

BCG Vaccination Modulates Long-Term TNF- α and sCD40L in COVID-19: An Exploratory Longitudinal Study

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Background: Bacillus Calmette–Guérin (BCG) vaccination exerts non-specific immunomodulatory effects through trained immunity, potentially modulating inflammatory responses in COVID-19. Tumor necrosis factor-alpha (TNF- α) and soluble CD40 ligand (sCD40L) are key inflammatory and pro-thrombotic mediators implicated in COVID-19 pathogenesis.

Methods: A randomized, placebo-controlled trial was conducted involving young, healthy adults with mild COVID-19 (BATTLE trial). Intradermal BCG vaccination or placebo was administered during the acute phase of infection. Plasma TNF- α and sCD40L levels were measured at days 7 and 45, and at 6 months post-intervention in a subset of participants, total n=13; BCG n=7; placebo n=6. The sCD40L/TNF- α ratio was calculated to explore the balance between adaptive immune activation and systemic inflammation.

Results: At day 7, BCG-vaccinated individuals exhibited higher TNF- α (p=0.01) and sCD40L (p=0.018) levels compared with the placebo group. In the placebo group, TNF- α showed a transient decline by day 45 (p=0.040), whereas sCD40L remained stable throughout follow-up (all p>0.05). In contrast, BCG recipients demonstrated a sustained reduction in both mediators from day 45 to 6 months (TNF- α : p=0.0012 at day 45 and p=0.0017 at 6 months; sCD40L: p=0.024 at day 45 and p=0.05 at 6 months). The sCD40L/TNF- α ratio increased transiently at day 45 in the BCG group (p=0.035), suggesting a temporary predominance of adaptive immune activation.

Conclusion: BCG vaccination induced a distinct and durable modulation of TNF- α and sCD40L in mild COVID-19, consistent with the concept of trained immunity. This immune profile may support faster resolution of inflammation and potentially reduce the risk of inflammatory complications. Larger and more diverse controlled trials are needed to confirm these findings and clarify their clinical implications.

Keywords: COVID-19, Bacillus Calmette–Guérin, trained immunity, TNF-alpha, sCD40L, inflammation, cytokines

Introduction

The COVID-19 pandemic, caused by the SARS-CoV-2 virus, emerged as one of the most significant public health crises in modern history, affecting millions of people worldwide and straining global healthcare systems.¹ At the onset of the pandemic, it became evident that an intense systemic inflammatory response is a key factor in determining disease severity and progression. This phenomenon, often referred to as a “cytokine storm”, is associated with severe complications, including acute respiratory distress syndrome, multiple organ failure, and death.^{2,3}

The human immune system responds to SARS-CoV-2 infection through a complex cascade of molecular and cellular events involving both innate and adaptive immunity.⁴ During the acute phase of infection, a massive release of

inflammatory mediators, including pro-inflammatory cytokines, chemokines, and other soluble factors, occurs, orchestrating the immune response.^{5,6} Among these mediators, tumor necrosis factor-alpha (TNF- α) and soluble CD40 ligand (sCD40L) are particularly relevant biomarkers in the pathogenesis of COVID-19.⁷⁻⁹

TNF- α is one of the most potent and well-characterized pro-inflammatory cytokines in the immune system, produced mainly by activated macrophages, dendritic cells, and T lymphocytes.¹⁰ This cytokine plays crucial roles in host defense against pathogens but can also contribute to tissue damage when produced in excess.¹¹ In COVID-19, elevated levels of TNF- α have been consistently associated with disease severity, progression to critical illness, and poor outcomes. It promotes endothelial activation, increases vascular permeability, induces the expression of adhesion molecules, and facilitates the recruitment of inflammatory cells to affected tissues.^{12,13}

Meanwhile, sCD40L is a multifunctional inflammatory mediator that plays important roles in platelet activation, endothelial function, and immune response.¹⁴ Initially identified as a regulator of B-cell activation and antibody production, sCD40L is primarily released by activated platelets and T cells, exerting both pro-inflammatory and pro-thrombotic effects.^{15,16} In the context of COVID-19, sCD40L has been implicated in the pathogenesis of the thrombotic complications that characterize many severe cases of the disease, contributing to endothelial dysfunction, thrombus formation, and the perpetuation of the inflammatory response, establishing a vicious cycle that worsens patient prognosis.^{9,17}

Understanding how these cytokines behave over time and in response to immunomodulatory interventions may provide valuable insights into the long-term inflammatory profile of individuals affected by COVID-19.¹⁸ Longitudinal studies have shown that the inflammatory response in COVID-19 is not limited to the acute phase of infection but may persist for weeks or months after the resolution of initial symptoms.^{19,20} This prolonged inflammation has been linked to the development of post-COVID-19 syndrome, also known as “long COVID”, characterized by persistent symptoms that significantly impact patients’ quality of life.²¹⁻²⁴

