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Perspective

Will bacille Calmette-Guerin immunization arrest the COVID-19 pandemic?

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a novel human coronavirus[1,2], has infected close to 22 million people and killed about 0.77 million people in more than 200 countries (as of August 18, 2020)[3]. Given the fact that SARS-CoV-2 poses an unprecedented threat in terms of transmission and mortality, the World Health Organization has geared up efforts to control, contain and prevent coronavirus disease 2019 (COVID-19). The development of a vaccine has a high attrition rate and involves linear steps of clinical trial and evaluation. For those systems that have been tested on humans previously, parallel testing can involve both animals and phase I human trials[4]. Although some of the potential COVID-19 vaccine candidates have made it through phase I and II clinical trials, mass availability of COVID-19 vaccine could only be possible by 2021[5]. Repurposing of the existing drugs and development of vaccines are thus feasible options to protect people from the severity of the COVID-19 pandemic.

Host immunity plays a crucial role in the elimination of viruses and prevents disease progression. Strategies to boost the same, especially during incubation and non-severe phase of SARS-CoV-2 infection, can be a viable option to check disease severity. Bacille Calmette-Guerin (BCG) induces non-specific protection against a range of bacteria and viruses[6].Therefore, it is worth exploring the immunostimulatory and protective potential of BCG against SARS-CoV-2 infection. BCG is a live attenuated strain of *Mycobacterium bovis* widely used as a vaccine for the prevention of tuberculosis (TB) caused by *Mycobacterium tuberculosis* (*Mtb*). Intradermal/subcutaneous delivery of BCG vaccine protects against disseminated forms of TB in children and provides variable protection against pulmonary TB in adults[7,8]. Recent reports suggest that the efficacy of BCG can be improved by selective delipidation of the outer membrane or alternative route of delivery[9,10]. BCG immunization elicits non-specific immunological outcomes that prevent general infections and reduce mortality caused by unrelated pathogens[6], besides amplifying responses to other paediatric vaccines[11]. BCG has long been employed as an immunotherapeutic agent or adjuvant for preventing recurrence and progression of bladder cancer[12]. A recent report[13] suggests that the potential of BCG in preventing SARS-CoV-2 infection may be validated among hyper-susceptible population subset such as the front line healthcare personnel and the elderly people. Clinical trials to test the efficacy of BCG in boosting the immune system against SARS-CoV-2, have been initiated in the Netherlands (NCT04417335), Australia (NCT04327206) and Columbia (NCT04362124) (*www.clinicaltrials.gov*). Host pattern recognition receptors (PRRs), expressed by innate immune cells, interact with pathogen-associated molecular patterns (PAMPs) of viruses and initiate innate immune response against viral pathogens. Many of the PAMPs are common among different species of bacteria and viruses. Multiple families of PRRs such as NOD-like receptors, RIG-l-like receptors and toll-like receptors detect viral proteins, which in turn induce interferons and cytokines, aiding in the elimination of the virus. It is tempting to speculate that immune response against BCG may cross-recognize SARS-CoV-2-associated PAMPs and confer immunity against this infection. Prior immunization with BCG or re-vaccination with BCG in adults can induce 'trained immunity' (innate immune memory), a term used to describe the possible mechanism(s) underlying heterologous protection induced by BCG against non-mycobacterial antigens. The trained immunity is a kind of conditioning of the innate immune cells, mainly monocytes/macrophages/natural killer cells, to undergo specific epigenetic changes (including de-methylation/de-acetylation) in the genes associated with mounting a robust

and non-specific immune response[14], resulting in a heightened recall response by primed innate immune cells upon a second encounter to the same/different/ broad range of unrelated microbial PAMPs. The degree of immunological response has been correlated with the chromatin accessibility at the genome regions controlling immune response[15]. Regulatory RNA species, including long non-coding RNAs and microRNAs, have also emerged as the regulators of epigenetic reprogramming of innate immune cells, besides substantial rewiring of host metabolic landscape with a predominant shift from oxidative phosphorylation to glycolysis[14].

In humans, limited documentation exists on the impact of unintended protective effects of BCG against viral infections. BCG-immunized mice exhibited higher resistance to influenza viral infection challenge as compared to the unvaccinated mice[16]. Arts *et al*[17] reported significantly low levels of viraemia in BCG-vaccinated human volunteers challenged with experimental yellow fever virus (attenuated strain), and the BCG-induced protection correlated with increased production of interleukin (IL)-1β. Consistent with these studies, BCG re-vaccination induced innate and adaptive immune responses in adult TB patients[18] also suggest its utility to renew response against mycobacteria and other unrelated pathogens possibly *via* induction of trained immunity. MTBVAC, a live attenuated *Mtb* vaccine strain, provides long-term non-specific immunological effect on innate trained immunity in addition to adaptive immune response[19]. Kleinnijenhuis *et al*[20] have demonstrated that the levels of pro-inflammatory cytokines such as IL-1β, tumour necrosis factor-α and IL-6 remain elevated up to one year post-BCG vaccination and subsequently waned.

