Heliyon 10 (2024) e32510

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

# Accelerated Bacille Calmette-Guérin reactions: More than meets the eye

Paola Villanueva [a,b,c,d,*], Nigel W. Crawford [a,b,d,e], Mariana Garcia Croda [f], Julio Croda [f,g,h], Margareth Dalcolmo [i], Bruno Araújo Jardim [j], Tyane de Almeida Pinto Jardim [j], Helen Marshall [k], Cristina Prat-Aymerich [l,m], Alice Sawka [n], Ketaki Sharma [o,p], Darren Troeman [l], Adilia Warris [q,r], Nicholas Wood [o,p,s], Nicole L. Messina [a,b,1], Laure F. Pittet [a,b,c,t,**,1], Nigel Curtis [a,b,c,1]

[a] *Department of Paediatrics, The University of Melbourne, Parkville, VIC, Australia*
[b] *Infection, Immunity and Global Health, Murdoch Children's Research Institute, Parkville, VIC, Australia*
[c] *Infectious Diseases, Royal Children's Hospital Melbourne, Parkville, VIC, Australia*
[d] *Department of General Medicine, Royal Children's Hospital Melbourne, Parkville, VIC, Australia*
[e] *Immunisation Service, Royal Children's Hospital Melbourne, Parkville, VIC, Australia*
[f] *School of Medicine, Federal University of Mato Grosso do Sul, Campo Grande, MS, Brazil*
[g] *Oswaldo Cruz Foundation, Campo Grande, Mato Grosso do Sul, Brazil*
[h] *Yale School of Public Health, New Haven, CT, USA*
[i] *Helio Fraga Reference Center, Oswaldo Cruz Foundation Ministry of Health, Rio de Janeiro, Brazil*
[j] *Carlos Borborema Clinical Research Institute, Fundação de Medicina Tropical Dr. Heitor Vieira Dourado, Manaus, Brazil*
[k] *Robinson Research Institute and Adelaide Medical School, The University of Adelaide and Department of Paediatrics, The Women's and Children's Health Network, Australia*
[l] *Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht University, Utrecht, Netherlands*
[m] *Institut d'Investigació Germans Trias i Pujol, Departament de Genètica i Microbiologia, CIBER de enfermedades respiratorias (CIBERES), Instituto de Salud Carlos III, Universitat Autònoma de Barcelona, Badalona, Catalunya, Spain*
[n] *Department of Thoracic Medicine, Royal Adelaide Hospital, Adelaide, SA, Australia*
[o] *National Centre for Immunisation Research and Surveillance, Westmead, NSW, Australia*
[p] *The Children's Hospital at Westmead, Westmead, NSW, Australia*
[q] *Medical Research Council Centre for Medical Mycology, University of Exeter, UK*
[r] *Great Ormond Street Hospital, London, UK*
[s] *The Children's Hospital at Westmead Clinical School, Faculty of Medicine and Health, University of Sydney, NSW, Australia*
[t] *Infectious Diseases Unit, Department of Paediatrics, Gynaecology and Obstetrics, Faculty of Medicine, University of Geneva and University Hospitals of Geneva, Geneva, Switzerland*

ARTICLE INFO

*Keywords:*
BCG
Accelerated BCG reaction
BCG test
Latent tuberculosis infection

ABSTRACT

An accelerated local injection site reaction following Bacille Calmette-Guérin (BCG) vaccination has been associated with underlying active tuberculosis (TB) in high TB-prevalence settings. The clinical significance of this accelerated BCG reaction in individuals without TB symptoms, particularly in low TB-prevalence countries, is unclear.

* Corresponding author. Department of Paediatrics, The University of Melbourne, Parkville, VIC 3052, Australia.
** Corresponding author. Infectious Diseases Unit, Department of Paediatrics, Gynaecology and Obstetrics, Faculty of Medicine, University of Geneva and University Hospitals of Geneva, Geneva, Switzerland.
*E-mail addresses:* paola.villanueva@rch.org.au (P. Villanueva), laure.pittet@mcri.edu.au (L.F. Pittet).
[1] joint senior authors.

https://doi.org/10.1016/j.heliyon.2024.e32510
Received 16 October 2022; Received in revised form 1 June 2024; Accepted 5 June 2024
Available online 6 June 2024

Using safety surveillance data and baseline interferon-gamma release assays (IGRA) within an international randomised trial of BCG vaccination in healthcare workers (the BRACE trial), we aimed to determine the incidence, and investigate for clinical implications, of an accelerated BCG reaction in asymptomatic adults in low and high TB-prevalence settings.

An accelerated BCG reaction occurred in 755/1984 (38 %) of BCG-vaccinees. Although more frequently painful, tender, erythematous and/or swollen within the first fourteen days of vaccination, compared with non-accelerated reactions, the majority of injection site reactions were mild and did not meet criteria for an adverse event.

Prior mycobacterial exposure, through prior BCG vaccination (OR 2.46, 95%CI 1.93–3.13, $p < 0.001$) or latent TB infection (OR 4.17, 95%CI 1.16–14.93, $p = 0.03$), and female sex (OR 1.27, 95%CI 1.03–1.57, $p = 0.02$), were key determinants for the occurrence of an accelerated BCG reaction.

The development of an accelerated local reaction to BCG vaccination in an individual without prior history of BCG vaccination, should prompt consideration of further investigations for potential underlying TB infection.

## 1. Introduction

The Bacille Calmette-Guérin (BCG) vaccine has been used globally for over one century to protect against tuberculosis (TB). Of recent times, there has been increasing interest in novel indications for the BCG vaccine, maximising its 'off-target' immunological effects to offer protection against other infectious and non-infectious diseases [1–3].