In this context, the search for therapeutic strategies that can modulate the inflammatory response in COVID-19 became a research priority.²⁵ Among the approaches investigated, vaccination with *Bacillus Calmette-Guérin* (BCG) emerged as a promising strategy due to its well-documented non-specific immunomodulatory effects.^{26,27} Originally developed as a vaccine against tuberculosis, BCG has demonstrated the ability to confer protection against a wide range of infections by enhancing innate immune memory, a phenomenon known as “trained immunity”.^{28,29}

Trained immunity refers to the innate immune system’s ability to develop a form of immunological memory, resulting in more effective responses to subsequent pathogen exposures.³⁰ This process involves the epigenetic and metabolic reprogramming of innate immune cells, particularly monocytes and macrophages, resulting in an enhanced capacity to produce pro-inflammatory cytokines in response to secondary stimuli.^{31,32} In the context of viral infections, including respiratory infections, both epidemiological and experimental studies have suggested that BCG vaccination may provide non-specific protection by reducing the incidence and severity of respiratory diseases.^{33,34}

Observational studies conducted during the COVID-19 pandemic identified inverse correlations between national BCG vaccination rates and the incidence, severity, and mortality of COVID-19.³⁵ While these observations generated considerable interest in the scientific community, the observational nature of the studies and the presence of multiple confounding factors have limited the ability to establish definitive causal relationships.³⁶ As a result, randomized controlled clinical trials became necessary to rigorously assess BCG’s therapeutic potential in COVID-19.^{37,38}

The mechanisms by which BCG can exert beneficial effects in COVID-19 are multiple and complex.³⁹ In addition to inducing trained immunity, BCG may modulate the immune response through dendritic cell activation, type I interferon production, stimulation of regulatory T cells, and modulation of the antibody response.^{40,41} These effects may lead to a more balanced immune response, reducing the excessive inflammation characteristic of severe COVID-19, while preserving the ability to control viral replication.⁴²

Despite growing interest in BCG’s immunomodulatory potential in COVID-19, although recent studies have investigated the immunological effects of BCG in COVID-19, longitudinal data specifically addressing the temporal modulation of key cytokines during active infection remain limited.^{43,44} Most studies published to date have focused on primary clinical outcomes such as hospitalization, mechanical ventilation, or mortality, with limited investigation of the underlying immunological mechanisms.^{45,46} Longitudinal analysis of inflammatory biomarkers in BCG-treated patients

could provide crucial insights into the mechanisms of action of this intervention and help identify potential predictors of therapeutic response.⁴⁷

Considering the importance of TNF- α and sCD40L in the pathogenesis of COVID-19 and the immunomodulatory potential of BCG, this exploratory study was designed to investigate whether BCG vaccination can modulate the systemic expression of these cytokines in patients with active COVID-19.⁴⁸ Through a preliminary longitudinal analysis of the acute phase of infection up to six months post-infection, we aim to characterize the temporal expression patterns of TNF- α and sCD40L and determine whether BCG exerts specific modulatory effects on these inflammatory mediators. This investigation may contribute to a better understanding of BCG's immunomodulatory mechanisms in the context of COVID-19.

Importantly, this study was conducted in a cohort of young adults, which imposes limitations on the extrapolation of findings to pediatric populations. This distinction is particularly relevant given that BCG is primarily administered during the neonatal period and that immune responses to BCG are known to be age dependent. In neonates and children, trained immunity has been associated with distinct cytokine profiles, including a more tightly regulated inflammatory response and reduced propensity for TNF- α hyperproduction, as highlighted in pediatric immunology studies.^{26,33,49} These developmental differences in immune ontogeny should be carefully considered when interpreting the present findings and their potential translational implications across age groups. Additionally, variability among BCG strains (eg, Russian versus Brazilian) and prior exposure to *Mycobacterium tuberculosis* or environmental mycobacteria may significantly influence the magnitude and consistency of trained immunity responses, thereby impacting the immunomodulatory efficacy of BCG, as highlighted in recent meta-analyses.^{26,34} Furthermore, it is important to consider potential variability in cytokine quantification, particularly for sCD40L and TNF- α in plasma, as differences between analytical assays may influence biomarker levels and their interpretation, as reported in cytokine biomarker studies.^{50,51}

