

Effects of Bacillus Calmette-Guérin (BCG Moscow) vaccination on white blood cell count: results of a randomized clinical trial

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ABSTRACT

Introduction: Bacillus Calmette-Guérin (BCG) vaccination induces innate and specific responses that protect against some severe forms of tuberculosis and have nonspecific effects against other infections. **Objective:** To evaluate whether revaccination with BCG Moscow is associated with serum increase in total and differential leukocytes. **Methods:** We conducted an analytical study on the white blood cell count of 156 participants (BCG revaccination group: 80; Control group: 76) of a randomized clinical trial investigating BCG revaccination for the prevention or reduction of complications associated with COVID-19. Blood samples were collected before randomization and after 15 days of intervention. Values were expressed as mean (μ) and standard deviation, using paired t-tests and Student's t-test. Results: BCG revaccination did not alter leukocyte levels between revaccinated (μ , 6019.74 \pm 1865.33) and non-revaccinated groups (μ , 6278.75 \pm 1823.87), $p=0.94$. Stratification by sex, obesity, and age did not significantly affect white blood cell levels. **Conclusion:** Revaccination with BCG Moscow did not stimulate leukocyte production.

Keywords: BCG vaccine; immunization, secondary; Leukocytes.

INTRODUCTION

The Bacillus Calmette-Guérin (BCG) vaccine, composed of attenuated *Mycobacterium bovis*, is widely used to prevent severe forms of disseminated tuberculosis and meningitis, particularly in children¹. The World Health Organization (WHO) recommends the BCG vaccine for newborns as part of an immunization program in tuberculosis (TB)-endemic countries. Despite its proven efficacy in preventing childhood tuberculosis, BCG does not seem to induce long-lasting immunity².

However, studies suggest pleiotropic effects on other infectious agents of a respiratory nature³⁻⁶. These effects are associated with an epigenetic, transcriptional, and

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functional reprogramming of innate immune cells that potentially increase cytokine production and promote a non-specific immunomodulatory effect, described by Netea *et al.*⁷ as “trained immunity,” which is associated with cross-protection against other infections⁸.

Coronavirus disease (COVID-19) is an emerging disease caused by a betacoronavirus that causes severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and, in 2019, was responsible for the COVID-19 pandemic. In general, the clinical spectrum of COVID-19 varies from asymptomatic to mild to moderate. However, some individuals develop severe forms of the disease^{9,10}.

Initially, epidemiological studies pointed out an association between BCG vaccination coverage in some countries and lower morbidity and mortality due to COVID-19, especially in children^{11,12}. Thus, owing to the lack of a specific vaccine against COVID-19 at that time, randomized clinical trials were conducted to evaluate the efficacy and safety of BCG vaccination in preventing and reducing the severity of COVID-19 infection¹³⁻¹⁵. None of these studies demonstrated the effectiveness of BCG revaccination in inducing protection or reducing the severity of COVID-19 infection. However, a study by Koeken *et al.*¹⁶ suggested that vaccination with BCG might induce changes in white blood cell (WBC) counts.

Given the above, this study was designed to evaluate whether revaccination with BCG is associated with an increase in serum leukocyte and differential counts of a population from the central west region of Brazil.

METHODS

This was a secondary analysis of the randomized clinical trial, a unicentric, parallel, phase II clinical trial conducted among healthcare workers (HCWs) with no prior COVID-19 infection conducted in the city of Goiânia (Goiás, Brazil), between August 20, 2020, and August 31, 2021¹⁴. The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Ethics Committee of *Comissão Nacional de Ética em Pesquisa* (CONEP), CAAE: 31783720.0.0000.5078. Informed consent was obtained from all participants involved in the study that was conducted by the requirements for Good Clinical Practice (ICH E6 - R2), as stated by Junqueira Kipnis *et al.*¹⁴.

Professionals with a history of BCG vaccination, without a previous diagnosis of COVID-19, and who work in person during the COVID-19 pandemic were eligible for the clinical trial. Individuals with prior known reaction to the BCG vaccine, fever prior, pregnant or breastfeeding women, suspected or confirmed viral infection including COVID-19 or bacterial infection, previous diagnosis of tuberculosis, vaccination in the previous four

weeks, medical diagnosis of immunosuppressive diseases, such as human immunodeficiency virus (HIV), and/or cancer in the previous two years and/or autoimmune disease and/or use of corticosteroids and/or antibiotics and/or chemotherapy, individuals with positive IgM and/or IgG for COVID-19 and/or neutrophil counts below 500/mm³ were excluded as highlighted by Junqueira Kipnis *et al.* and Anjos *et al.*^{14,17}.