The normal expected reaction to intradermal BCG vaccination is the formation of a small, red papule at the injection site within 2–3 weeks. The papule usually softens, turning into a small ulcer, which heals over several weeks to months into a small flat scar [4,5]. However, in some people, an 'accelerated local reaction' may occur, defined as the early development of an induration or swelling (at least 0.5 cm in children or 1.0 cm in adults) at the injection site, starting within 24–72 h of vaccination [6–10]. The earlier onset is also usually followed by earlier healing within 10–15 days [4].

Studies have shown an association between an accelerated BCG reaction and prior mycobacterial exposure, such as prior BCG vaccination ('revaccination') [11] or exposure to non-tuberculous mycobacteria [12]. An accelerated BCG reaction was first proposed as a possible TB diagnostic test in 1949 [13] and was found to be more sensitive than tuberculin skin test (TST) for diagnosing TB [9]. In a meta-analysis of children with symptoms suggestive of TB in high TB prevalence settings (≥40 cases per 100 000 people), the sensitivity of an accelerated reaction for the diagnosis of active TB (confirmed or probable) was 87 % (95%CI: 81 %–91 %) and specificity 90 % (95%CI: 85 %–94 %) [14]. In adults, this 'BCG test' has been used as a rule-out test in symptomatic patients with suspected pulmonary TB whose sputum tests negative [10]. The significance of an accelerated BCG reaction in asymptomatic individuals is less clear.

No studies have been done in low TB-prevalence countries and none have explored a potential association between accelerated BCG reaction and latent TB infection (LTBI).

Using vaccine safety surveillance data from an international randomised controlled trial of BCG vaccination to reduce the impact of COVID-19 in healthcare workers (the BRACE trial; ClinicalTrials.gov NCT04327206; registration date March 31, 2020), we aimed to determine the incidence and clinical implication of an accelerated BCG reaction in asymptomatic individuals in low and high TB-prevalence settings.

## 2. Methods

### 2.1. Participants and setting

The BRACE trial Stage 2 recruited healthcare workers (HCW) in both high (Brazil [15]) and low (Australia, The Netherlands, Spain and the UK) TB-prevalence countries, from May 2020 to April 2021. HCW were eligible if they worked in healthcare settings or had face-to-face contact with patients during the COVID-19 pandemic. The trial protocol is described elsewhere in detail [16]. Briefly, HCW recruited in 25 sites were randomised in a one-to-one ratio and blind design to receive BCG vaccine or placebo intradermal saline injection. Exclusion criteria encompassed any contraindication to BCG (such as immunosuppression or history of TB disease), previous significant local adverse reaction to BCG, BCG vaccine received within the previous 12 months, any other live-attenuated vaccine received within the last month or indicated in the next month, or any COVID-19-specific vaccine administered. Prior known LTBI was not an exclusion criterion.

For participants in Rio de Janeiro and Mato Grosso do Sul in Brazil, blood collected at recruitment was tested for LTBI using an interferon-gamma release assay (IGRA). Participants with a positive IGRA were referred to local health services for further assessment and management; they were not excluded from participation.

### 2.2. Intervention

Inidividuals randomised to BCG received one dose of BCG-Denmark (AJ Vaccines, Copenhagen), 0.1 ml (corresponding to 2–8 ×

$10^5$ colony-forming units of *Mycobacterium bovis*, Danish strain 1331) [17] via intradermal injection in the upper arm, using a 10 mm bevel needle (25 G to 30 G).

### 2.3. Data collection

The REDCap web application [18] was used to collect data, including details on demographics, any prior history of BCG vaccination, previous TST, and previous known LTBI. Presence or absence of a post-injection site wheal was documented by trained immunisers following vaccine administration. Information on the evolution of the injection site (pain, tenderness, erythema and/or swelling), the size of any injection site swelling or erythema, the occurrence of regional lymphadenopathy, as well as serial injection site photographs, were captured through daily web-based questionnaires for two weeks following vaccination (vaccine diary) and a questionnaire at three months following vaccination (see Supplementary Material 1). Participants could additionally contact the investigators by telephone or e-mail with any concerns about their injection site.

The QuantiFERON-TB Gold Plus (QFT-Plus) assay (Qiagen, Hilden, Germany) was used for LTBI screening among participants in Brazil. Results of IGRA testing were assessed according to the manufacturer's criteria, using Qiagen software version 2.71.2. Indeterminate results were re-run (as per manufacturer recommendations).

### 2.4. Active safety surveillance

Participants who reported a possible adverse event following immunisation (AEFI) were actively followed up by designated safety medical doctors (SMD). Adverse events (including regional lymphadenopathy) occurring within three months of vaccination were recorded on standard forms and are reported elsewhere. Accelerated local reactions were not considered adverse events, unless

