2020.05.05.20092395; doi: <https://doi.org/10.1101/2020.05.05.20092395>
Now published in : *International Journal of Psychosocial Rehabilitation*. 24(10) ;2020 : 3679- 3690

¹⁷ Singh S, Maurya RP, Singh RK (2020) “Trained immunity” from *Mycobacterium* spp. exposure or BCG vaccination and COVID-19 outcomes. *PLOS Pathogens* 16(10): e1008969. <https://doi.org/10.1371/journal.ppat.1008969>

¹⁸ Takahashi, H. Role of latent tuberculosis infections in reduced COVID-19 mortality: Evidence from an instrumental variable method analysis. *Medical Hypotheses*. 2020. :144110214, <https://doi.org/10.1016/j.mehy.2020.110214.657-8501>, Japan

¹⁹ Banerjee S, Saha A .Finding Tentative Causes for the Reduced Impact of Covid-19 on the Health Systems of Poorer and Developing Nations: An Ecological Study of the Effect of Demographic, Climatological and Health Related Factors on the Global Spread of Covid-19 .medRxiv preprint doi: <https://doi.org/10.1101/2020.05.25.20113092>. posted May 26, 2020.

²⁰ Al-Momen H, Raham TF, Daher AM. Tuberculosis versus COVID-19 Mortality: A New Evidence. *Open Access Maced J Med Sci* [Internet]. 2020Sep.25 [cited 2021Jan.19];8(T1):179-83. Available from: <https://www.id-press.eu/mjms/article/view/5248>

²¹ Singh BR, Gandharva R, Karthikeyan R, Singh SV, Yadav A, Vinodh Kumar OR et al. Epidemiological determinants of acute respiratory syndrome coronavirus-2 disease pandemic and the role of the Bacille-CalmetteGuerin vaccine in reducing morbidity and mortality. *J Pure Appl Microbiol*. 2020;14.

²² Raham TF .Influence of Malaria Edemicity and Standardized TB Prevalence to BCG Coverage on Covid-19 Mortality medRxiv 2020.09.09.20191684; doi: <https://doi.org/10.1101/2020.09.09.20191684>

²³ Singh S. BCG Vaccines May Not Reduce Covid-19 Mortality Rates. medRxiv 2020;2020.04.11.20062232. Available from: <http://medrxiv.org/content/early/2020/04/17/2020.04.11.20062232>. <https://doi.org/10.1101/2020.04.11.20062232>

²⁴ Comstock GW, Livesay VT, Woolpert SF. *The prognosis of a positive tuberculin reaction in childhood and adolescence*. *Am J Epidemiol*. 1974;99:131-8

²⁵ Soheila Alyasin, Zahra Kannejad, Hossein Esmaeilzadeh, Hesamedin Nabavizadeh , Mohammad Amin Ghatee , and Reza Amin. Relationship between Bacillus Calmette Guerin Vaccination Policy and Coronavirus Disease-2019 (COVID-19) Incidence . SHORT PERSPECTIVE.. 2021: In Press Iran J Allergy Asthma Immunol In press. PUBLISHED: 2021-01-01

²⁶ Raham TF. Malaria Elimination Date Correlation to COVID-19 Mortality: New Evidence (November 21, 2020). SSRN: <https://ssrn.com/abstract=3735069> or <http://dx.doi.org/10.2139/ssrn.3735069>

²⁷ WHO .Malaria . Certification process. Last update: 13 March 2018

<https://www.who.int/malaria/areas/elimination/certification/en/>

accessed :1/9/2020

²⁸ Fukui M, Kawaguchi K, Matsuura H.Does TB Vaccination Reduce COVID-19 Infection? No Evidence from a Regression Discontinuity Analysis

medRxiv 2020.04.13.20064287; doi: <https://doi.org/10.1101/2020.04.13.20064287>

²⁹ Riccò M, Gualerzi G, Ranzieri S, Bragazzi NL. Stop playing with data: there is no sound evidence that Bacille Calmette-Guérin may avoid SARS-CoV-2 infection (for now). *Acta Bio-medica : Atenei Parmensis*. 2020 May;91(2):207-213. DOI: 10.23750/abm.v91i2.9700.

³⁰ Chimoyi L, Velen K, Churchyard GJ, Wallis R, Lewis JJ, *et al.* (2020) An ecological study to evaluate the association of Bacillus Calmette-Guerin (BCG) vaccination on cases of SARS-CoV2 infection and mortality from COVID-19. *PLOS ONE* 15(12): e0243707. <https://doi.org/10.1371/journal.pone.0243707>

³¹ Aksu K, Naziroğlu T, Özkan P. Factors determining COVID-19 pneumonia severity in a country with routine BCG vaccination. *Clin Exp Immunol*. 2020 Nov;202(2):220-225

³² Clément de Chaisemartin, Luc de Chaisemartin. BCG vaccination in infancy does not protect against COVID-19. Evidence from a natural experiment in Sweden.