Blood samples were collected from all participants on day 1 (before randomization). Blood (10 mL) was collected in a tube with ethylenediaminetetraacetic acid (EDTA) and analyzed using an ABX Micros 60 (HORIBA ABX SAS, Grabels, France). The samples were submitted for blood count. Afterward, participants were randomized into one of two groups: 1) revaccination with Russian strain BCG (Moscow) group and 2) a control group composed of unvaccinated individuals. Fifteen days after the intervention, a new blood count was performed to evaluate immune response, and WBC count results were analyzed in this study.

Participant data

Participants from both groups of the clinical trial (revaccination with BCG and control groups) were included in this analysis. Participants with an abnormal blood count at the time of inclusion in the clinical trial, those who did not attend the second blood count collection 15 days after the randomization, and those who presented with inconsistencies in the report of a previous BCG vaccination were excluded. In this study, we analyzed WBC counts and differential counts of participants from both groups of the clinical trial at two time points, day 1 (D1 = randomization) and day 15 (D15 = 15 days after randomization), of the intervention.

Statistical analysis

All analyses were performed using SPSS v26.0 (IBM Corporation, Armonk, NY, USA). The level of significance was set at 5% ($p < 0.05$)¹⁸. Categorical variables were reported as absolute and relative frequencies. Continuous variables were reported as means (μ), standard deviations, and confidence intervals (CIs). Data parametricity was verified using a normalized Q-Q plot and a histogram of standardized residuals¹⁸. The homogeneity of the groups (control: -BCG and revaccinated with BCG: +BCG) was evaluated using Pearson's chi-square test. Laboratory tests before the intervention (D1) and 15 days after the intervention (D15) were compared using paired t-tests. The delta (Δ) was calculated for each laboratory test. These values were used to compare the mean variation of the tests between vaccinated and unvaccinated patients using Student's t-test. Data were stratified by sex, obesity, and age. Participants with a body mass index (BMI = weight/height²) greater than or equal to 30 were considered obese¹⁹.

RESULTS

Baseline Characteristics

A total of 156 participants were included in this analysis: 80 in the BCG revaccination group and 76 in the control group.

The baseline characteristics of the participants according to the allocated groups (-BCG and +BCG) are presented in Table 1. In the -BCG and +BCG groups, the sample consisted of young patients with an average age of 36 and 39 years, respectively. In the -BCG group, 67.1% of the participants were female (n=51) and

Table 1: Baseline characteristics according to allocated group (-BCG and +BCG).

Characteristic	Group		Total (n=156)	p-Value*
	-BCG (n=76)	+BCG (n=80)		
Age distribution (years), n (%)				
18 - 39	36 (47.4)	39 (48.8)	75 (48.1)	0.92
40 - 59	34 (44.7)	36 (45.0)	70 (44.9)	
60 - 69	6 (7.9)	5 (6.2)	11 (7.1)	
Sex, n (%)				
Female	51 (67.1)	41 (51.3)	92 (59.0)	0.06
Male	25 (32.9)	39 (48.8)	64 (41.0)	
Obesity, n (%)				
No	60 (78.9)	67 (83.8)	127 (81.4)	0.44
Yes	16 (21.1)	13 (16.3)	29 (18.6)	
Degree of education, n (%)				
Middle school	1 (1.3)	4 (5.0)	5 (3.2)	0.56
Incomplete graduation	33 (43.4)	33 (41.3)	66 (42.3)	
Full graduation	24 (31.6)	22 (27.5)	46 (29.5)	
Postgraduated	18 (23.7)	21 (26.3)	39 (25.0)	
Professional category, n (%)				
HCW	56 (73.7)	57 (71.3)	113 (72.4)	0.73
Non-HCW	20 (26.3)	23 (28.8)	43 (27.6)	
Economic class, n (%)				
A or B	27 (35.5)	20 (25.0)	47 (30.1)	0.35
C	31 (40.8)	38 (47.5)	69 (44.2)	
D or E	18 (23.7)	22 (27.5)	40 (25.6)	
Number of contacts with suspected COVID-19 patients, n (%)				
≤ 20 people	35 (46.1)	44 (55.0)	79 (50.6)	0.51
21 to 80 people	26 (34.2)	24 (30.0)	50 (32.1)	
> 80 people	15 (19.7)	12 (15.0)	27 (17.3)	
BCG vaccine scar, n (%)				
No	4 (5.3)	5 (6.3)	9 (5.8)	0.79
Yes	72 (94.7)	75 (93.8)	147 (94.2)	
Current smoking, n (%)				
No	75 (98.7)	73 (91.3)	148 (94.9)	0.08
Yes	1 (1.3)	7 (8.8)	8 (5.1)	
Alcohol addiction, n (%)				
No	68 (89.5)	70 (87.5)	138 (88.5)	0.70
Yes	8 (10.5)	10 (12.5)	18 (11.5)	
Comorbidity profile [#]				
Comorbidity	12 (15.8)	12 (15.0)	24 (15.4)	0.89
Hypertension	9 (11.8)	10 (12.5)	19 (12.2)	0.90
Diabetes	2 (2.6)	0 (0.0)	2 (1.3)	0.14
Cardiac insufficiency	2 (2.6)	2 (2.5)	4 (2.6)	0.96