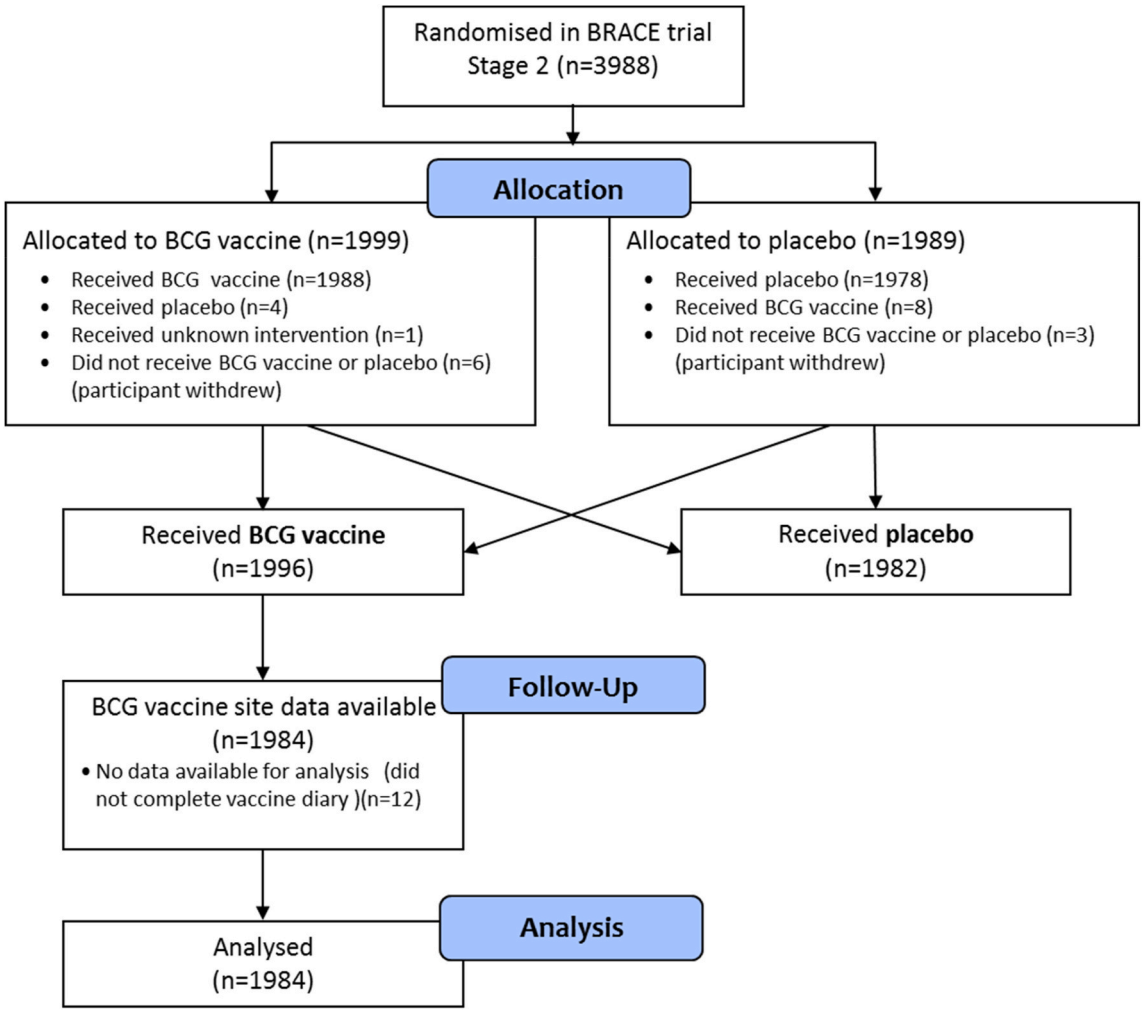


**Fig. 1.** BRACE participants who received BCG in Stage 2
Abbreviations: BCG, Bacille Calmette-Guérin.

associated with severe (grade 3 or 4) pain, tenderness, swelling or erythema at the injection site, in accordance with the FDA toxicity grading scale [19] (Table S1).

### 2.5. Case definitions

Accelerated local BCG reaction: swelling of 1.0 cm in diameter or more at the BCG injection site, beginning within 24–72 h of BCG vaccination. BCG-associated lymphadenopathy: palpable regional (ipsilateral axilla or neck) lymph node enlargement, within three months of vaccination. Post-injection site wheal: skin wheal of at least 7 mm diameter, documented by a vaccinator at the time of vaccine administration. BCG-revaccination: BCG vaccination in a participant who had any prior BCG vaccination history. BCG-naïve: BCG vaccination in a participant who had no prior BCG vaccination history.

### 2.6. Statistical analysis

Statistical analysis was done using StataIC 17.0 (Statacorp LP, College Station, TX, USA). The incidence of accelerated BCG reactions was calculated among participants who received BCG vaccination in the trial and provided vaccine safety data in their vaccine diaries. Local BCG injection site reactions (pain, tenderness, erythema, swelling) were categorised according to the US Food and Drug Administration (FDA) toxicity grading scale [19] (Table S1). Local reaction grades at the injection site were compared between participants with and without an accelerated BCG reaction (excluding participants without any BCG injection site reaction), using Chi-square or Fisher exact tests. The onset of local reactions was compared using Mann-Whitney test. To identify factors associated with an accelerated BCG reaction, odds ratio (OR) and 95 % confidence intervals (CI) were calculated using univariate logistic regression, with and without adjustment for revaccination. Significant associated factors (p value $< 0.2$) resulting from the univariate logistic regression analysis were included as possible covariates in a multivariate model. Backward stepwise exclusion of factors with p value $> 0.05$ was used to create the model, through sequential model testing.

