Relationship between Bacillus Calmette Guerin Vaccination Policy and Coronavirus Disease-2019 (COVID-19) Incidence

Soheila Alyasin\textsuperscript{1,2}, Zahra Kanannejad\textsuperscript{1}, Hossein Esmaeilzadeh\textsuperscript{1,2}, Hesamedin Nabavizadeh\textsuperscript{1,2}, Mohammad Amin Ghaee\textsuperscript{3}, and Reza Amin\textsuperscript{1,2}

\textsuperscript{1} Allergy Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
\textsuperscript{2} Department of Allergy and Clinical Immunology, Namazi Hospital, Shiraz University of Medical Sciences, Shiraz, Iran
\textsuperscript{3} Cellular and Molecular Research Center, Yasuj University of Medical Sciences, Yasuj, Iran

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ABSTRACT

Bacillus Calmette Guerin (BCG) was designed for protecting children against tuberculosis. Also, it can protect against other infectious diseases through the induction of trained immunity. Due to its heterologous protective effects, the BCG vaccine has been proposed as a treatment option for coronavirus disease-2019 (COVID-19). Epidemiological studies have found that countries without BCG vaccination policy have experienced higher mortality rates related to COVID-19 infection than those with BCG vaccination policy. However, there are some confounding factors such as age, population intensity, immigration, the pandemic phase, and data accuracy that may affect these results. Therefore, this hypothesis should be evaluated by clinical trial studies. Large-scale clinical trials are in progress to investigate if the BCG vaccine could be used as a useful tool for protection against COVID-19 infection.

Keywords: COVID-19; Mycobacterium bovis

INTRODUCTION

Currently, the world has an encounter with coronavirus disease-2019 (COVID-19) infection caused by severe acute respiratory syndrome (SARS) coronavirus-2 (SARS-CoV-2).\textsuperscript{1} It belongs to a positive-sense single-stranded RNA genome known as Coronaviridae family.\textsuperscript{2} This virus induces respiratory tract infection in human with cold, sneezing, pneumonia, and coughing, while in the animals it leads to some respiratory infection and diarrhea.\textsuperscript{2} It can be released through droplets of saliva or from the nose of the infected person and transmitted to other individuals.\textsuperscript{3} For the first time, it was isolated from a pneumonia patient who lives in Wuhan, China, and then spread around the world.\textsuperscript{3} Due to rapid spread, WHO reported it as a public health emergency of international concern (PHEIC) on 30 January 2020.\textsuperscript{4} At the time of writing, its incidence was about 3,344 per million population, with a fatality rate of 0.23 per million. Currently, there is no certain antiviral medicine or preventive vaccine for COVID-19. At the moment, treatment options for COVID-19 include drugs that have been approved for the treatment of SARS, Ebola,
Middle East respiratory syndrome (MERS), influenza, and human immunodeficiency virus (HIV). Also, six vaccines in clinical evaluation and about 70 in the pre-clinical evaluation exist for COVID-19.

According to non-specific immune response created by Bacillus Calmette-Guerin (BCG) vaccination, some investigators have proposed it in COVID-19 pandemic to fight against SARS-CoV-2. Epidemiological studies have been performed to investigate the BCG vaccination effects on COVID-19 incidence in countries where people have taken BCG compared to those without BCG immunization.

**BCG Vaccination and Trained Immunity**

In 1921, the BCG vaccine is designed for the treatment of tuberculosis (TB) caused by *Mycobacterium tuberculosis* (Mt) at the Institute Pasteur in Paris. BCG is a live attenuated strain obtained from *Mycobacterium bovis* isolate. Pieces of evidence showed that neonatal BCG vaccination can decrease the mortality rate in the group of children who received it and not received it. Studies have shown that some BCG strains not only prevent TB but also provide effective immune responses against non-related infections and diseases. A study in Guinea Bissau country, in the West-African, showed that vaccinated children experienced a 50% lower risk of overall mortality and morbidity. This effect cannot be limited to protection against tuberculosis alone but it may be related to protection against other infectious diseases such as respiratory tract infections or neonatal sepsis. A study in South Africa showed that using BCG in adolescence was connected with lower respiratory tract infections. Also, it has been suggested by some studies that BCG vaccine can create protection against other viral infections including Influenza, RAS, and HSV2. In addition to defending against viral infections, BCG vaccination also has a protective role against yellow fever, bladder cancer, malaria, leishmaniasis, and asthma.