In line with previous reports showing beneficial non-specific immune potentiating effects of BCG vaccine, one can logically speculate that BCG vaccination (or re-vaccination in countries with universal BCG immunization) may lead to improved clinical outcomes in viral or other respiratory tract infections, including SARS-CoV-2. A recent ecological study has suggested that countries where BCG is part of the immunization schedule, the extent of mortality and morbidity due to COVID-19 is significantly reduced, and may be able to contain the spread of SARS-CoV-2 better than those countries which did not have BCG vaccination[21]. The differences in the number of SARS-CoV-2-infected cases between countries adhering to the universal BCG vaccination policy and those where universal BCG vaccination is not a policy were evaluated, based on the data of COVID-19 cases across the globe (*https://www.coronavirus.jhu.edu/map.html*) and BCG vaccination status from BCG World Atlas database[22]. It was observed that in countries where BCG was a part of the immunization schedule, COVID-19 cases/100,000 population were almost similar to those where BCG was not a part of universal vaccination schedule (Figure A). However, the cause-specific death rate/100,000 population was significantly lower in countries having BCG immunization policy, compared to those where a universal BCG vaccination policy did not exist (Figure B). This suggests that BCG vaccination-induced non-specific immunity may be associated with the mitigation of disease severity in COVID-19-pandemic areas.

We hypothesized that prior BCG vaccination status was associated with the extent of the COVID-19 epidemic. Because COVID-19 cases started or peaked at different time periods and the infection or death rate stabilized differently among various countries, it posed a limitation to the current study. Our results, therefore, are a pointer rather than final conclusion about the role of BCG in arresting the COVID-19 pandemic. To have a more definitive picture, the association of BCG vaccination with the protection or recovery from the SARS-CoV-2 infection must be reinforced with the data available at the individual level. A more comprehensive comparison of BCG-vaccinated individuals on the basis of age at the time of vaccination or re-vaccination may provide critical evidence and logical conclusion for ascertaining the use of BCG in the prevention of COVID-19.

A significant amount of work has been done to engineer BCG to enhance its immune-boosting and protective properties. VPM1002, a genetically engineered BCG, exhibits improved immunogenicity[23], has completed phase I clinical trial in Germany (NCT00749034) and phase II clinical trial in South Africa (NCT01479972) and is currently in phase III clinical trials in India (NCT03152903) for assessing the prevalence of TB recurrence in drug-treated individuals. A separate phase III trial of VPM1002 (NCT04387409) has also been initiated to assess healthcare professionals' absenteeism during the COVID-19 pandemic in Germany (Table). VPM1002 was engineered to survive within the