**Table 1**
Factors investigated for association with accelerated BCG reaction.

| **Factor** | accelerated BCG reaction | | | | |
|---|---|---|---|---|---|
| | **Total BCG** | | Univariate | Univariate | Multivariate |
| | **n = 1984** | **n = 755 (%)** | OR (95 % CI) | Adjusted[1] OR (95 % CI) | OR (95 % CI) |
| Sex | | | | | |
| Male | 547 | 189 (25.0) | 1 (reference) | 1 (reference) | 1 (reference) |
| Female | 1437 | 566 (75.0) | 1.23 (1.00–1.51), p = 0.05 | 1.26 (1.03–1.56), p = 0.03 | 1.27 (1.03–1.57), p = 0.02 |
| Age | | | | | |
| 18-49y | 1426 | 536 (71.0) | 1 (reference) | 1 (reference) | – |
| 50y+ | 558 | 219 (29.0) | 1.07 (0.88–1.31), p = 0.5 | 1.14 (0.93–1.39), p = 0.2 | – |
| Study country | | | | | |
| Australia | 210 | 88 (11.7) | 1.20 (0.89–1.60), p = 0.3 | 1.51 (1.12–2.05), p = 0.01 | – |
| Brazil | 1278 | 508 (67.3) | 1.23 (1.01–1.48), p = 0.04 | 0.68 (0.53–0.87), p < 0.01 | – |
| Netherlands | 291 | 76 (10.1) | 0.53 (0.40–0.70), p < 0.001 | 0.92 (0.65–1.30), p = 0.6 | – |
| Spain | 118 | 36 (4.8) | 0.70 (0.47–1.05), p = 0.1 | 0.95 (0.62–1.44), p = 0.8 | – |
| UK | 87 | 47 (6.2) | 1.97 (1.28–3.04), p < 0.01 | 1.82 (1.18–2.81), p < 0.01 | – |
| BCG history | | | | | |
| BCG naïve | 453 | 107 (14.2) | 1 (reference) | – | 1 (reference) |
| BCG revaccination | 1531 | 648 (85.8) | 2.37 (1.87–3.01), p < 0.001 | – | 2.46 (1.93–3.13), p < 0.001 |
| Lived in TB endemic country | | | | | |
| No | 656 | 226 (29.9) | 1 (reference) | 1 (reference) | – |
| Yes | 1324 | 528 (69.9) | 1.26 (1.04–1.53), p = 0.02 | 0.72 (0.56–0.92), p < 0.01 | – |
| Unknown | 4 | 1 (0.1) | 0.63 (0.07–6.13), p = 0.7 | 0.55 (0.05–5.69), p = 0.6 | – |
| Previous known LTBI | | | | | |
| No | 1966 | 744 (98.5) | 1 (reference) | 1 (reference) | 1 (reference) |
| Yes | 11 | 7 (0.5) | 2.87 (0.84–9.85), p = 0.1 | 4.00 (1.13–14.12), p = 0.03 | 4.17 (1.16–14.93), p = 0.03 |
| Unknown | 7 | 4 (0.9) | 2.19 (0.49–9.81), p = 0.3 | 2.08 (0.46–9.49), p = 0.3 | – |
| Previous TST | | | | | |
| Negative/None | 1779 | 656 (86.9) | 1 (reference) | 1 (reference) | – |
| Positive (>5 mm) | 101 | 48 (6.4) | 1.55 (1.04–2.31), p = 0.03 | 1.56 (1.04–2.35), p = 0.03 | – |
| Unknown | 104 | 51 (6.8) | 1.65 (1.11–2.45), p = 0.01 | 1.55 (1.04–2.31), p = 0.03 | – |
| Post-injection wheal | | | | | |
| Yes | 1957 | 744 (98.5) | 1 (reference) | 1 (reference) | – |
| No | 27 | 11 (1.5) | 1.12 (0.52–2.43), p = 0.8 | 1.40 (0.64–3.10), p = 0.4 | – |
| IGRA (Brazil) | Total BCG | | | | |
| Negative | 931 | 346 | 1 (reference) | 1 (reference) | |
| Positive | 120 | 57 | 1.53 (1.04–2.24), p = 0.03 | 1.52 (1.04–2.23), p = 0.03 | |

Abbreviations: BCG, Bacille Calmette-Guérin vaccine; IGRA, interferon-gamma release assay; LTBI, latent tuberculosis infection; OR, odds ratio; TB, tuberculosis; TST, tuberculin skin test.

[1] Adjusted for revaccination.

Subgroup analyses were done amongst participants with an IGRA test result available (Brazil), as well as those who were BCG-naïve at recruitment. In addition, for the Brazil participants who had an IGRA test, demographics were compared those with a positive and negative IGRA result using Chi-square test.

### 2.7. Ethics statement

Ethics approval was obtained from The Royal Children's Hospital (RCH) Human Research Ethics Committee (HREC 62586), with additional ethics and governance approvals at each participating site. All participants provided written informed consent to participate in the study prior to enrolment.

## 3. Results

Of 1996 HCW who received BCG in BRACE Stage 2, 1984 (99 %) provided vaccine safety data (Fig. 1). Their age ranged from 18 to 78 years old (median 41) and most were recruited in Brazil (64 %; Table 1). The majority were female (72 %) and had a prior BCG vaccination history (77 %).