Materials and Methods

Patients and Ethics Statement

This study is a secondary analysis of samples and data derived from the BATTLE clinical trial (BCG Against COVID-19: A Double-Blind, Placebo-Controlled Trial), a multicenter, prospective, randomized (1:1), double-blind, parallel-group, placebo-controlled Phase III trial. The trial was registered at ClinicalTrials.gov (NCT04369794) and approved by the Brazilian National Research Ethics Commission (CONEP; approval no. 31049320.7.1001.5404). All participants provided written informed consent before enrollment.⁵²

Recruitment occurred between October 2020 and December 2021. Eligible participants were adults aged 18 years or older, of both sexes, with a confirmed SARS-CoV-2 infection diagnosed within the preceding 14 days by RT-qPCR from nasopharyngeal swabs. Participants were randomly assigned to receive an intradermal injection of 0.1 mL of BCG (BCG Group) or a placebo (0.9% saline, Control Group) within 14 days of symptom onset. Patients were followed up at 7 (T1), 45 (T2), and 180 (T3) days post-injection. The exclusion criteria were pregnancy, a history of compromised immunity, and inability or unwillingness to provide informed consent.

For this secondary analysis, a subset of participants was selected from the original trial population based on the availability of complete plasma samples at all predefined time points (days 7, 45, and 180). To minimize selection bias, samples were randomly selected among participants who completed follow-up through the final time point and had samples available for all study visits. No additional clinical or demographic selection criteria were applied beyond sample completeness. This approach ensured that the analyzed subset remained representative of the study population that completed longitudinal follow-up, while enabling a consistent temporal evaluation of inflammatory markers. Table 1 summarizes the clinical and demographic characteristics of the participants included in this secondary analysis. This distinction ensures that the present study provides a novel mechanistic perspective within the broader BATTLE research framework.

Table 1 Clinical and Demographic Characteristics of the Patients Included in the Study

	Placebo-Control Group	BCG Group
Number of patients	6	7
Gender (male/female)	3/3	3/4
Age (median–min/max)	34 years old (24–46)	33 years old (28–48)
BMI	23.5 (21.34–30.76)	23.8 (21.5–27.16)
Smoker (Yes/No)	1/5	0/7
Light Alcohol consumption (Yes/No)	4/2	6/1
Physical activity level (Sedentary/Light/Moderate/Vigorous)	1/0/1/4	1/0/3/3

Note: Numerical variables are described as the median [min, max], and categorical variables are expressed as absolute frequencies.

Abbreviation: BMI, Body mass index.

Cytokines Quantification

Peripheral blood was collected from patients, and plasma samples were obtained by centrifugation at 3500 rpm for 15 minutes at 4 °C. The plasma aliquots were snap-frozen and stored at –80 °C until analysis. Samples were kept at room temperature only during the assay. Cytokine concentrations, including CD40L and TNF- α , were quantified from patient plasma samples using the Bio-Plex Pro Human Th17 Cytokine Assay Kit (catalog number 171AA001M, Bio-Rad Laboratories, Hercules, CA, USA), following the manufacturer's instructions.

The 96-well plates were pre-wetted and washed, then incubated with magnetic beads, standards, and plasma samples for 60 minutes. After adding detection antibodies and a 30-minute incubation, streptavidin-PE was applied, followed by a wash, and the sample was incubated for 10 minutes at room temperature. Following a final wash, the samples were analyzed on a Luminex system using Bio-Plex Manager software (version 6.0) at the Life Sciences Core Facility (LaCTAD) at the University of Campinas (Unicamp).

Statistics

Statistical analyses were performed using appropriate parametric or nonparametric tests based on data distribution, assessed by the Shapiro–Wilk test. Between-group comparisons were performed using Student's *t*-test or Mann–Whitney *U*-test, as appropriate. A *p*-value <0.05 was considered statistically significant. Given the exploratory nature and small sample size of this study, no formal power calculation was performed, and no adjustments for multiple comparisons were applied. Results should therefore be interpreted as hypothesis-generating.

Results

Temporal Modulation of TNF- α Levels Following BCG Vaccination in COVID-19 Patients

The systemic inflammatory response was assessed by quantifying plasma TNF- α levels at different time points following administration of BCG or placebo in patients with COVID-19 (Figure 1).

After seven days of intervention, patients vaccinated with BCG showed significantly higher levels of TNF- α compared to those in the placebo group (Figure 1A; *p* = 0.01), indicating an initial activation of the inflammatory response associated with vaccination.

In the placebo group, a significant reduction in TNF- α levels was observed at 45 days compared to day 7 (Figure 1B; *p* = 0.040). However, no statistical differences were found between TNF- α levels at 6 months and those at either 45 days (*p* = 0.22) or 7 days (*p* = 0.22), suggesting that inflammatory markers stabilized after the second month (Figure 1B).