BCG: Bacillus Calmette-Guérin; COVID-19: Coronavirus disease; -BCG: control group; +BCG: revaccinated with BCG; HCW, health care workers; * Pearson's chi-squared test; n= absolute frequency, % = relative frequency, n (%); [#] Only prevalence presented

32.9% were male (n=25), whereas in the +BCG group, 51.3% were female (n=41) and 48.8% were male (n=39), which indicated a similar sex distribution profile between the groups ($p=0.06$). In the -BCG group, 78.9% (n=60) were not obese, and 21.1% (n=16) declared their weight to be overweight, according to BMI¹⁹. Likewise, in the +BCG group, 83.8 (n=67) individuals were not obese, and 16.3 (n=13) declared their weight to be overweight, which does not indicate a statistically significant difference ($p=0.44$) between -BCG and +BCG. 73.7% (n=56 of the individuals in the -BCG group were HCWs, and 26.3% (n=20) were non-HCWs, while 71.3% (n=57) of the individuals in the +BCG group were healthcare professionals and 28.8% (n=23) were not HCWs, which indicates a non-significant statistical difference ($p=0.73$). Other sociodemographic and clinical variables were similar between the two groups, with no statistically significant differences ($p>0.05$). The sociodemographic profiles of the two groups (-BCG and +BCG) were stratified by sex into female and male categories and exhibited equal homogeneity for the observed variables (Supplemental material, Table S1).

Evaluation of WBCs counts after BCG revaccination.

WBC counts were compared between D1 and D15 in both groups (-BCG and +BCG). All laboratory values at both time points were within normal clinical reference intervals in the adult population. As shown in Table 2, revaccination with BCG did not induce any changes in the leukogram.

To assess whether sex can interfere with leukocyte changes after revaccination with BCG, we stratified participants according to sex, and a significant difference was found only in the unvaccinated male group, which showed a decrease in basophil levels (Δ : -22.44; 95% CI: -41.74; -3.14; $p=0.02$). Revaccination with

BCG Moscow did not alter the blood cell profile, regardless of sex (Supplemental material, Table S2).

Stratification of the same parameters about age was performed (Supplemental material, S3), with a cutoff point of 50 years old, <50 years old (n=120), and ≥ 50 years old (n=36). In the unvaccinated group, among individuals aged ≥ 50 years, a variation in lymphocyte count was observed (Δ : 363.70; 95% CI: 96.51; 630.89; $p=0.01$) and monocytes (Δ : -69.19; 95% CI: -22.32; -15.88; $p=0.02$). Revaccination with BCG caused a reduction in eosinophil levels in individuals aged <50 years (Δ : -32.27; 95% CI: -64.97; -9.56; $p=0.04$), whereas in individuals aged ≥ 50 years, no difference was found in serum WBC counts ($p>0.05$). However, lymphocytes showed a non-significant reduction on D15 (Δ : -229.44; 95% CI: -525.63; -66.76; $p=0.06$), generating a significant difference when we compared the mean variation between the two groups (-BCG and +BCG), $p<0.01$.

Obesity was stratified to evaluate its effect on leukocyte changes caused by BCG revaccination. Revaccination with BCG induced a reduction in eosinophil levels (Δ : -32.72; 95% CI: -57.95; -7.48; $p=0.04$) and lymphocytes (Δ : -174.06; 95% CI: -328.13; -19.99; $p=0.01$) in non-obese individuals (Table 3). There were no changes in cell levels in the obese group.