**Table 2**
Characteristics of accelerated BCG reactions.

| | Total BCG | Accelerated | Non-accelerated | p-value |
|---|---|---|---|---|
| | n = 1934 | n = 755 | n = 1179 | |
| **Pain** | 980 (50.7 %) | 482 (63.8 %) | 498 (42.2 %) | <0.001 |
| None | 954 (49.3 %) | 273 (36.2 %) | 681 (57.8 %) | |
| Grade 1 | 861 (44.5 %) | 413 (54.7 %) | 448 (38.0 %) | 0.1† |
| Grade 2 | 113 (5.8 %) | 65 (8.6 %) | 48 (4.1 %) | |
| Grade 3 | 5 (0.3 %) | 3 (0.4 %) | 2 (0.2 %) | |
| Grade 4 | 1 (0.1 %) | 1 (0.1 %) | 0 (0.0 %) | |
| Onset, days | 2 (1–3) | 2 (1–2) | 2 (1–4) | 0.8 |
| Mean [SD] | 2.9 [3.5] | 2.5 [2.6] | 3.3 [4.1] | |
| **Tenderness** | 1239 (64.0 %) | 559 (74.0 %) | 680 (57.7 %) | <0.001 |
| None | 695 (36.0 %) | 196 (26.0 %) | 499 (42.3 %) | |
| Grade 1 | 945 (48.9 %) | 389 (51.5 %) | 556 (47.2 %) | <0.001† |
| Grade 2 | 212 (11.0 %) | 121 (16.0 %) | 91 (7.7 %) | |
| Grade 3 | 81 (4.2 %) | 48 (6.4 %) | 33 (2.8 %) | |
| Grade 4 | 1 (0.1 %) | 1 (0.1 %) | 0 (0.0 %) | |
| Onset, days | 2 (1–3) | 2 (1–2) | 2 (1–3) | 0.01 |
| Mean [SD] | 2.7 [3.3] | 2.2 [2.3] | 3.0 [3.8] | |
| **Erythema** | 1872 (96.8 %) | 749 (99.2 %) | 1123 (95.2 %) | <0.001 |
| None | 62 (3.2 %) | 6 (0.8 %) | 56 (4.8 %) | |
| Grade 1 | 1820 (94.1 %) | 709 (93.9 %) | 1111 (94.2 %) | <0.001† |
| Grade 2 | 51 (2.6 %) | 39 (5.2 %) | 12 (1.0 %) | |
| Grade 3 | 1 (0.1 %) | 1 (0.1 %) | 0 (0.0 %) | |
| Grade 4 | 0 (0.0 %) | 0 (0.0 %) | 0 (0.0 %) | |
| Onset, days | 1 (1–2) | 2 (1–2) | 1 (1–2) | 0.03 |
| Mean [SD] | 2.0 [2.1] | 1.7 [1.0] | 2.2 [2.6] | |
| Maximal diameter, cm | 1.5 (1.0, 2.0) | 2.0 (1.3, 3.0) | 1.0 (1.0, 2.0) | <0.001 |
| **Swelling** | 1583 (81.8 %) | 755 (100.0 %) | 828 (70.2 %) | <0.001 |
| None | 351 (18.2 %) | 0 (0.0 %) | 351 (29.8 %) | |
| Grade 1 | 1454 (75.1 %) | 670 (88.7 %) | 784 (66.5 %) | <0.001† |
| Grade 2 | 125 (6.5 %) | 82 (10.9 %) | 43 (3.6 %) | |
| Grade 3 | 4 (0.2 %) | 3 (0.4 %) | 1 (0.1 %) | |
| Grade 4 | 0 (0.0 %) | 0 (0.0 %) | 0 (0.0 %) | |
| Onset, days | 2 (1–3) | 2 (1–2) | 2 (1–4) | <0.001 |
| Mean [SD] | 2.5 [2.9] | 1.7 [0.7] | 3.2 [3.8] | |
| Maximal diameter, cm | 1.0 (0.5, 1.5) | 1.5 (1.0, 2.0) | 0.5 (0.5, 1.0) | <0.001 |
| **Regional lymphadenopathy** | 53 (2.7 %) | 30 (4.0 %) | 23 (1.9 %) | <0.01 |
| Onset, days | 4 (2–7) | 4 (2–7) | 5 (3–7) | 0.6 |
| Mean [SD] | 6.7 [7.7] | 6.5 [7.4] | 6.8 [8.3] | |
| Duration, days | 3 (2–6) | 3 (2–6) | 2 (2–5) | 0.4 |
| Mean [SD] | 4.1 [3.4] | 4.2 [3.1] | 4.0 [3.7] | |
| Maximal diameter, cm | 1.0 (1.0, 2.0) | 1.0 (1.0, 2.0) | 1.0 (0.5, 2.0) | 0.2 |
| Mean [SD] | 1.3 [0.8] | 1.5 [0.9] | 1.2 [0.6] | |

Categorical variables are reported as number (%), continuous variables are reported as median (range).
†p-value comparing reaction severity Grades 1 to 4, between accelerated and non-accelerated groups.

Overall, an accelerated BCG reaction occurred in 755/1984 (38 %) participants. It was more common in BCG-revaccinated participants than BCG-naïve participants (648/1531 [42 %] vs 107/453 [24 %], $p < 0.001$).

The incidence of an accelerated BCG reaction varied between countries: Brazil (a high TB-prevalence country) 508/1278 [40 %], Netherlands 76/291 [26 %], Spain 36/118 [31 %], Australia 88/210 [42 %], UK 47/87 [54 %].

### 3.1. Characteristics of an accelerated BCG reaction

Of the 1984 BCG recipients who provided vaccine safety data, 1934 (97 %) reported an injection site reaction (pain, tenderness, erythema and/or swelling). A higher proportion of participants in the accelerated BCG reaction group compared with the non-accelerated reaction group, experienced pain (482/755 [64 %] vs 498/1179 [42 %], $p < 0.001$), tenderness (559/755 [74 %] vs 680/1179 [58 %], $p < 0.001$), erythema (749/755 [99 %] vs 1123/1179 [95 %], $p < 0.001$) or swelling (755/755 [100 %] vs 828/1179 [70 %], $p < 0.001$) within two weeks, at the BCG injection site (Table 2).

Tenderness, erythema or swelling occurred earlier and were more severe in the accelerated group compared with the non-accelerated group (Table 2; Fig. 2). The size of erythema and swelling was larger in the accelerated group. Regional lymphadenopathy was more likely in the accelerated group (OR 2.08, 95%CI 1.20–3.61, $p < 0.01$; OR adjusted for revaccination 2.28, 95%CI 1.30–4.00, $p < 0.01$). Time to onset, duration and size of lymphadenopathy were similar between both accelerated and non-accelerated groups (Table 2).

### 3.2. Factors associated with an accelerated BCG reaction

In the univariate analysis (Table 1), an accelerated BCG reaction was more likely among BCG-revaccinated compared with BCG-naïve participants, females compared with males, participants in Brazil and UK (each country compared with the other four countries), and those with a prior positive TST. When adjusted for revaccination, participants in Brazil and those with a history of living in a TB endemic country, were less likely to develop an accelerated BCG reaction. An accelerated BCG reaction was more likely in participants with previous known history of LTBI.

In the multivariate analysis, BCG-revaccination (OR 2.46, 95%CI 1.93–3.13, $p < 0.001$), previous known LTBI (OR 4.17, 95%CI 1.16–14.9, $p = 0.03$) and female sex (OR 1.27, 95%CI 1.03–1.57, $p = 0.02$) were the only three factors associated with an accelerated BCG reaction.

In a sub-analysis of the BCG-naïve participants ($n = 453$), previous known LTBI was the only factor associated with an accelerated BCG reaction (OR 6.75, 95%CI 1.22–37.36, $p = 0.03$; Supplemental Table 2).