It is believed that two types of immunological mechanisms mediate these beneficial effects of BCG. First, memory T cells (CD4+ and CD8+) have been activated by some stimuli such as cytokines in an antigen-independent manner, a process which is named heterologous immunity. Second, BCG vaccination is associated with inducing a process known as trained immunity. It refers to some characteristics of adaptive immune responses performed by innate immune cells like NK-cells and macrophages. In this way, innate immune cells are primed through the identification of pathogen-associated molecular patterns (PAMPs) and induce a high protective inflammatory response against secondary detection of PAMPs introduced by other pathogens (cross-protection). Innate immune cells can be trained through two important mechanisms including epigenetic and metabolic reprogramming to act effectively over a second exposure.

Epigenetic modification is introduced as some chemical modifications including DNA and its tone methylation, and acetylation which leads to increase chromatin accessibility, followed by easier transcription of some genes that are important in improved cell function and antimicrobial responses. This mechanism does not affect the DNA sequence, but it can be transferred to the offspring. Studies have shown that the epigenetic reprogramming of monocytes at the promoter regions of inflammatory cytokine genes induced by BCG is accompanied by increased pro-inflammatory cytokines production like tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6. In metabolic reprogramming certain metabolites, which have a role as co-factors for some enzymes and also have a role in the epigenetic change, are accumulated or depleted. Glycolysis and glutamine metabolisms are identified as two essential factors for inducing trained immunity in monocytes by BCG vaccination.

It has been revealed that some long lifespan cells such as stem cells, intestinal stromal cells, fibroblast, and microglial cells can transfer trained immunity. A study has shown that BCG induces hematopoietic stem cells (HSC) and multipotent progenitors (MPPs) reprogramming in the bone marrow which leads to the generation of trained monocytes/macrophages for long-lasting immunological memory. The exact duration of protection created by BCG against TB or other diseases remains unknown. Some studies have indicated that the BCG vaccine creates protection against tuberculosis for 50-60 years. However; the exact duration of such protection against heterologous infections is unclear.

**Does BCG Vaccination Policies Lead to Reduce COVID-19 Incidence?**

According to these data, it has been proposed that whether BCG vaccination may lead to a protective response against COVID-19 infection and may decrease its incidence. Epidemiological studies have
shown that countries with mandated BCG vaccination have a lower number of patients with COVID-19 infection and mortality rates compared to countries without BCG vaccination. In these epidemiological studies COVID-19 incidence and mortality rates have been compared between BCG-vaccinated countries and non-BCG vaccinated countries.

The first epidemiological study in this field was performed by Miller et al, 2020 who suggested that high rates of COVID-19 in countries such as Italy, the Netherlands, and the USA, could be related to non-universal BCG vaccination. To test this hypothesis, they evaluated and compared a large number of countries for BCG vaccination policy and COVID-19 incidence. Their results showed that countries without BCG vaccination policy (Italy, Nederland, USA) had higher mortality rates than countries with mandatory BCG policy (South Korea and Japan). Also, they pointed out that countries where have adopted BCG vaccination program recently (e.g. Iran started from, 1984) had an increased mortality rate, compatible with the idea that BCG protects the vaccinated elderly population. Thereafter, this hypothesis has been tested by other epidemiological studies worldwide. The results of some studies were consistent with this hypothesis while no significant correlation was seen between BCG vaccination policy and protection against COVID-19 by other studies (Table 1). Although these epidemiological data propose BCG vaccination as a protective mean against COVID-19 infection, because of several confounding factors these studies cannot be used as reliable evidence. There are some confounding factors in these epidemiological studies including differences in non-pharmaceutical interventions policy such as quarantine or social distancing, the demographic and genetic structure of the populations.