In addition, we analyzed the monocyte/lymphocyte ratio (MLR) between both groups D1 and D15 (Figure 1). Despite a slight increase in the MLR in the +BCG group, this data did not have a statistically significant difference (Δ : 0.16 ± 0.12 , $p=0.24$).

DISCUSSION

This study evaluated the WBC count changes in two cohorts of individuals included in a clinical trial investigating the efficacy and safety of BCG revaccination in preventing and reducing

Table 2: Result of the comparison of the leukogram parameters at baseline and 15 days after the intervention, considering the - BCG and + BCG groups in the total sample (n=156).

Variable (mm ³)	-BCG		Δ (-BCG)	+BCG		Δ (+BCG)	p^a	p^b	p^*
	D1	D15	Mean - CI 95%	D1	D15	Mean - CI 95%			
Leucocytes	6031.58 \pm 1470.89	6019.74 \pm 1865.33	-11.84 (-389.74 – 366.06)	6287.50 \pm 1733.76	6278.75 \pm 1823.87	-8.75 (-260.60 – 243.10)	0.95	0.95	0.94
Neutrophils	3453.75 \pm 1315.96	3433.75 \pm 1470.72	-20.00 (-382.57 – 342.57)	3480.93 \pm 1314.84	3596.21 \pm 1399.24	115.29 (-135.53 – 366.11)	0.91	0.36	0.79
Eosinophils	132.09 \pm 113.34	131.38 \pm 88.52	-0.71 (-25.57 – 24.15)	140.88 \pm 122.20	119.16 \pm 92.33	-21.71 (-48.66 – 5.23)	0.95	0.11	0.28
Basophils	12.61 \pm 30.87	5.63 \pm 18.25	-6.97 (-14.89 – 0.94)	13.35 \pm 29.82	11.59 \pm 29.32	-1.76 (-9.51 – 5.99)	0.08	0.65	0.38
Lymphocytes	2128.74 \pm 647.00	2139.50 \pm 659.79	10.76 (-134.48 – 156.01)	2327.44 \pm 717.21	2217.33 \pm 710.05	-110.11 (-249.22 – 28.99)	0.88	0.12	0.23
Monocytes	299.29 \pm 126.63	306.89 \pm 142.84	7.61 (-30.41 – 45.62)	321.91 \pm 176.09	332.51 \pm 192.76	10.60 (-39.13 – 60.33)	0.69	0.67	0.86

- BCG: control group; + BCG: revaccinated with BCG.

Paired t-test: ^a -BCG; ^b +BCG; *Student's t-test for comparison between groups

Descriptive statistics: Mean \pm standard deviation or mean and 95% confidence

the severity of COVID-19 during the pandemic¹⁷. No significant quantitative difference was found in the leukogram after BCG revaccination of professionals exposed to SARS-CoV-2. Considering the nonspecific effects involved in trained immunity induced by the BCG vaccine⁷, it was hypothesized that this could occur through changes in the proportion of different WBCs.

BCG may affect the functional and qualitative aspects of leukocyte subsets and their response to cytokine production⁵, which were not evaluated in this study. The clinical trial that provided the data for this analysis had the secondary objective of evaluating the activation of innate immunity caused by BCG through the activation of natural killer cells (NK) 15–20 days after the intervention about the day of inclusion; however, there were no significant differences between the groups¹⁷.

Previous studies on primary vaccination with BCG among the pediatric population have shown discordant results, with samples obtained at various times and in different populations. In a randomized clinical trial with low-birthweight neonates in Guinea-Bissau, BCG Danish strain vaccination was associated with increased total leukocyte, monocyte, and basophil counts 4 weeks

post-vaccination only in female children²⁰. On the other hand, another randomized, controlled clinical trial conducted in healthy Danish children did not find significant differences between the BCG Danish vaccinated group and the control group at three time

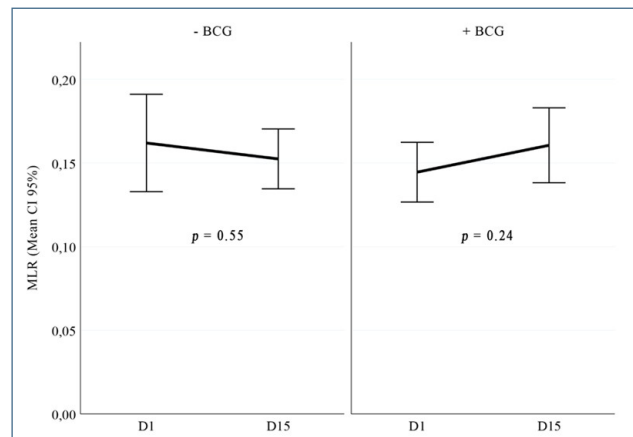


Figure 1: Comparison of monocyte/lymphocyte ratio (MLR) at D1 and D15, considering the -BCG and +BCG groups in the total sample (n=156).