Amongst the subset of 1051 participants in Brazil who had a baseline IGRA test done (none of whom had a previous known history of LTBI), the test result was positive in 120 (11 %; Table 3). An accelerated BCG reaction was more common in participants with a positive IGRA compared with those with a negative IGRA (OR 1.53, 95%CI 1.04–2.24, $p = 0.03$); this association remained significant when adjusted for revaccination (OR 1.52, 95%CI 1.04–2.23, $p = 0.03$; Table 1).

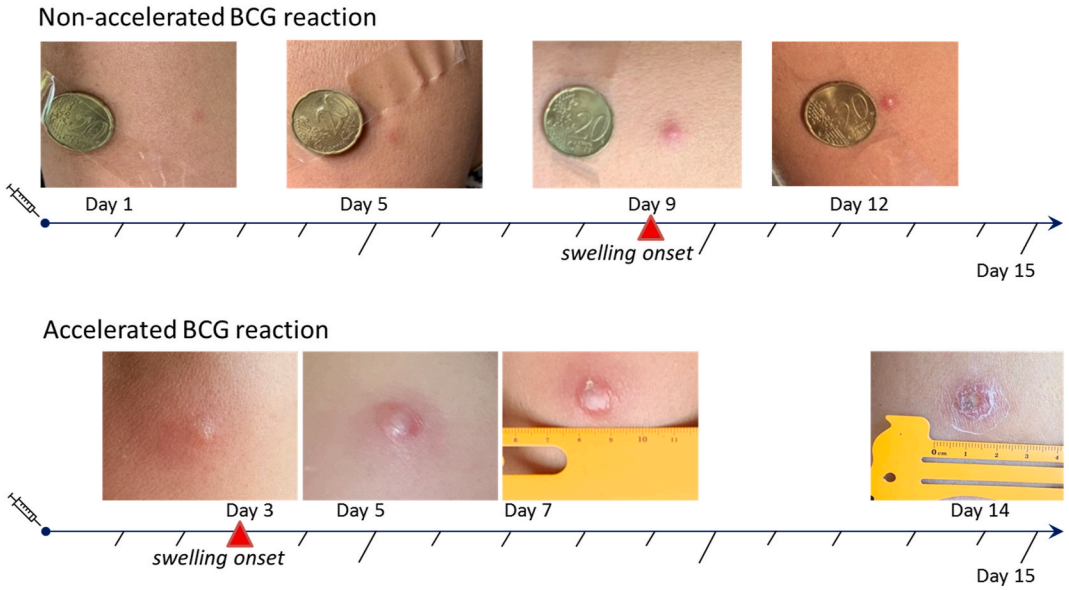


**Fig. 2.** Accelerated vs non-accelerated BCG reaction
Upper panel: Injection site photographs from a participant with a non-accelerated BCG reaction; injection site swelling onset on day 9.
Lower panel: Injection site photographs from a participant with an accelerated BCG reaction; injection site swelling onset on day 3, followed by pustule formation and healing by day 14.

In the multivariate analysis amongst these participants in Brazil (Table 4), BCG-revaccination (OR 2.59, 95%CI 1.12–6.03, p = 0.03), positive IGRA (OR 1.57, 95%CI 1.07–2.31, p = 0.02) and female sex (OR 1.56, 95%CI 1.17–2.07, p < 0.01) were the only three factors associated with an accelerated BCG reaction.

## 4. Discussion

This is the first study to show that accelerated BCG reactions occur in both low and high TB-prevalence settings. Prior mycobacterial exposure (through prior BCG vaccination or LTBI) was the key determinant of an accelerated BCG reaction, regardless of setting, in the multivariate analysis. Vaccine administration technique, as assessed by the presence of a post-injection skin wheal, did not affect the development of an accelerated BCG reaction.

Previous history of BCG is associated with accelerated reactions in asymptomatic children [14]. In two studies, an accelerated reaction was more common in BCG-revaccinated compared with BCG-naïve children (29 % vs 17 %, p = 0.05 [20]; 78 % vs 18 %, p < 0.001 [11]). Consistent with this, in our study, an accelerated BCG reaction was more common in adults who had prior BCG vaccination compared to those who were BCG-naïve at recruitment (42 % vs 24 %). Interestingly, almost all had received their first BCG vaccine in infancy, indicating that a time interval of decades since primary BCG vaccination still increases the risk of accelerated reaction.

Supporting the hypothesis that mycobacterial exposure influences the development of an accelerated BCG reaction in asymptomatic individuals, a history of prior LTBI was significantly associated with an accelerated BCG reaction in BCG-naïve adults. Similarly, a positive IGRA was found to increase the likelihood of an accelerated reaction. This finding suggests that an accelerated skin reaction involves an immunological memory response. This also has clinical implications for the management of BCG-naïve individuals who develop an accelerated BCG reaction, as they may need further tests for potential underlying TB infection.

The prevalence of a positive IGRA in our study (11 %) was within range of global LTBI prevalence in HCW [21], but lower than that reported (27 %) in a previous study of LTBI prevalence using IGRA in HCW in Brazil [22]. This may reflect the latter study incorporating only HCW in community services, different time points and different regions within Brazil.

The association with female sex in the multivariate analysis is more challenging to explain. Only one previous study has assessed the frequency of accelerated BCG reactions by sex and found no significant difference amongst 1095 BCG-revaccinated children in Burma [7]. In our study in adults, there may be a difference in the frequency of reporting of injection site reactions between the sexes, as men have been known to underreport on health-related matters [23,24]. Nevertheless, true biological sex differences in underlying immune responses is also plausible [25–27].