Table 1. Epidemiological studies on the association between Bacillus Calmette Guerin (BCG) vaccination and Coronavirus Disease-2019 (COVID-19) incidence

| Study             | Time of study       | Findings                                                                 |
|-------------------|---------------------|--------------------------------------------------------------------------|
| Miller et al      | March 21st, 2020    | Decreased morbidity rate in countries with BCG vaccination policy.       |
| Sala et al        | March 28st, 2020    | BCG vaccination may protect SARS-CoV-2.                                   |
| Berg et al        | April 1, 2020       | A significant flattening of increase in COVID-mortality associated with BCG vaccination. |
| Dayal et al       | April 5, 2020       | The difference in the mean CFR between vaccinated and non-vaccinated countries. |
| Akiyama et al     | April 5, 2020       | A significant difference between the DT of the death between "BCG" and "non-BCG" countries. Using the “Tokyo 172-1” strain in BCG vaccination is associated with a longer DT of death. |
| Singh et al       | April 4, 2020       | A negative correlation between COVID-19 morbidity and BCG vaccination.   |
| Hegarty et al     | March 22, 2020      | A correlation between BCG vaccination and reduced COVID-19 mortality rates. |
| Shet et al        | March 29, 2020      | A correlation between BCG vaccination and reduced COVID-19 incidence.    |
| Ozdemir et al     | April 16, 2020      | Reduced COVID-19-mortality among BCG-using countries.                    |
| Singh et al       | April 4, 2020       | The lower mean of cases per population ratio in BCG-vaccinated countries. |
| Aksu et al        | April 7, 2020       | Decreased the incidence rates of COVID-19 with an increase in % latent TB infection. |
| Hamiel et al      | March 1 to April 5, 2020 | BCG vaccination in childhood had not a protective effect against COVID-19 in adulthood. |

Abbreviation: CFR=case fatality rate; DT=doubling time
Table 2. Clinical trial studies of Bacillus Calmette Guerin (BCG) for Coronavirus Disease-2019 (COVID-19)

| Identifier | Status | Intervention | BCG strain | Phase | Sample size | Study population | Location |
|------------|--------|--------------|------------|-------|-------------|------------------|----------|
| NCT04534803 | Not yet recruiting | BCG/Placebo | Tokyo-172 | 3 | 2100 | Elderly people (70 y≤) | U.S.A |
| NCT04328441 | Active | BCG/Placebo | Danish 1331 | 3 | 1500 | HCW (18y≤) | Netherland |
| NCT04362124 | Not yet recruiting | BCG/Placebo | BCG(freeze-dried) | 3 | 1000 | HCW(18-65 y) | Colombia |
| NCT04379336 | Recruiting | BCG/Placebo | Danish 1331 | 3 | 500 | HCW (18 y≤) | South Africa |
| NCT 04417335 | Active | BCG/Placebo | Danish 1331 | 4 | 2014 | Elderly people (60 y≤) | Netherland |
| NCT04350931 | Not yet recruiting | BCG/Placebo | Danish 1331 | 3 | 900 | HCW (18 y≤) | Egypt |
| NCT04537663 | Recruiting | BCG/Placebo | Danish 1331 | 4 | 5200 | Elderly people (60y≤) | Netherland |
| NCT04475302 | Recruiting | BCG/Control | BCG(freeze-dried) | 3 | 1450 | Elderly people (60-80 y) | India |
| NCT04461379 | Not yet recruiting | BCG/Placebo | Tokyo-172 | 3 | 908 | HCW (18 years and older) | Mexico |
| NCT04327206 (BRACE) | Recruiting | BCG/Placebo | Danish 1331 | 3 | 10078 | HCW (18 y≤) | Australia |
| NCT04369794 (BATTLE) | Recruiting | BCG/Placebo | BCG (freeze-dried) | 4 | 1000 | Patient with COVID-19(18 y≤) | Brazil |
| NCT04444267 (ACTIVATEI) | Recruiting | BCG/Placebo | BCG (freeze-dried) | 4 | 900 | Elderly people (50 y≤) | Greece |
| NCT04384549 | Recruiting | BCG/Placebo | Danish 1331 | 3 | 1120 | HCW (18 y≤) | France |
| NCT04373291 | Not yet recruiting | BCG/Placebo | Danish 1331 | 3 | 1500 | HCW(18-100 y) | Denmark |
| NCT04542330 | Not yet recruiting | BCG/Placebo | Danish 1331 | 3 | 1900 | Elderly people (65-110 y) | Denmark |
| NCT04348370 (BADAS) | Recruiting | BCG/Placebo | Tice | 4 | 1800 | HCW (18-75 y) | U.S.A |
| NCT04453579 | Recruiting | rBCG/Placebo | VPM1002 | 3 | 2038 | Elderly people (60 y≤) | Germany |
| NCT04439045 (COBRA) | Recruiting | rBCG/Placebo | VPM1002 | 3 | 3626 | Front-line employees (18 y≤) | Canada |
| NCT04387409 | Recruiting | rBCG/Placebo | VPM1002 | 3 | 1200 | HCW (18 y≤) | Germany |