Table 3: Result of the comparison of the absolute values of the leukogram at baseline and 15 days after the intervention, considering the -BCG and + BCG groups in the total sample, stratified for non-obese and obese.

Variable (mm³)	-BCG		Δ (-BCG)	+BCG		Δ (+BCG)	p ^a	p ^b	p [*]
	D1	D15	Mean - CI 95%	D1	D15	Mean - CI 95%			
Non obese (n = 127)									
Leucocytes	6103.33 ± 1529.70	5958.33 ± 1591.21	-145.00 (-514.16 – 224.16)	6140.30 ± 1756.12	6034.33 ± 1726.49	-105.97 (-380.78 – 168.84)	0.69	0.30	0.72
Neutrophils	3514.55 ± 1395.27	3383.48 ± 1129.30	-131.07 (-507.68 – 245.55)	3346.94 ± 1319.13	3438.21 ± 1330.60	91.27 (-173.30 – 355.83)	0.99	0.68	0.79
Eosinophils	136.52 ± 112.42	134.30 ± 91.12	-2.22 (-30.58 – 26.15)	143.52 ± 125.66	110.81 ± 73.60	-32.72 (-57.95 - -7.48)	0.92	0.04	0.22
Basophils	15.97 ± 34.01	7.13 ± 20.31	-8.83 (-18.87 – 1.20)	13.79 ± 30.49	11.70 ± 29.95	-2.09 (-10.90 – 6.72)	0.12	0.59	0.40
Lymphocytes	2136.23 ± 699.40	2126.22 ± 707.26	-10.02 (-182.20 – 162.16)	2315.79 ± 692.75	2141.73 ± 694.81	-174.06 (-328.13 - -19.99)	0.98	0.01	0.14
Monocytes	293.60 ± 126.67	305.10 ± 137.28	11.50 (-30.92 – 53.92)	316.67 ± 174.18	330.37 ± 195.50	13.70 (-42.95 – 70.35)	0.94	0.73	0.89
Obese (n = 29)									
Leucocytes	5762.50 ± 1231.19	6250.00 ± 2714.16	487.50 (-739.84 – 1714.84)	7046.15 ± 1443.15	7538.46 ± 1858.11	492.31 (-148.86 – 1133.48)	0.70	0.14	0.44
Neutrophils	3225.75 ± 964.96	3622.25 ± 2398.10	396.50 (-668.25 – 1461.25)	4171.46 ± 1092.69	4410.54 ± 1514.53	239.08 (-587.84 – 1066.00)	0.68	0.60	0.83
Eosinophils	115.50 ± 118.91	120.44 ± 79.71	4.94 (-52.70 – 62.57)	127.23 ± 105.89	162.23 ± 154.69	35.00 (-75.57 – 145.57)	0.64	0.58	0.66
Basophils	0.00 ± 0.00	0.00 ± 0.00	0.00 (0.00 – 0.00)	11.08 ± 27.05	11.00 ± 26.98	-0.08 (-17.32 – 17.16)	1.00	1.00	0.51
Lymphocytes	2100.63 ± 409.82	2189.31 ± 453.65	88.69 (-185.33 – 362.70)	2387.46 ± 861.58	2606.92 ± 683.77	219.46 (-75.06 – 513.98)	0.76	0.15	0.40
Monocytes	320.63 ± 128.21	313.63 ± 166.83	-7.00 (-102.37 – 88.37)	348.92 ± 190.56	343.54 ± 185.06	-5.38 (-112.98 – 102.21)	0.26	0.62	0.90

- BCG: control group; + BCG: revaccinated with BCG

Paired t-test: ^a -BCG; ^b +BCG; ^{*} Student's t-test for comparison between groups

Descriptive statistics: Mean ± standard deviation or mean and 95% confidence interval

points, 4 days, 3 months, and 13 months after immunization, in total leukocytes or differential white cell counts²¹.