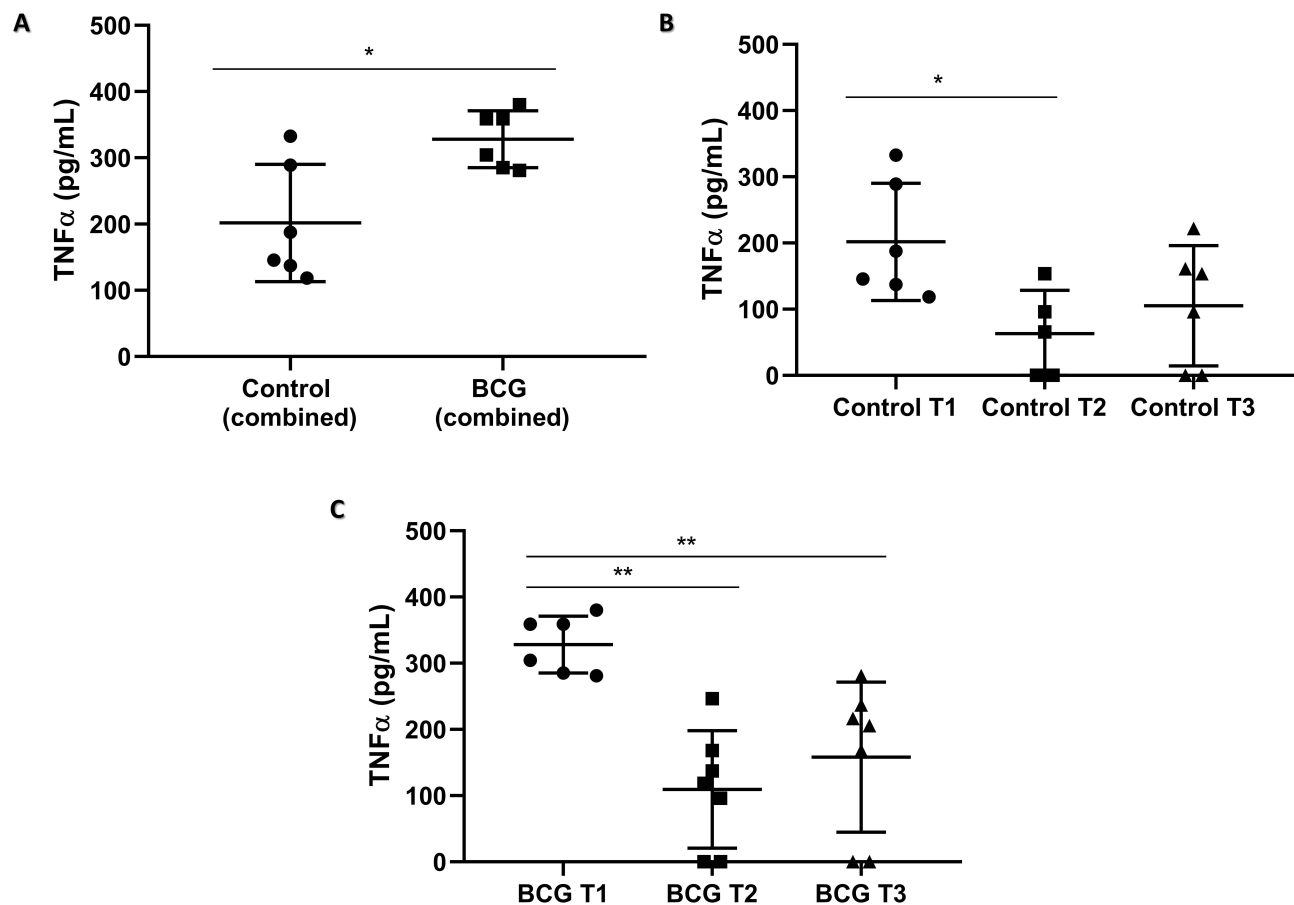


Figure 1 Modulation of plasma TNF- α levels in response to BCG vaccination. Plasma levels of TNF- α were measured at multiple time points following BCG or placebo administration in COVID-19 patients. **(A)** Combined analysis of all time points shows that patients vaccinated with BCG exhibited higher TNF- α levels compared to the placebo group. **(B)** In the placebo group, TNF- α levels significantly decreased on day 45 compared to day 7, with no further differences observed between day 45 and 6 months or between day 7 and 6 months. **(C)** In the BCG-vaccinated group, TNF- α levels significantly declined by day 45 and remained reduced at 6 months, indicating sustained modulation of systemic inflammation induced by BCG vaccination. Control Group $n=6$. BCG Group $n=7$. Control Group = Patients who received placebo injection. BCG Group = Patients who received BCG vaccination. Control T1 = Placebo group evaluated on day 7. Control T2 = Placebo group evaluated on day 45. Control T3 = Placebo group evaluated at 6 months. BCG T1 = BCG group evaluated at day 7. BCG T2 = BCG group evaluated on day 45. BCG T3 = BCG group evaluated at 6 months. * $p < 0.05$; ** $p < 0.01$.

