‘Trained immunity’ from *Mycobacterium* spp. exposure or BCG vaccination and COVID-19 outcomes

Samer Singh, PhD 1,2#; Rajendra P. Maurya, MBBS, MD, PhD3; Rakesh K. Singh, PhD4

1Centre of Experimental Medicine & Surgery, Institute of Medical Sciences, Banaras Hindu University, Varanasi – 221005, India
2Department of Microbial Biotechnology, Panjab University, Chandigarh – 160014, India
3Regional Institute of Ophthalmology, Institute of Medical Sciences, Banaras Hindu University, Varanasi – 221005, India
4Department of Biochemistry, Institute of Science, Banaras Hindu University, Varanasi – 221005, India

# Corresponding Author’s E-mail: samer.singh10@bhu.ac.in; samer@pu.ac.in; Tel.: +91-9161111173

ABSTRACT:

Protective variables for COVID-19 are unknown. ‘Trained immunity’ of the populace as a result of mandatory BCG immunization policy implementation by countries has been suggested as one of the factors responsible for the differential impact of COVID-19 on different countries. Several trials are underway to evaluate the potential protective role of BCG vaccination in COVID-19. However, the lack of clarity on the use of appropriate controls concerning ‘trained immunity’ has been a cause of concern leading to more confusion as exemplified by a recently concluded trial in Israel that failed to find any correlation. Whereas, when we analyze the COVID-19 data of European countries without any mandatory BCG vaccination policy but with similar age distribution, comparable confounding variables, and the stage of the pandemic, the prevalence of *Mycobacterium* spp. (including BCG vaccine) exposure of the populations is consistently negatively correlated with COVID-19 infections per million population at all the time points evaluated \([r(20): -0.5511\text{ to } -0.6338; \text{p-value: } 0.0118\text{ to } 0.0027]\). The results indicate that the on-going and future studies evaluating the effect of BCG vaccination on COVID-19 outcomes should consider the inclusion of ‘controls’ for underlying ‘trained immunity’ prevalence or that resulting from the intervention (BCG vaccine) in such trials to arrive at more dependable conclusions.

Key Words: BCG vaccine; COVID-19; Europe; Latent Tuberculosis Infection; SARS-CoV-2; *Mycobacterium* spp.; Trained Immunity
INTRODUCTION:

The implementation of mandatory BCG vaccination policy by countries in the past and the BCG immunization coverage of the populations have been associated with the lower COVID-19 cases and mortality. However, the confounding variables among countries such as the stage of the outbreak, population age distribution, health infrastructure, management practices, the testing/screening, and reporting guidelines, etc. make the comparisons tenuous[1,2]. Since the ‘trained immunity’ status conferred by BCG vaccination in childhood wanes rapidly within years in the absence of ‘booster’ Mycobacterium spp. exposures[3], the prevalent actual ‘trained immunity’ of the population could be a better evaluable predictive parameter of populations’ response to COVID-19 infections, if any, rather than relying on BCG vaccination in the childhood or the national policy as recently reported in JAMA by Hamiel and colleagues[4].

Tuberculin Sensitivity Test (TST) combined with Interferon Gamma Release Assay (IGRA) is generally used to assess the presence of immune response against Mycobacteria spp. (environmental or BCG vaccine). In the absence of IGRA positivity, it is referred to as ‘Latent Tuberculosis Infection’ (LTBI) broadly signifying the absence of tuberculosis disease but the presence of active immunity against the pathogen. As about 1-5% of the LTBI positive individuals could develop Tuberculosis in their lifetime due to either subsequent infection after the ‘trained immunity’ wanes or when the immunity is compromised for any reason (e.g., HIV infection, cancer, immunosuppressant therapy) this ‘LTBI’ nomenclature has been retained though not without causing confusion and general aversion of its use as a measure of ‘trained immunity’. We reason, if indeed ‘trained immunity’ status could help reduce infections in a population, the estimated prevalence of Latent Tuberculosis Infection (%LTBI) of resident populations would more closely correlate with COVID-19 infection and mortality rates. The European countries with no mandatory BCG vaccination policy, comparable medical infrastructure, mobility, exposure to SARS-CoV-2, and other confounding variables, currently at a similar stage of epidemic-curve but differential ‘trained immunity’ status as reflected in %LTBI offer an excellent opportunity to evaluate such assertion.

METHODS:

The populations from 20 European countries with a differential prevalence of %LTBI[5] (published by the ‘Institute for Health Metrics and Evaluation (IHME)’ 2018) and comparable confounding variables, including the stage of the pandemic (infections peak) (Table 1) are assessed for any correlation with COVID-19 cases and mortality data without any exclusion criterion (e.g., age, sex, ethnicity) at different stages, i.e., 8 April, 12 May, and 26 May 2020 from https://www.worldometers.info/coronavirus/[6] of the ongoing pandemic, as done previously for Vitamin D [7].
RESULTS:

The ‘trained immunity’ prevalence correlates of the populations (i.e., %LTBI) indicates a strong correlation with COVID-19 infections (Table 1). The analysis of the COVID-19 data reveals a consistently negative covariation of the cases per million with population’s %LTBI at all the time points evaluated \[ r(20): -0.5511 \text{ to } -0.6338; \text{ p-value: } 0.0118 \text{ to } 0.0027 \] both pre- and post-infections peak [8 April to 26 May 2020], whereas the negative covariation of deaths per million population observed \[ r(20) - 0.2836 \text{ to } -0.3283 \] remained insignificant (p-values >0.05) (Table 1), similar to previously observed by us[1]. See Figure 1 for the potential predictive correlative inference of the data on May 26, 2020.