Among the studies involving adults, Koeken *et al.*¹⁶ described the results of a 300-BCG cohort, a follow-up of young adults vaccinated with the BCG Bulgarian strain in the Netherlands. They showed stability in WBC counts after vaccination; however, in differential counts, BCG caused an increase in lymphocytes and monocytes in the samples collected 2 weeks after immunization. Moreover, in a study of 75 young women (mean age, 23 years) in the Netherlands, the BCG Danish strain caused an increase in total WBC between one and four days after vaccination due to an increase in neutrophils and monocytes. There were no effects on lymphocytes, and normalization of cell levels was observed after two weeks²².

In older individuals (over 65 years old), a randomized clinical trial evaluated the non-specific effects of the BCG Danish strain compared to a placebo in preventing infections. They reported no differences in WBC counts between two weeks or three months after the intervention⁵.

This study differs from the studies in several respects. First, the effects of BCG on leukocytes increasing after revaccination with the BCG Moscow strain have not been evaluated. Brazil has a high burden of TB; and therefore, newborns are routinely vaccinated²³ using the BCG Moreau strain produced by the *Fundação Ataufo de Paiva* (FAP), Rio de Janeiro, for several years. However, due to the suspension of operations at this institution by the National Health Surveillance Agency (ANVISA), production of BCG Moreau was interrupted in 2021. Since then, the Ministry of Health has been importing BCG Moscow from the Serum Institute of India to prevent shortages of the BCG vaccine in Brazil²⁴. Thus, in this study, revaccination was performed with a different strain from that previously used in the primary vaccination, demonstrating the results' originality.

The initial BCG strain was created over 100 years ago by Calmette and Guérin. The Pasteur Institute manufactured BCG until 1961. Due to the increasing demand for vaccine production at that time, the institute distributed strains to other laboratories. These laboratories cultured the strains in non-standardized media, resulting in the development of various strains in different regions²⁵. Since the strains have biological differences, this may result in varying levels of protection and contribute to the highly variable protective efficacy of BCG against tuberculosis observed in clinical trials²⁴. Similarly, the immune response elicited by the vaccine varies depending on the strain used²⁴. The BCG Danish strain has been most frequently studied in the context of evaluating the nonspecific effects of BCG, with BCG Moscow being less immunogenic than others^{26,27}.

This study analyzed the variation in WBC counts only at one time, two weeks after BCG. In the present study, the choice of blood count analysis in this interval agreed with the secondary

objective of the clinical trial that provided data for the analysis: activation of NK cells 15–20 days after the intervention¹⁷. Among studies conducted in the adult age group that identified changes in WBC count, the study by Koeken *et al.*¹⁶ differs from the current study not making a comparison with the control group and in not evaluating revaccination. The study by Blok *et al.*²², which analyzed only females, found changes at earlier times compared to the current study. Other factors associated with BCG immunogenicity, such as nutritional status and age at vaccination²¹, may explain the different results found in clinical trials conducted in children.

The nonspecific effects of vaccines composed of living microorganisms have been described differently between sexes, with females being more susceptible to these immunomodulatory effects²⁸. A clinical trial by Jensen *et al.*²⁰ in low-birthweight newborns in Guinea-Bissau showed a tendency for the specific and non-specific effects of BCG to be more potent in females than in male infants. Three randomized studies that evaluated the effects of neonatal BCG on all-cause mortality showed differences in the temporality of benefits between female and male infants. In the first week after vaccination, male infants showed a greater beneficial effect in reducing mortality, whereas in female infants, the most pronounced effect occurred after the first week of vaccination³.

Considering the different immunological responses to BCG between males and females, stratification of the analyses was performed for both sexes. However, no difference was found in WBC counts associated with BCG. The only significant variation occurred in the unvaccinated group, in which there was a reduction in basophil levels, which was unrelated to the BCG. A study by Jensen *et al.*²¹ in healthy Dutch children also found no differences in WBC counts during sex stratification. Studies conducted in adult age groups did not present stratification results according to sex.

Our study involved economically active professionals working face-to-face during the COVID-19 pandemic. Therefore, they were more vulnerable to viral contamination. Thus, most of the study population was comprised of young adults. Aging leads to a gradual decline in immune function, called immunosenescence, which contributes to a lower vaccine response in older individuals, making them more susceptible to infections, inflammatory diseases, malignancies, and autoimmune disorders²⁹.