Among patients who received BCG, TNF- α levels significantly decreased by day 45 post-vaccination (Figure 1C; $p = 0.0012$) and remained low at 6 months (Figure 1C; $p = 0.0017$), indicating a sustained modulation of the systemic inflammatory response induced by BCG over time.

Longitudinal Dynamics of sCD40L Levels After BCG Vaccination in COVID-19 Patients

The time-dependent modulation of the immune response was further investigated by quantifying plasma sCD40L levels at various time points following the administration of BCG or placebo in patients with COVID-19 (Figure 2).

At day 7 post-intervention, patients who received BCG exhibited a significant increase in sCD40L levels compared to the placebo group (Figure 2A; $p = 0.018$), suggesting that vaccination induced an early upregulation of this mediator. In the placebo group, sCD40L concentrations remained stable over time, with no significant differences between days 7, 45, and 6 months (Figure 2B; $p = 0.47$ T2 vs T1 and $p = 0.99$ T3 vs T1), indicating that infection with SARS-CoV-2 alone did not alter systemic sCD40L levels.

In contrast, in the BCG group, the elevated sCD40L levels observed on day 7 declined markedly by day 45 and remained low at 6 months (Figure 2C; $p = 0.024$). At the 6-month time point, sCD40L values approached those of the

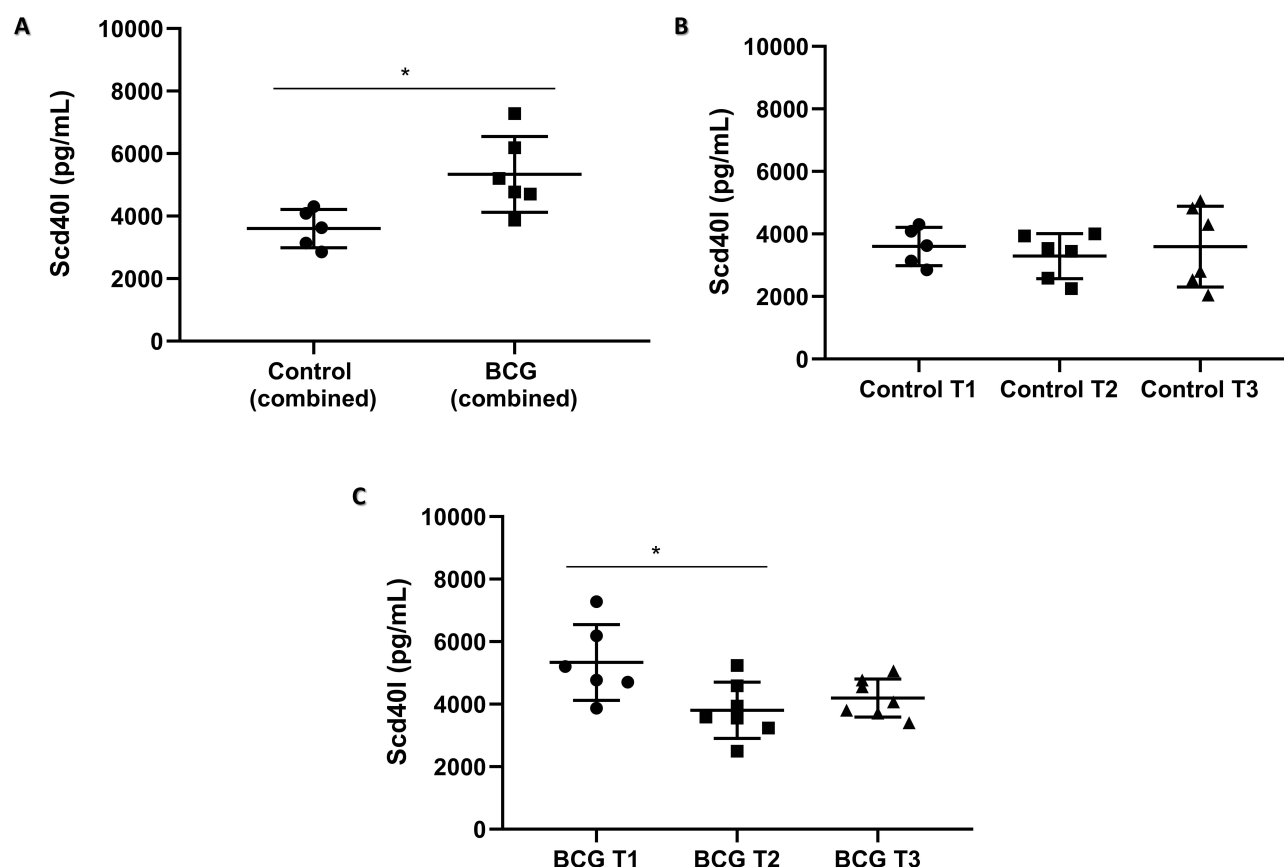


Figure 2 Modulation of plasma sCD40L levels in response to BCG vaccination. Plasma levels of sCD40L were measured at multiple time points following BCG or placebo administration in COVID-19 patients. **(A)** Combined analysis of all time points shows that patients vaccinated with BCG exhibited higher sCD40L levels compared to the placebo group. **(B)** In the placebo group, sCD40L concentrations remained stable over time, with no significant differences between day 7, day 45, and 6 months. **(C)** In the