medRxiv 2020.06.22.20137802; doi: <https://doi.org/10.1101/2020.06.22.20137802>

Now published in *Clinical Infectious Diseases* doi: [10.1093/cid/ciaa1223](https://doi.org/10.1093/cid/ciaa1223)

³³ Hamiel U, Kozer E, Youngster I. SARS-CoV-2 Rates in BCG-Vaccinated and Unvaccinated Young Adults. *JAMA*. 2020;323(22):2340–2341. doi:10.1001/jama.2020.8189

³⁴ Asahara M. The effect of BCG vaccination on COVID-19 examined by a statistical approach: no positive results from the Diamond Princess and cross-national differences previously reported by world-wide comparisons are flawed in several ways

medRxiv 2020.04.17.20068601; doi: <https://doi.org/10.1101/2020.04.17.20068601>

³⁵ Hensel, J., McAndrews, K.M., McGrail, D.J. *et al.* Protection against SARS-CoV-2 by BCG vaccination is not supported by epidemiological analyses. *Sci Rep* 10, 18377 (2020). <https://doi.org/10.1038/s41598-020-75491-x>

³⁶ Pearl J. *Causality. Models, reasoning and inference.* New York: Cambridge University Press, 2000.

³⁷ Sonis J. A closer look at confounding. *Fam Med.* 1998 Sep;30(8):584-8. PMID: 9773290.

³⁸ WHO. Report on BCG vaccine use for protection against mycobacterial infections including tuberculosis, leprosy, and other nontuberculous mycobacteria (NTM) infections. Prepared by the SAGE Working Group on BCG Vaccines and WHO Secretariat 22. September 2017.p24 https://www.who.int/immunization/sage/meetings/2017/october/1_BCG_report_revised_version_online.pdf?ua=1

³⁹World Health Organization. Implementing the WHO Stop TB Strategy: A Handbook for National Tuberculosis Control Programmes. Geneva. 2008. 8, BCG vaccination. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK310750/>

⁴⁰ Marcel B A, Paul E H, Lalita R. Is Mycobacterium tuberculosis infection life long? *BMJ* 2019; 367 :l5770

⁴¹ EuroTB (InVS/KNCV) and the national coordinators for tuberculosis surveillance in the WHO European Region. Surveillance of tuberculosis in Europe. Report on tuberculosis cases notified in 2003, Institut de Veille Sanitaire, Saint-Maurice, France. September 2005.

⁴² Infuso A, Falzon D, on behalf of the Euro-TB network. European survey of BCG vaccination policies and surveillance in children, 2005. Euro Surveill. 2006;11(3):pii=604. <https://doi.org/10.2807/esm.11.03.00604-en>

⁴³ Sharma SK, Vashishtha R, Chauhan LS, Sreenivas V, Seth D (2017) Comparison of TST and IGRA in Diagnosis of Latent Tuberculosis Infection in a High TB-Burden Setting. *PLoS ONE* 12(1): e0169539. <https://doi.org/10.1371/journal.pone.0169539>

Appendix A :TB Prevalence 2011-2018 and COVID-19 deaths/Million reported on September 2, 2020 in different classification according to BCG vaccination policy

| Country | Covid-19 Deaths/M 2/September 2020 | TB Prevalence highest available figure in 2011- 2018. |
|---|---------------------------------------|--|
| JSC1-BCG : Just Single Current BCG no Previous Booster | | |
| Algeria | 34 | 75 |
| Lesotho | 0 | 788 |
| Mauritius | 8 | 22 |
| Jordan | 1 | 530 |
| Iraq | 182 | 43 |
| Libya | 35 | 40 |
| Morocco | 31 | 107 |
| Qatar | 71 | 760 |
| United Arab Emirates | 39 | 140 |
| Albania | 101 | 550 |
| Greece | 26 | 490 |
| Hungary | 64 | 9 |
| Lithuania | 32 | 56 |
| Kyrgyzstan Kyrgyz Republic | 162 | 144 |
| Turkmenistan | 0 | 70 |
| Cuba | 8 | 7 |
| Jamaica | 7 | 5 |
| Paraguay | 46 | 43 |
| Sri Lanka | 144 | 65 |
| JSC1-BCG : Just Single Current with Previous Booster | | |
| Bosnia and Herzegovina | 189 | 37 |
| Poland | 54 | 19 |
| Japan | 10 | 17 |
| Mongolia | 111 | 428 |
| Kuwait | 125 | 23 |
| Singapore | 5 | 47 |
| Argentina | 193 | 27 |
| Uruguay | 13 | 33 |
| Estonia | 48 | 18 |
| Latvia | 18 | 29 |
| Romania | 192 | 84 |
| Chile | 591 | 18 |
| Serbia | 82 | 17 |
| Tunisia | 7 | 35 |
| Croatia | 46 | 8 |
| Belarus | 72 | 55 |
| MC-BCG: Multiple Current BCG | | |
| Armenia | 297 | 41 |
| Kazakhstan | 84 | 89 |
| Republic of Moldova | 250 | 152 |
| Republic of North Macedonia | 290 | 13 |
| Uzbekistan | 10 | 79 |
| Russian Federation | 119 | 80 |