A study conducted with a 13-valent pneumococcal conjugate vaccine showed a better vaccine response in individuals vaccinated at a younger age, between 50 and 59 years, with higher production of serotypes, about the application at an older age, between 60 and 64 years old³⁰. During the COVID-19 pandemic, individuals aged ≥ 65 years were included in the risk group and were associated with a worse prognosis^{31,32}. Thus, we stratified the analysis by age, with a cutoff point of 50 years. The only difference found was in the age group <50 years, in which there was a reduction in eosinophils 2 weeks after revaccination with BCG. Koeken *et al.*¹⁶ observed an increase in eosinophils in blood samples collected three months after

BCG. Other studies did not find the alterations observed in the current study, and further studies are required to explain this effect.

Interestingly, studies have also observed suboptimal vaccine immune responses in obese individuals, owing to mechanisms that have not yet been fully elucidated³³, in addition to increased susceptibility to infections³⁴. During the COVID-19 pandemic, obesity was associated with a higher risk of hospitalization, ICU admission, and death^{35,36}. In our stratification considering obesity with a BMI cutoff of 30 kg/m², BCG caused a significant reduction in eosinophil and lymphocyte counts in non-obese individuals. However, these effects have not been evaluated in other studies of BCG.

Changes in WBC results can occur for distinct reasons unrelated to vaccination, such as stress, allergic reactions, inflammation of another nature, and even chronic inflammation³⁷. Furthermore, the significant results of our study appeared in stratified groups that contained a smaller number of individuals and, therefore, we recommend caution when interpreting our results and reinforcing that deeper investigations need to be conducted to verify the association with the leukogram³⁷.

The proportions of different leukocytes have been studied as potential biomarkers in various conditions. The MLR in the bloodstream may reflect the body's ability to mount an effective immune response, which has already been associated with the inhibition of mycobacterial growth *in vitro*³⁸ and active tuberculosis³⁹. Considering the potential of MLR as a biomarker associated with mycobacteria, we investigated the effect of BCG Moscow on this ratio; however, we found no association between MLR and BCG Moscow.

Additionally, our study did not use a placebo for non-revaccinated HWs, as to date, there is no simulated vaccine available that causes reactions like BCG. Furthermore, we believe that being HWs, these professionals would easily detect that they were allocated to the control group (using placebo). Finally, it is noteworthy that other randomized clinical trials evaluated the effect of BCG on the differential leukocyte count measured in peripheral blood using or not using a placebo for the control group^{5,21}. The results of these studies also did not find any effect of BCG in differential leukocyte counts at any time, which suggests that the absence of a placebo in the control group does not interfere with the inferences obtained. Despite this, we recommend caution when interpreting our findings.

A strength of this study is that it considered data from a clinical trial in which randomization ensured homogeneity between the

revaccinated and unvaccinated groups. Moreover, highly qualified professionals performed blood collection and laboratory tests, and the professionals who performed laboratory tests and data analysis were blinded.

However, we highlight that the analysis of the leukogram comparing only two moments (day 1 and day 15) may have represented a limitation of the study and, therefore, we recommend carrying out additional investigations, including at more moments. Moreover, it is important to note the limitations of blood count. As a screening test for infectious or inflammatory conditions, blood count does not always reflect events at the vaccination site and has a large degree of individual and temporal variation.

Studies using more complex immunological assays than WBC analysis may be useful to verify the effects of the BCG vaccine in preventing or reducing the severity of different infections. Therefore, recently, our group further investigated the immunological response to BCG vaccination in participants included in the BCG-COVID-19 clinical trial. In this approach, participants were divided into diverse groups according to the variation in the expression of IFN- γ by NK cells between days 1 and 15 after BCG vaccination. In conclusion, it was shown that CD314 expression by NK cells before BCG vaccination influences their IFN- γ responses, the generation of NK subpopulations, and the specific T immune response at 15 days after vaccination. Despite this, we recommend further investigations to confirm whether revaccination with BCG induces an increase in serum leukocyte counts and differential counts, especially in other populations. Ours and other studies are particularly important to improve understanding of the immune response generated by BCG and its association with leukocyte changes⁴⁰.

In the present study, revaccination with BCG Moscow did not stimulate white cell production 15 days post-vaccination. This may be because the putative protective mechanisms related to BCG immunity training were unrelated to the macroscopic response observed in the blood count.

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