BCG group, the elevated sCD40L levels observed on day 7 markedly declined by day 45 and remained reduced at 6 months, approaching values observed in the placebo group. Control Group $n=6$. BCG Group $n=7$. Control Group = Patients who received placebo injection. BCG Group = Patients who received BCG vaccination. Control T1 = Placebo group evaluated on day 7. Control T2 = Placebo group evaluated on day 45. Control T3 = Placebo group evaluated at 6 months. BCG T1 = BCG group evaluated at day 7. BCG T2 = BCG group evaluated on day 45. BCG T3 = BCG group evaluated at 6 months. * $p < 0.05$; ** $p < 0.01$.

placebo group (Figure 2C; $p = 0.05$), supporting the interpretation that the observed early increase was attributable to BCG vaccination rather than to COVID-19 itself.

Relative Dynamics of Adaptive Immune Activation versus Systemic Inflammation (sCD40L/TNF Ratio) Following BCG Vaccination in COVID-19 Patients

No significant changes in the sCD40L/TNF ratio were observed in the placebo group across the different study time points (Figure 3A; $p = 0.31$ for T1 vs. T2; $p = 0.97$ for T3 vs. T1).

In contrast, in the BCG-vaccinated group, the sCD40L/TNF ratio significantly increased at day 45 compared with day 7 post-vaccination (Figure 3B; $p = 0.035$), suggesting a transient predominance of adaptive immune activation over systemic inflammation. However, this effect was no longer evident in 6 months, when the ratio returned to levels comparable to those observed on day 7 (Figure 3B; $p = 0.37$).

Discussion

This study evaluated the long-term immunomodulatory effects of BCG vaccination in a cohort of young adults with mild COVID-19. The homogeneity of the sample, characterized by the absence of comorbidities, balanced demographics, and mild clinical presentations, provided a controlled environment to investigate subtle immunological changes without the