Abbreviation: HCW=health care worker

Case identification, population density, age distribution of population, wealth, health care system, varied BCG strains, the beginning time of study which may affect the accurate comparison of COVID-19 mortality rate between different places. However, in some studies, the association between BCG vaccination and COVID-19 occurrence remained unchanged after adjusting for some confounding factors.
factors. This association was seen by Berg et al after adjusting for geographic region and net migration rate, population density and size, median age, and by Shet et al for the time of the epidemic, country income status, and age. In addition, for mitigating the effect of such confounding factors, this hypothesis should be comprehensively tested by three approaches including meta-analysis investigations, randomized control trials, and large prospective cohort studies. Two meta-analysis studies investigated effect of BCG vaccination on the respiratory infection. A meta-analysis suggested that BCG could affect the mortality rate related to pneumonia and sepsis in children under 5 years old in low-income countries. Another study reported that administration of BCG vaccine is connected with increased antibody levels against other virus vaccines such as influenza A virus and pneumococcus. In addition, several clinical trial studies are ongoing to evaluate the efficacy of BCG vaccination on COVID-19 protection in some populations with a high risk of infection such as hospital care workers (HCW) or older individuals. A list of active, recruiting, and not yet recruiting clinical trials for BCG vaccination was prepared in table 2.

There are about 13 active and recruiting and 5 not yet recruiting clinical trials in this field. These studies are varied for BCG strain and characteristic features of the study population that can help choose an effective BCG vaccination route against COVID-19 infection. Different BCG strains including TICE (Chicago), Danish 1331 (Denmark) and, Tokyo-172 (Japan) have been used in these studies. In addition to BCG strains, two studies in Germany (NCT04435379 and NCT04387409) and one in Canada (NCT04439045) are evaluating the effect of VPM1002, a recombinant vaccine from BCG. Based on gene analysis results, the BCG vaccine has two subgroups as follows early strain, including BCGs Russia, Birkhaug, Japan, Sweden, and Moreau, and the late strains, including BCGs Danish, Glaxo, Pasteur, and Prague which are different genetically and phenotypically. Some studies have reported that early and late strains have different abilities for inducing immune responses. It has been shown that BCG Japan can induce more strong proliferation of T cells, higher Th1 cytokine production including IL-2, interferon (IFN)-γ, TNF-α, and IL-2, and lower Th2 cytokine production including IL-4 compared with BCG Denmark. In the context of the COVID-19 pandemic, it seems that using BCG Japan and Russia strains (early strain) are associated with a lower COVID-19 mortality rate compared with using BCG Denmark (late strain). Therefore, BCG strain may affect the result of these clinical trials and should be considered in the conclusion. Another difference factor between BCG clinical trial studies is the varied characteristic features of the study population. Most studies have been performed on HCW (aged≥18 y) or elderly people (aged≥50 y) except for one study in Brazil (NCT04369794) which was performed on a patient with laboratory or clinical-epidemiological confirmation of COVID-19 (aged≥18 y). HCW and elderly individuals are more susceptible to COVID-19 infection but, selecting these groups may affect the results of the study. Due to the effects of aging on the immune system, the results of such studies can be imply assigned to old individuals, not young populations. HCW have more potential risks to health in their work associated with many pathogens which may induce occupational diseases and develop abnormalities of the immune system. We suppose that long-term activation of inflammatory immune responses in the HCW leads to bias in the results of the study and wrong conclusion. In addition, some clinical trials have been conducted in BCG-implemented countries such as Brazil, Colombia, Egypt, Greece, India, and Mexico. Some studies have demonstrated that BCG revaccination increases adaptive Th1, Th17, and innate effectors. Therefore, the results of studies in such countries may be different from non-implemented countries. In most clinical trial studies, the sample size ranging is from 500 to 2000 except for Australia (NCT04327206) and Netherland (NCT04537663) that are about 10,000 and 5000, respectively. As less than 10 out of 1,000 individuals are estimated to be engaged with COVID-19 infection during the pandemic, such small-scale clinical trials with a small number of participants cannot be suitable for providing a clear-cut answer. Despite the defects of such clinical trial studies, those seem to be necessary for approving BCG as a protective mean for HCW and elderly people against COVID-19 until approval of a specific vaccine.