| | | |
|--|-----|----|
| Ukraine | 60 | 91 |
| JP-BCG: Just Previous BCG | | |
| Italy | 587 | 7 |
| UK and Northern Ireland | 611 | 10 |
| Sweden | 575 | 9 |
| Switzerland | 232 | 7 |
| Canada | 242 | 6 |
| Austria | 81 | 8 |
| Spain | 622 | 12 |
| Slovakia | 6 | 7 |
| Slovenia | 64 | 7 |
| Czechia | 40 | 18 |
| Denmark | 108 | 6 |
| Finland | 61 | 6 |
| France (Metropolitan) | 469 | 9 |
| Germany | 112 | 8 |
| Ireland | 359 | 7 |
| Israel | 104 | 8 |
| Trinidad and Tobago | 18 | 21 |
| Australia | 26 | 7 |
| Norway | 49 | 6 |
| Portugal | 179 | 24 |
| New Zealand | 4 | 65 |
| NP/C-BCG: BCG : 0 = No Previous or Current BCG (The Controlled Group) | | |
| Lebanon | 26 | 13 |
| Bahrain | 111 | 18 |
| Netherlands | 363 | 6 |
| Belgium | 853 | 9 |
| United States of America | 567 | 7 |
| Cyprus | 17 | 6 |

TB Prevalent Groups: (Low: ≤ 15), (Moderate: 16-49), and (High: ≥ 50).

Appendix B : references for data

| Category | References |
|--|--|
| Covid-19 deaths/M as it is in September,2,2020 | Worldmeter. Coronavirus Accessed: 2/9/2020 https://www.worldometers.info/coronavirus/ |

| | |
|---------------------------------------|---|
| <p>Malaria elimination references</p> | <p>1-https://www.who.int/malaria/areas/elimination/malaria-free-countries/en/</p> <p>2-CDC Malaria Information and Prophylaxis, by Country [E]</p> <p>3- World malaria report 2019. Geneva: World Health Organization; 2019. Licence: CC BY-NC-SA 3.0 IGO. file:///C:/Users/zz/Downloads/9789241565721-eng.pdf</p> <p>4- The World Bank. Data. Incidence of Malaria https://data.worldbank.org/indicator/SH.MLR.INCD.P3</p> <p>5-WHO. Global Health Observatory data repository. By Category. Malaria .Cases https://apps.who.int/gho/data/node.main.MALARIAINCIDENCE?lang=en</p> <p>6- Roser M, Ritchie H. Our World in Datta. Malaria . last revised in October 2019. https://ourworldindata.org/malaria Accessed: 3/9/202</p> <p>6-Constantinou K. Anopheles (malaria) eradication in Cyprus. Parassitologia. 1998 Jun;40(1-2):131-5. PMID: 9653740.</p> <p>7-Bulletin of the World Health Organization</p> <ul style="list-style-type: none"> • Past issues Volume 86: 2008 • Volume 86, Number 2, February 2008, 81-160 • 8- Chapter 5. Elimination of malaria in: World Malaria Report 2009. P55 <p>9- Countries and territories certified malaria-free by WHO Last update: 22 May 2019</p> |
| <p>BCG and TB references</p> | <p>1- http://www.bcgatlas.org/ttp://www.bcgatlas.org/</p> <p>2- https://worldhealthorg.shinyapps.io/tb_profiles/?inputs=&lan=%22EN%22&iso2=%22AU%22</p> <p>3- https://data.worldbank.org/indicator/SH.TBS.INCD</p> <p>4- WHO. Global Health Observatory data repository. By category > Immunization. BCG. Immunization coverage estimates by country.https://apps.who.int/gho/data/view.main.80500?lang=en ccessed 3/9/2020</p> <p>5- Zwerling A, Behr MA, Verma A, Brewer TF, Menzies D, Pai M. The BCG World Atlas: a database of global BCG vaccination policies and practices. <i>PLoS Med</i>. 2011;8(3):e1001012. doi:10.1371/journal.pmed.1001012</p> |

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| Exclusion list | (excluded list of Countries and territories with less than 1 million populations) These include: Montenegro, Seychelles, Iceland, La Réunion, Malta, Monaco, San Marino, Antigua and Barbuda, Luxembourg, Bahamas, Barbados, Dominica, Fiji, Nauru, Grenada, Tuvalu, Tonga, Samoa, Palau, Niue, Saint Kitts and Nevis, Saint Lucia, Micronesia (Federated States of), Marshall Islands, Kiribati, Cook Islands, Brunei Darussalam, Andorra and Maldives, Micronesia, Saint Vincent and the Grenadines. |
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UNDER PEER REVIEW