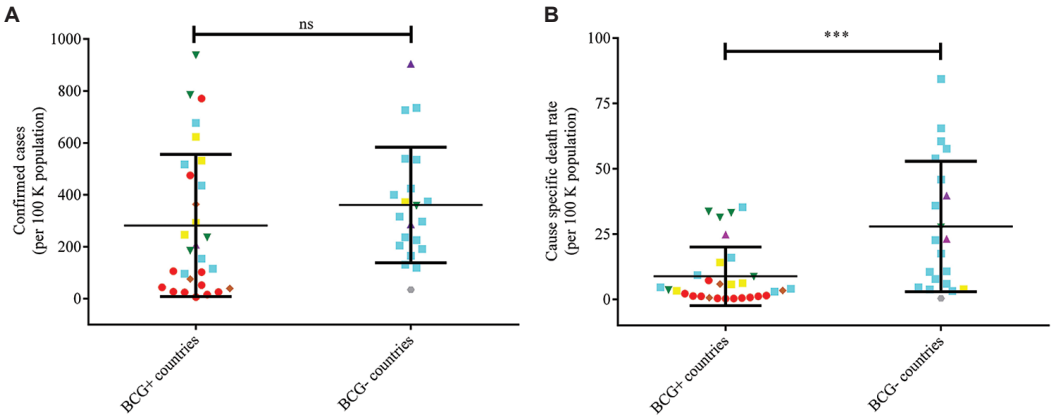

Figure: Scatter plot showing differences in the number of confirmed cases of SARS-CoV-2 infection (**A**) and cause-specific death rate (**B**) per 100,000 population between countries having universal BCG vaccination policy (BCG+ countries) and countries that do not have a universal BCG vaccination policy (BCG− countries). The description of the different colour and symbols is as follows: European countries (sky blue square), Asian countries (red circle), Middle East countries (yellow square), African countries (brown diamond), North American countries (purple upright triangle), South American countries (green inverted triangle) and Australia (grey hexagon). The countries represented in the graph are those with >3000 confirmed COVID-19 cases, as of July 9, 2020. The significance of the two data sets was tested using unpaired, non-parametric Student's t test using GraphPad Prism software version 6.7 (San Diego, CA, USA) and is shown on the top of the plot (ns, non-significant; $^{***}P<0.001$). *Source*: *www.bcgatlas.org*; *www.gisanddata.maps.arcgis.com*.

Table. Role of bacille Calmette-Guerin (BCG), recombinant BCG, attenuated *Mycobacterium tuberculosis* (*Mtb*) or *M. indicus pranii* (MIP) as immunotherapy and possible intervention against COVID-19

Strategy	Study type	Outcome	References
Delipidated BCG	Pre-clinical *in vivo*	Improved protection against *Mtb* infection in mice	9
Alternative route of administration of BCG	Pre-clinical *in vivo*	Improved protection to *Mtb* in *Macaca mulatta* (non-human primates)	10
BCG	Phase III/IV clinical trials against COVID-19	-	NCT04362124 NCT04369794 NCT04348370 NCT04373291 NCT04379336 NCT04327206 NCT04384549 NCT04328441
Recombinant BCG (VPM1002)	Phase III clinical trials against COVID-19	-	NCT04387409 NCT04439045 NCT04435379
BCG	Re-vaccination	Improved protection against pulmonary infection	18
Live attenuated *Mtb* (MTBVAC)	Pre-clinical *in vivo*	Provides protection to mice against bacterial pneumonia	19
Recombinant BCG (STING)	Pre-clinical *in vivo*	Protection of mice and guinea pigs against *Mtb*	24
MIP	Pre-clinical *in vivo*/phase III clinical trial	Protection against leprosy (Phase III)	25
		Protection against category II TB (Phase III)	26
		Tumour regression in mice (pre-clinical)	27
MIP	Phase III clinical trials against COVID-19	-	NCT04358809 NCT04353518

phagosome (unlike BCG), and was equipped with listeriolysin (from *Listeria*) to perforate phagosomal membrane. VPM1002 has also been reported to prevent recurrence of bladder tumours, highlighting its non-specific benefits. Another recombinant BCG strain overexpressing STING (stimulator of interferon genes)-agonist has shown significantly augmented pro-inflammatory cytokine response and protective efficacy in mice and guinea pigs challenged with *Mtb*[24].

Alternatively, immunogenic components of BCG, such as muramyl dipeptide, can also be tested as an adjunct therapy for immune stimulation in those at high-risk to be affected with COVID-19. In addition to being broadly protective, safe and immunogenic, BCG is cost-effective, easy to produce in bulk and, therefore, may be suitable in terms of both availability and affordability. However, before exploiting BCG-induced training of innate immune responses against infections by unrelated pathogens, several potentially confounding factors such as host genetic polymorphisms, endemicity to other viral/bacterial infections and route of immunization need to be examined. Of particular note is the association of BCG vaccination, with some adverse effects such as formation of abscess and lymphadenitis and local cutaneous inflammation[28,29]. Intravesical BCG therapy for the treatment of non-invasive bladder cancer has resulted in short period of fever and discomfort in majority of the patients[30]. Clinical trials for testing the efficacy of BCG, administered (or re-administration) through a conventional or alternate route[9,10], against SARS-CoV-2 can be initiated as an interim intervention against COVID-19. The pros and cons of diverting the stock of BCG as a temporary measure for non-specific protection till actual vaccines for COVID-19 are commercially available must be deliberated upon, as it should not limit the supply of BCG for the people in TB-infected endemic regions. Therefore, it is important to explore agents similar to BCG that can act as immunomodulator. In this regard, it is equally tempting to suggest another mycobacteria discovered in India, *Mycobacterium indicus pranii* (MIP)[31], earlier known as *Mw*. MIP has been found to be a strong immunomodulator with proven utility as an adjunct therapy for leprosy treatment[25], category II TB[26] in humans and in inducing tumour regression[27], and possibly functions by invoking trained immunity (Table).

***Conflicts of Interest*:** None.

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