In conclusion, BCG vaccination is described to induce its heterologous protection effects through the process known as trained immunity. Some epidemiological studies have suggested that BCG-vaccinated countries have lower mortality rates due to COVID-19 compared to those without BCG.
vaccination policy. However, such epidemiological studies are prone to be affected by many confounder factors, including differences in the stage of the pandemic in each country, national demographics, testing rates for COVID-19 virus infections. Therefore, this hypothesis needs to be tested by large-scale clinical trial studies. To assess BCG efficacy, some clinical trials are ongoing to investigate whether BCG vaccination could be effective for COVID-19 infection. Although current clinical trial studies in this field have some defects, its performance may be useful for confirming BCG as a suitable mean to protect especially vulnerable population such as HCW against infection with SARS-CoV-2 during current COVID-19 pandemic.

CONFLICT OF INTEREST
The authors declare no conflicts of interest.

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Not applicable

REFERENCES
1. Lai CC, Shih TP, Ko WC, Tang HJ, Hsueh PR. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease-2019 (COVID-19): the epidemic and the challenges. Int J Antimicrob Agents. 2020;105924.
2. Hassan SA, Sheikh FN, Jamal S, Ezeh JK, Akhtar A. Coronavirus (COVID-19): a review of clinical features, diagnosis, and treatment. Cureus. 2020;12(3).
3. Rothan HA, Byrareddy SN. The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. J Autoimmun. 2020;12(3).
4. Dzobo M, Chitungo I, Dzinamarira T. COVID-19: a perspective for lifting lockdown in Zimbabwe. Pan Afr Med J. 2020;35(13).
5. Guo YR, Cao QD, Hong ZS, Tan YY, Chen SD, Jin HJ, et al. The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak—an update on the status. Mil Med Res. 2020;7(1):1-0.
6. Le TT, Andreadakis Z, Kumar A, Roman RG, Tollefsen S, Saville M, et al. The COVID-19 vaccine development landscape. Nat Rev Drug Discov. 2020;19:305-6.
7. Miller A, Reandelaer 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.
8. Sala G, Miyakawa T. Association of BCG vaccination policy with prevalence and mortality of COVID-19. MedRxiv. 2020.
9. 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.
10. Dayal D, Gupta S. Connecting BCG vaccination and COVID-19: additional data. MedRxiv. 2020.
11. Thysen SM, Benn CS, Gomes VF, Rudolf F, Wejse C, Roth A, et al. Neonatal BCG vaccination and child survival in TB-exposed and TB-unexposed children: a prospective cohort study. BMJ open.2020;10(2):e035595.
12. Moorlag SJ, Arts RJ, van Crevel R, Netae MG. Non-specific effects of BCG vaccine on viral infections. Clin Microbiol Infect. 2019;25(12):1473-8.
13. Kristensen I, Fine P, Aaby P, Jensen H. Routine vaccinations and child survival: follow up study in Guinea-Bissau, West Africa. Commentary: an unexpected finding that needs confirmation or rejection. BMJ. 2000;321(7274):1435.
14. Nemes E, Geldenhuys H, Rozot V, et al. Prevention of M. tuberculosis Infection with H4:IC31 Vaccine or BCG Revaccination. N Engl J Med. 2018;379(2):138-49.
15. O'Neill LAJ, Netae MG. BCG-induced trained immunity: can it offer protection against COVID-19? Nat Rev Immunol. 2020;20(6):335-37.