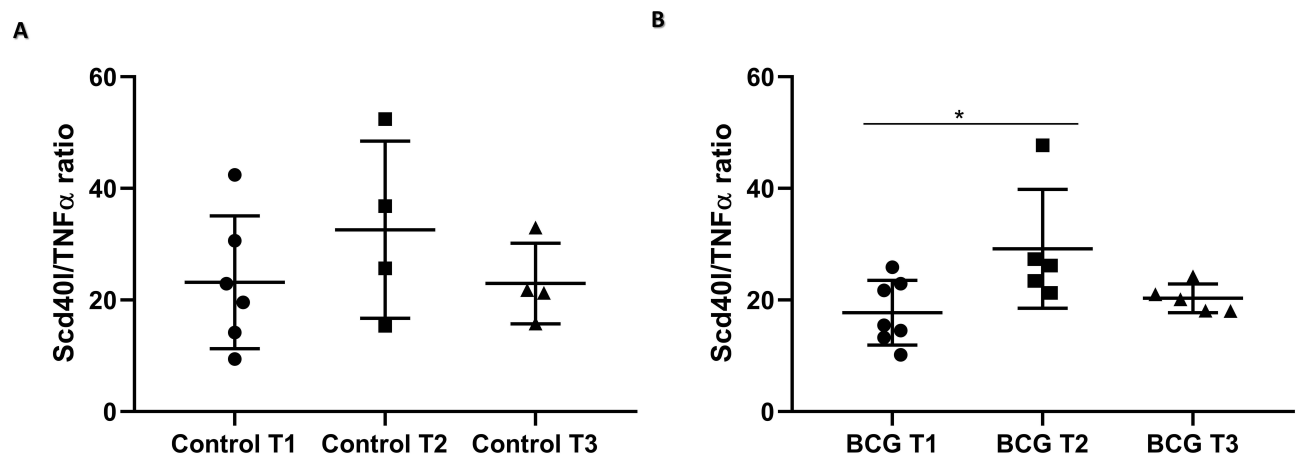


Figure 3 sCD40L/TNF Ratio as an Indicator of Adaptive Immune Modulation Relative to Inflammation. The sCD40L/TNF ratio was measured at multiple time points following BCG or placebo administration in COVID-19 patients. **(A)** In the placebo group, no significant changes in the sCD40L/TNF ratio were detected across the evaluated time points. **(B)** In the BCG group, the sCD40L/TNF ratio significantly increased at day 45 compared with day 7 post-vaccination, indicating a transient predominance of adaptive immune modulation over systemic inflammation. This effect was lost in 6 months, when the ratio returned to values comparable to those observed on day 7. Control Group $n = 6$. BCG Group $n = 7$. Control Group = Patients who received placebo injection. BCG Group = Patients who received BCG vaccination. Control T1 = Placebo group evaluated on day 7. Control T2 = Placebo group evaluated on day 45. Control T3 = Placebo group evaluated at 6 months. BCG T1 = BCG group evaluated at day 7. BCG T2 = BCG group evaluated on day 45. BCG T3 = BCG group evaluated at 6 months. * $p < 0.05$; ** $p < 0.01$.

confounding influence of severe disease. Although this limits the generalizability of our findings, it enables a focused analysis of BCG's impact on systemic inflammatory mediators within the context of a self-limited viral infection.

The results of this longitudinal study provided evidence on the immunomodulatory potential of BCG vaccination in COVID-19 patients, demonstrating specific effects on key inflammatory cytokines over six months, consistent with the concept of trained immunity.^{26–29} The analysis of temporal patterns of TNF- α and sCD40L revealed potential mechanisms by which BCG may influence systemic inflammatory responses during COVID-19, contributing to the understanding of its immunomodulatory effects.

The significant reduction in TNF- α levels observed at 45 days in the control group, compared to 7 days post-infection, reflects the natural progression of the inflammatory response after the acute phase of COVID-19.^{2,4,6,7} This pattern, previously described in the literature, is associated with a gradual decline in pro-inflammatory cytokines predominantly produced by alveolar macrophages and dendritic cells in response to viral infection.^{10–12}

The most relevant finding, however, was the distinct modulation observed in BCG-vaccinated patients. In this group, a more pronounced initial elevation of TNF- α was followed by a sustained reduction at day 45, with low levels maintained throughout six months. This behavior suggested that BCG induces not only a more robust early inflammatory response but also a more orchestrated and prolonged resolution of systemic inflammation. Such effects are consistent with the epigenetic reprogramming of innate immune cells, a hallmark of trained immunity, which promotes more balanced subsequent responses. This reprogramming involves histone modifications and DNA methylation, as well as the potential expansion of regulatory T cells (Tregs), which play a key role in controlling excessive inflammatory responses.^{31,34,41}

The analysis of sCD40L further supports the specificity of the modulation induced by BCG. In the control group, no significant changes in this molecule were observed during follow-up, suggesting that its modulation is not part of the natural course of mild COVID-19. In contrast, the BCG group exhibited a significant reduction in sCD40L levels both at day 45 and six months after immunization. Since sCD40L is released by activated platelets and effector T cells and is involved in endothelial activation and thrombus formation, the BCG-induced reduction may have important implications for preventing thrombotic complications associated with COVID-19.^{9,15,17}

The assessment of the sCD40L/TNF ratio allowed exploration of the relationship between adaptive immune activation and systemic inflammation. In vaccinated patients, a transient increase in this ratio was observed at day 45, suggesting a temporary predominance of adaptive immune activation over inflammation, consistent with the known kinetics of BCG-induced activation. By six months, the ratio had returned to baseline levels, indicating that this adaptive

profile does not persist in the long term. The absence of significant changes in the control group further supports the notion that the observed modulation is attributable to vaccination, rather than SARS-CoV-2 infection itself.

From a clinical perspective, the sustained modulation of TNF- α and sCD40L by BCG may be associated with improved resolution of inflammatory responses, although this study was not designed to assess clinical outcomes directly, such as post-COVID-19 syndrome.^{18,22,34,41} In this study, all patients experienced rapid clinical recovery, with no need for hospitalization or progression to long COVID, consistent with the hypothesis that BCG promotes a more regulated immune profile.

The findings of this study align with the concept of trained immunity, characterized by epigenetic and metabolic reprogramming of innate immune cells, resulting in modified responses to secondary challenges.⁵¹ The observed patterns of TNF- α and sCD40L modulation support the hypothesis that BCG induces a state of more controlled and efficient inflammatory response, with potential clinical benefits in COVID-19.