DISCUSSION:

In the differentially ‘trained immunity’ populations of European countries, the COVID-19 cases per million population negatively correlate with the %LTBI prevalence of the population at each analyzed stage of the pandemic. However, an insignificant negative correlation is observed with deaths per million populations as would be expected with individuals/populations without sufficient ‘trained immunity’ being vulnerable to getting infected.

The strength of the assertion lies in the large affected study population, i.e., 1,413,367 COVID-19 patients (about 25% of total worldwide on 26.05.2020)[6]; representing 20 different ‘trained immunity’ level (%LTBI prevalence) groups/populations from countries without a mandatory BCG vaccination policy; comparable health infrastructure, screening and reporting guidelines along with similar age and sex distribution and other confounding variables. The correlation analysis consistently indicates the same association at all 3 different time points of the epidemic curve evaluated. In the light of observation presented, it may be suggested that ongoing trials/studies evaluating the effect of BCG vaccination on COVID-19 infections[2], including the one recently concluded in Israel and reported in JAMA[4] could provide more objective conclusions on inclusions of the estimates about the ‘trained immunity’ of study participants/populations.

Dedicated studies using the available patient records or epidemiological surveys backed by follow-up clinical trials with suitable controls for the ‘trained immunity’ correlates are advisable to assess the biological significance of the observed correlation and arrive at a more meaningful conclusion about BCG vaccination conferred ‘trained immunity’ and COVID-19 control.
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Compliance with ethical standards

Conflict of interest: There is no conflict of interest to disclose.

Ethical statement: The study complied with the existing ethical standards.

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Table 1: COVID-19 (SARS-CoV-2) infections in European countries with different prevalence of Latent Tuberculosis Infection (% LTBI) and the Correlation Analysis

| Countries  | Most countries before infections peak | 8 April 2020 | 12 May 2020 | 26 May 2020 | % LTBI |
|------------|--------------------------------------|--------------|-------------|-------------|--------|
|            | Cases per Million Pop. | Deaths per Million Pop. | Cases per Million Pop. | Deaths per Million Pop. | Cases per Million Pop. | Deaths per Million Pop. |        |
| Spain      | 3137 | 314 | 5735 | 572 | 6060 | 580 | 6.06 |
| Iceland    | 4736 | 18 | 5278 | 29 | 5290 | 29 | 7.67 |
| Ireland    | 1230 | 48 | 4685 | 297 | 5015 | 327 | 8.14 |
| Belgium    | 2019 | 193 | 4612 | 751 | 4959 | 806 | 8.75 |
| UK         | 895 | 105 | 3286 | 472 | 3909 | 546 | 9.55 |
| Italy      | 2306 | 292 | 3636 | 508 | 3813 | 545 | 12.87 |
| Switzerland | 2686 | 103 | 3506 | 213 | 3557 | 221 | 8.42 |
| Sweden     | 834 | 68 | 2641 | 322 | 3412 | 409 | 10.07 |
| Portugal   | 1289 | 37 | 2715 | 112 | 3040 | 132 | 10.33 |
| France     | 1671 | 167 | 2718 | 408 | 2800 | 437 | 8.86 |
| Netherlands | 1199 | 131 | 2497 | 318 | 2661 | 342 | 8.29 |
| Germany    | 1309 | 25 | 2060 | 91 | 2164 | 101 | 9.20 |
| Denmark    | 933 | 38 | 1815 | 92 | 1974 | 97 | 8.81 |
| Turkey     | 453 | 10 | 1657 | 46 | 1884 | 52 | 12.46 |
| Norway     | 1123 | 19 | 1500 | 41 | 1547 | 43 | 8.46 |
| Estonia    | 893 | 18 | 1312 | 46 | 1383 | 49 | 12.03 |
| Finland    | 449 | 7 | 1080 | 49 | 1196 | 56 | 8.28 |
| Czechia    | 488 | 9 | 763 | 26 | 845 | 30 | 11.41 |
| Hungary    | 93 | 6 | 340 | 44 | 390 | 52 | 13.03 |
| Slovakia   | 125 | 0.4 | 267 | 5 | 277 | 5 | 12.70 |
| Average    | 1393.40 | 80.42 | 2605.15 | 222.10 | 2808.80 | 242.95 | 9.77 |
| STDEV      | 1129.98 | 94.61 | 1600.99 | 221.41 | 1681.89 | 238.78 | 2.00 |

**CORRELATION ANALYSIS**

| Metric | Cases Per Million Pop. vs %LTBI | Deaths Per Million Pop. vs %LTBI |
|--------|---------------------------------|---------------------------------|
|        | $r(20)$: -0.5511; $p$ – value: 0.0118 | $r(20)$: -0.2836; $p$ – value: 0.2256 |
|        | $r(20)$: -0.6338; $p$ – value: 0.0027 | $r(20)$: -0.3283; $p$ – value: 0.1576 |
|        | $r(20)$: -0.6194; $p$ – value: 0.0036 | $r(20)$: -0.3088; $p$ – value: 0.1853 |

*Note: The values rounded off to the indicated decimal places.*
Figure 1. The trained immunity prevalence (%LTBI) of European populations vs COVID-19 cases per million