16. Arts RJ, Novakovic B, ter Horst R, Carvalho A, Bekkering S, Lachmandas E, et al. Glutaminolysis and fumarate accumulation integrate immunometabolic and epigenetic programs in trained immunity. Cell Metab. 2016;24(6):807-19.
17. Buffen K, Oosting M, Quintin J, Ng A, Kleinmijenhuis J, Kumar V, et al. Autophagy controls BCG-induced trained immunity and the response to intravesical BCG therapy for bladder cancer. PLoS Pathog. 2014;10(10).
18. Berendsen ML, Van Gijzel SW, Smits J, De Mast Q, Aaby P, Benn CS, et al. BCG vaccination is associated with reduced malaria prevalence in children under the age of 5 years in sub-Saharan Africa. BMJ Glob Health. 2019;4(6).
19. Pereira LI, Dorta ML, Pereira AJ, Bastos RP, Oliveira MA, Pinto SA, et al. Increase of NK cells and proinflammatory monocytes are associated with the clinical improvement of diffuse cutaneous leishmaniasis after immunochemothapy with BCG/Leishmania
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antigens. Am J Trop Med Hyg. 2009;81(3):378-83.
20. Alyasin S, Katibeh P, Asadi S. The relationship between tuberculin response, BCG vaccine scar and asthma. Iran J Allergy Asthma Immunol. 2009;8(4):205-10.
21. Berg RE, Cordes CJ, Forman J. Contribution of CD8+ T cells to innate immunity: IFN-γ secretion induced by IL-12 and IL-18. Eur J Immunol. 2002;32(10):2807-16.
22. Berg RE, Crossley E, Murray S, Forman J. Memory CD8+ T cells provide innate immune protection against Listeria monocytogenes in the absence of cognate antigen. J Exp Med. 2003;198(10):1583-93.
23. Mathurin KS, Martens GW, Kornfeld H, Welsh RM. CD4 T-cell-mediated heterologous immunity between mycobacteria and poxviruses. J Virol. 2009;83(8):3528-39.
24. Kleinnijenhuis J, Quintin J, Preijers F, Joosten LA, Ifflin DC, Saeed S, et al. Bacille Calmette-Guerin induces NOD2-dependent nonspecific protection from reinfection via epigenetic reprogramming of monocytes. PNAS. 2012;109(43):17537-42.
25. Netea MG, Joosten LA, Latz E, Mills KH, Natoli G, Stunnenberg HG, et al. Trained immunity: a program of innate immune memory in health and disease. Science. 2016;352(6284):aaf1098.
26. Hamada A, Torre C, Drancourt M, Ghigo E. Trained immunity carried by non-immune cells. Front Microbiol. 2019;9:3225.
27. Netea MG, Latz E, Mills KH, O'neill LA. Innate immune memory: a paradigm shift in understanding host defense. Nat Immunol. 2015;16:675-99.
28. Saeed S, Quintin J, Kerstens HH, Rao NA, Aghajanirefah A, Matarese F, et al. Epigenetic programming of monocyte-to-macrophage differentiation and trained innate immunity. Science. 2014;345(6204):1251086.
29. Arts RJ, Moorlag SJ, Novakovic B, Li Y, Wang SY, Oosting M, et al. BCG vaccination protects against experimental viral infection in humans through the induction of cytokines associated with trained immunity. Cell Host Microbe. 2018;23(1):89-100.
30. Arts RJ, Carvalho A, La Rocca C, Palma C, Rodrigues F, Silvestre R, et al. Immunometabolic pathways in BCG-induced trained immunity. Cell Rep. 2016;17(10):2562-71.
31. Kaufmann E, Sanz J, Dunn JL, Khan N, Mendonça LE, Pacis A, et al. BCG educates hematopoietic stem cells to generate protective innate immunity against tuberculosis. Cell. 2018;172(1-2):176-90.
32. Aronson NE, Santosham M, Comstock GW, Howard RS, Moulton LH, Rhoades ER, et al. Long-term efficacy of BCG vaccine in American Indians and Alaska Natives: a 60-year follow-up study. JAMA. 2004;291(17):2086-91.