**Table 3**
Demographics of participants in Brazil with an IGRA result.

| | Total IGRA | IGRA pos | IGRA neg | p-value |
|---|---|---|---|---|
| | n = 1051 (%) | n = 120 (%) | n = 931 (%) | |
| Sex | | | | 0.1 |
| Female | 754 (71.7) | 79 (65.8) | 675 (72.5) | |
| Male | 297 (28.3) | 41 (34.2) | 256 (27.5) | |
| Age | | | | <0.001 |
| 18-49y | 819 (77.9) | 72 (60.0) | 747 (80.2) | |
| 50y+ | 232 (22.1) | 48 (40.0) | 184 (19.8) | |
| Role | | | | 0.3 |
| Allied health | 261 (24.8) | 26 (21.7) | 235 (25.2) | |
| PSA/hospital maintenance | 258 (24.6) | 32 (26.7) | 226 (24.3) | |
| Administrative/clerical staff | 170 (16.2) | 16 (13.3) | 154 (16.5) | |
| Nurse/midwife | 107 (10.2) | 15 (12.5) | 92 (9.9) | |
| Community health agent | 87 (8.3) | 9 (7.5) | 78 (8.4) | |
| Scientist (medical/research) | 43 (4.1) | 3 (2.5) | 40 (4.3) | |
| Doctor | 42 (4.0) | 10 (8.3) | 32 (3.4) | |
| Dentist/dental therapy | 20 (1.9) | 1 (0.8) | 19 (2.0) | |
| Carer | 7 (0.7) | 1 (0.8) | 6 (0.6) | |
| Other role | 56 (5.3) | 7 (5.8) | 49 (5.3) | |
| BCG history | | | | 0.8 |
| BCG naïve | 34 (3.2) | 3 (2.5) | 31 (3.3) | |
| BCG revaccination | 1017 (96.8) | 117 (97.5) | 900 (96.7) | |
| Previous known LTBI | | | | 0.2 |
| No | 1048 (99.7) | 119 (99.2) | 929 (99.8) | |
| Yes | 0 (0.0) | 0 (0.0) | 0 (0.0) | |
| Unknown | 3 (0.3) | 1 (0.8) | 2 (0.2) | |
| Previous TST | | | | <0.001 |
| Negative/None | 986 (93.8) | 103 (85.8) | 883 (94.8) | |
| Positive (>5 mm) | 27 (2.6) | 16 (13.3) | 22 (2.4) | |
| Unknown | 38 (3.6) | 1 (0.8) | 26 (2.8) | |

Abbreviations: BCG, Bacille Calmette-Guérin vaccine; LTBI, latent tuberculosis infection; neg, negative; pos, positive; PSA, patient services assistant; TB, tuberculosis; TST, tuberculin skin test.

**Table 4**
Factors investigated for association with accelerated BCG reaction (Brazil only).

| Factor | accelerated BCG reaction | | | | |
|---|---|---|---|---|---|
| | Total BCG | n = 403 (%) | Univariate | Univariate | Multivariate |
| | n = 1051 | | OR (95 % CI) | Adjusted[1] OR (95 % CI) | OR (95 % CI) |
| Sex | | | | | |
| Male | 297 | 94 (23.3) | 1 (reference) | 1 (reference) | 1 (reference) |
| Female | 754 | 309 (76.7) | 1.50 (1.13–1.99), p < 0.01 | 1.53 (1.15–2.03), p < 0.01 | 1.56 (1.17–2.07), p < 0.01 |
| Age | | | | | |
| 18-49y | 819 | 321 (79.7) | 1 (reference) | 1 (reference) | – |
| 50y+ | 232 | 82 (20.3) | 0.85 (0.63–1.15), p = 0.3 | 0.65 (0.92–1.19), p = 0.4 | – |
| BCG history | | | | | |
| BCG naïve | 34 | 7 (1.7) | 1 (reference) | – | 1 (reference) |
| BCG revaccination | 1017 | 396 (98.3) | 2.46 (1.06–5.70), p = 0.04 | – | 2.59 (1.12–6.03), p = 0.03 |
| Previous TST | | | | | |
| Negative/None | 986 | 380 (94.3) | 1 (reference) | 1 (reference) | – |
| Positive (>5 mm) | 38 | 15 (3.7) | 1.04 (0.54–2.02), p = 0.9 | 1.06 (0.54–2.06), p = 0.9 | – |
| Unknown | 27 | 8 (2.0) | 0.67 (0.29–1.55), p = 0.4 | 0.71 (0.31–1.65), p = 0.4 | – |
| Post-injection wheal | | | | | |
| Yes | 1051 | 403 (100.0) | – | – | – |
| No | 0 | 0 (0.0) | – | – | – |
| Previous known LTBI | | | | | |
| No | 1048 | 402 (99.8) | 1 (reference) | 1 (reference) | – |
| Yes | 0 | 0 (0.0) | – | – | – |
| Unknown | 3 | 1 (0.2) | 0.80 (0.07–8.89), p = 0.9 | 0.78 (0.07–8.67), p = 0.8 | – |
| IGRA | | | | | |
| Negative | 931 | 346 (85.9) | 1 (reference) | 1 (reference) | 1 (reference) |
| Positive | 120 | 57 (14.1) | 1.53 (1.04–2.24), p = 0.03 | 1.52 (1.04–2.23), p = 0.03 | 1.57 (1.07–2.31), p = 0.02 |

Abbreviations: BCG, Bacille Calmette-Guérin vaccine; IGRA, interferon-gamma release assay; LTBI, latent tuberculosis infection; OR, odds ratio; TB, tuberculosis; TST, tuberculin skin test; y, years.
[1] Adjusted for revaccination.

Regional lymphadenopathy following BCG vaccination was twice as likely in those with an accelerated reaction compared to those without an accelerated reaction. This difference remained significant when adjusted for revaccination, which has previously been shown to increase the risk of regional lymphadenopathy [28].

Although an accelerated BCG reaction was more frequently painful, tender, erythematous and/or swollen within the first fourteen days of vaccination, compared with non-accelerated reactions, the majority of injection site reactions were mild (consistent with expected BCG injection site reactions) and did not meet criteria for an adverse event. In addition, as cases of BCG-associated regional lymphadenopathy self-resolved in a median time of three days, this study confirms an accelerated BCG reaction is not a clinically serious adverse event following immunisation. As the incidence of an accelerated BCG reaction was found to be up to 54 %, it would be important for clinicians and the medical community to be aware of this phenomenon, especially as BCG revaccination is increasingly being considered for broader indications.