Despite the relevance of these results, some limitations must be acknowledged. The sample size, although sufficient to detect differences in the analyzed cytokines, limits generalizability to other populations. The analysis focused on key targets TNF- α and sCD40L. The safety of BCG re-vaccination in convalescent COVID-19 patients was previously established.⁵² Tuberculin skin tests or interferon-gamma release assays were not performed before vaccination, which could have provided additional information on baseline immune status. Furthermore, the study included only young and healthy individuals with mild COVID-19, precluding extrapolation to severe cases or populations with comorbidities.

The relatively small and homogeneous sample, composed exclusively of young, healthy adults with mild disease, limits the external validity of the findings and precludes direct extrapolation to other populations, particularly pediatric cohorts. This limitation is especially relevant given that BCG is primarily administered during the neonatal period and that immune responses to this vaccine are strongly age dependent. Differences in immune ontogeny may result in distinct trained immunity profiles in children, including more tightly regulated inflammatory responses and altered cytokine production dynamics, such as reduced propensity for TNF- α hyperproduction. These factors should be carefully considered when interpreting the translational implications of the present findings across age groups.^{26,27}

In addition, the absence of baseline assessment of prior exposure to *Mycobacterium tuberculosis* or environmental mycobacteria (eg, interferon-gamma release assays) may have contributed to inter-individual variability in trained immunity responses, representing a potential source of confounding.^{34,44} Furthermore, the quantification of sCD40L in plasma warrants cautious interpretation, as this mediator is predominantly derived from activated platelets and may be influenced by pre-analytical variables, including platelet contamination. Although standardized sample processing protocols were applied, the lack of validation using platelet-poor plasma constitutes a methodological limitation.^{9,17} Finally, the exploratory nature of this secondary analysis, including the absence of a priori power calculation and adjustments for multiple comparisons, reinforces that these findings should be interpreted as hypothesis-generating rather than confirmatory.

The results reinforce the therapeutic potential of BCG as a modulator of inflammatory and prothrombotic mediators in COVID-19.^{53,54,55} Future studies should include larger and more heterogeneous cohorts, evaluate different BCG strains and administration protocols, correlate biomarkers with clinical outcomes, and investigate whether sCD40L reduction translates into lower incidence of thromboembolic events. Such efforts are justified to further explore BCG's role in both the acute phase and the prevention of long-term COVID-19 complications.

Importantly, this study did not directly evaluate clinical outcomes such as thromboembolic events or long COVID. Therefore, any clinical implications should be interpreted cautiously and considered hypothesis-generating.

Conclusion

BCG vaccination in young, healthy adults with mild COVID-19 was associated with a distinct and sustained modulation of TNF- α and sCD40L, consistent with mechanisms of trained immunity. Vaccinated individuals exhibited an early pro-inflammatory peak followed by a prolonged reduction in these mediators, a temporal pattern not observed in the placebo group. These findings provide novel longitudinal in vivo evidence that BCG may reshape the trajectory of key inflammatory and prothrombotic mediators during and after SARS-CoV-2 infection. In addition, the exploratory

assessment of the sCD40L/TNF- α ratio suggests its potential as an integrative marker reflecting the dynamic balance between adaptive immune activation and systemic inflammation.

However, these results should be interpreted with caution. The small sample size, homogeneous study population, and exploratory design limit the robustness and generalizability of the findings. Moreover, the absence of baseline assessment of prior mycobacterial exposure and methodological considerations related to sCD40L quantification may have contributed to inter-individual variability and potential bias. Importantly, this study was not designed to assess clinical outcomes, and therefore the observed immunological effects cannot be directly translated into clinical benefit.

Overall, these findings should be considered hypothesis-generating and provide mechanistic support to the broader clinical observations from the BATTLE trial. Further studies in larger and more heterogeneous populations - including different age groups, particularly pediatric cohorts, as well as variations in BCG strains and standardized immunological assessments - are required to validate these results and determine their clinical relevance.

Data Sharing Statement

The original contributions presented in this study are included in the article. Further inquiries can be directed at the corresponding author.

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patients to publish this paper.

Author Contributions

C.F. is responsible for collecting and organizing patient data, as well as collecting and storing samples. C.F. and G. B. assisted with experiments and contributed to the final writing of the manuscript. G.B. also contributed to sample storage. S.F.S., V.P., D.M., and L.B.P. participated in manuscript writing and review. C.F., V.P., and L.B.P. also performed the ELISA assay. L.O.R. conceived and designed the study, was responsible for the samples, sources, and funding, and oversaw the overall coordination of the research. All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no conflicts of interest in this work.

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