33. Nguidop-Djomo P, Heldal E, Rodrigues LC, Abubakar I, Mangtani P. Duration of BCG protection against tuberculosis and change in effectiveness with time since vaccination in Norway: a retrospective population-based cohort study. Lancet Infect Dis. 2016;16(2):219-26.
34. Akiyama Y, Ishida T. Relationship between COVID-19 death toll doubling time and national BCG vaccination policy. MedRxiv. 2020.
35. 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-Calmette-Guerin vaccine in reducing morbidity and mortality. J Pure Appl Microbiol. 2020;14.
36. 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.
37. Hegarty PK, Sfakianos JP, Giannarini G, DiNardo AR, Kamat AM. COVID-19 and Bacillus Calmette-Guerin: What is the link?. European Urology Oncology. Eur Urol Oncol. 2020;3(3):259-61.
38. Shet A, Ray D, Malavigne N, Santosham M, Bar-Zeev N. Differential COVID-19-attributable mortality and BCG vaccine use in countries. MedRxiv. 2020.
39. Ozdemir C, Kucuksezer UC, Tamay ZU. Is BCG vaccination effecting the spread and severity of COVID-19?. Allergy. 2020;75(7):1824-1827.
40. Singh S. BCG vaccines may not reduce COVID-19 mortality rates. MedRxiv. 2020.
41. Aksu K, Naziroğlu T, Özkaran P. Factors determining COVID-19 pneumonia severity in a country with routine BCG vaccination. Clin Exp Immunol. 2020 Nov;202(2):220-225
42. Hensel J, McGrail DJ, McAndrews KM, Dowlatshahi D, LeHue VS, Kalluri R. Exercising caution in correlating COVID-19 incidence and mortality rates with BCG vaccination policies due to variable rates of SARS CoV-2 testing. MedRxiv. 2020.
43. Hamiel U, Kozer E, Youngster I. SARS-CoV-2 Rates in BCG-Vaccinated and Unvaccinated Young Adults. JAMA. 2020;323(22):2340-2341.
44. Cruz CT, Almeida B, Troster E. Systematic Review of the Non-Specific Effects of Bacillus Calmette-Guerin Vaccine on Child. Mortality. J Infec Dis Treat. 2017;3:1.
45. Zimmermann P, Curtis N. The influence of BCG on vaccine responses - a systematic review. Expert Rev...
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Vaccines. 2018;17(6):547-554.
46. Clinicaltrials.gov.https://clinicaltrials.gov/ct2/results?cond=Covid (accessed 20 September 2020)
47. Miyazaki J, Onozawa M, Takaoka E, Yano I. Bacillus Calmette-Guérin strain differences as the basis for immunotherapies against bladder cancer. Int J Urol. 2018;25(5):405-413.
48. Ladefoged A, Bunch-Christensen K, Guld J. Tuberculin sensitivity in guinea-pigs after vaccination with varying doses of BCG of 12 different strains. Bull World Health Organ. 1976;53(4):435.
49. Davids V, Hanekom WA, Mansoor N, Gamieldien H, Sebastian JG, Hawkridge A, et al. The effect of bacille Calmette-Guerin vaccine strain and route of administration on induced immune responses in vaccinated infants. J Infect Dis. 2000;193(4):531-6.
50. Miyasaka M. Is BCG vaccination causally related to reduced COVID-19 mortality? EMBO Mol Med. 2020;12(6):e12661.
51. Weyand CM, Goronzy JJ. Aging of the Immune System. Mechanisms and Therapeutic Targets. Ann Am Thorac Soc. 2016;5(Suppl 5):S422-S428.
52. Brewczyńska A, Depczyńska D, Borecka A, Winnicka I, Kubiak L, Skopińska-Różewska E, et al. The influence of the workplace-related biological agents on the immune systems of emergency medical personnel. Cent Eur J Immunol. 2015;40(2):243.
53. Rakshit S, Ahmed A, Adiga V, Sundararaj BK, Sahoo PN, Kenneth J, et al. BCG revaccination boosts adaptive polyfunctional Th1/Th17 and innate effectors in IGRA+ and IGRA–Indian adults. JCI Insight. 2019;4(24).