Our study has some limitations. First, characterisation of local reactions relied on participant self-report. However, all were HCW and vaccine diary data was available for 99 % of participants. Second, daily vaccine diary questionnaire data was sought only until day 14, whereas the normal papule (swelling) formation following BCG vaccination occurs up to 3 weeks [4] after vaccination. Third, baseline IGRAs were only done for the subset of participants in Brazil and could not be included in the multivariate analysis. Strengths of our study include the assessment of accelerated BCG reactions in a large population of almost 2000 BCG recipients in both high and low TB prevalence countries. This prospective data collection enabled the characterisation and evaluation of the factors contributing to accelerated BCG reactions in asymptomatic individuals. Future research may explore the immunological responses underlying accelerated and normal BCG reactions, and how these reactions correlate with vaccine efficacy.

In conclusion, we found that an accelerated reaction to BCG vaccination was associated with prior mycobacterial exposure through prior BCG vaccination or latent TB infection. The development of an accelerated local reaction to BCG vaccination in an individual without prior history of BCG vaccination, should prompt consideration of further investigations for potential underlying TB infection.

## Data availability statement

The authors declare that data supporting the study findings are available within the main tables and figures. All de-identified participant data are available on request and completion of a signed data access agreement. Requests can be addressed in writing to braceresearch@mcri.edu.au.

## CRediT authorship contribution statement

**Paola Villanueva:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Nigel**

**W. Crawford:** Writing – review & editing. **Mariana Garcia Croda:** Writing – review & editing. **Julio Croda:** Writing – review & editing. **Margareth Dalcolmo:** Writing – review & editing. **Bruno Araújo Jardim:** Writing – review & editing. **Tyane de Almeida Pinto Jardim:** Writing – review & editing. **Helen Marshall:** Writing – review & editing. **Cristina Prat-Aymerich:** Writing – review & editing. **Alice Sawka:** Writing – review & editing. **Ketaki Sharma:** Writing – review & editing. **Darren Troeman:** Writing – review & editing. **Adilia Warris:** Writing – review & editing. **Nicholas Wood:** Writing – review & editing. **Nicole L. Messina:** Writing – review & editing, Supervision, Conceptualization. **Laure F. Pittet:** Writing – review & editing, Supervision, Conceptualization. **Nigel Curtis:** Writing – review & editing, Supervision, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

We are grateful to the BRACE trial participants for making this study possible and to the researchers involved in forming the BRACE trial (please see Supplementary Material 2 for the BRACE trial Consortium Group).

The Murdoch Children's Research Institute (MCRI) leads the BRACE trial. It is supported by the Victorian Government's Operational Infrastructure Support Programme. The trial is also supported by the Bill & Melinda Gates Foundation [INV-017302], the Minderoo Foundation [COV-001], Sarah and Lachlan Murdoch, the Royal Children's Hospital Foundation [2020-1263 BRACE Trial], Health Services Union NSW, the Peter Sowerby Foundation, the Ministry of Health Government of South Australia, the NAB Foundation, the Calvert-Jones Foundation, the Modara Pines Charitable Foundation, the UHG Foundation Pty Ltd, Epworth Healthcare and individual donors. The sponsors had no role in the collection, analysis and interpretation of data or in the preparation, review or approval of the manuscript.

P.V. is supported by the Australian Government Research Training Programme Scholarship provided by the Australian Commonwealth Government and the University of Melbourne, and an MCRI Ph.D. Top-Up Scholarship. L.F.P. is supported by the Swiss National Science Foundation [Early Postdoc Mobility Grant, P2GEP3_178155]. N.C. is supported by a National Health and Medical Research Council Investigator Grant [GNT1197117].

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e32510.

## References

[1] N. Fritschi, N. Curtis, N. Ritz, Bacille Calmette Guérin (BCG) and new TB vaccines: specific, cross-mycobacterial and off-target effects, Paediatr. Respir. Rev. 36 (2020) 57–64.
[2] N.L. Messina, et al., Off-target effects of bacillus Calmette-Guérin vaccination on immune responses to SARS-CoV-2: implications for protection against severe COVID-19, Clinical & translational immunology 11 (2022) e1387.
[3] N. Curtis, A. Sparrow, T.A. Ghebreyesus, M.G. Netea, Considering BCG vaccination to reduce the impact of COVID-19, Lancet 395 (2020) 1545–1546.
[4] Australian Technical Advisory Group on Immunisation (ATAGI), Australian Immunisation Handbook: BCG Vaccination Procedures, 2018.
[5] H.J. Ustvedt, Local reaction in BCG vaccination, Bull. World Health Organ. 2 (1950) 441–468.
[6] G.H. Bothamley, E. Cooper, D. Shingadia, A. Mellanby, Tuberculin testing before BCG vaccination, BMJ 327 (2003) 243–244.
[7] T.T. Myint, Y. Yin, M.M. Yi, H.H. Aye, BCG test reaction in previously BCG vaccinated children, Ann. Trop. Paediatr. 5 (1) (1985) 29–31.
[8] S.N.H. Bokhari, Accelerated BCG response: diagnostic value among adults in an endemic area, Specialist 15 (3) (1999) 181–184.
[9] E. Sonmez, et al., Diagnosis of tuberculosis: PPD or BCG test, J. Trop. Pediatr. 44 (1) (1998) 40–42.
[10] M.L. Mathur, S. Raman, Potential utility of BCG test in excluding tuberculosis in adults, Respir. Med. 97 (2003) 528–532.
[11] M. Singla, V. Sahai, S. Sodhi, R.P. Gupta, BCG skin reaction in Mantoux-negative healthy children, BMC Infect. Dis. 5 (2005).
[12] T.F. Collins, Limitations of the accelerated BCG reaction as a test for previous infection with the tubercle bacillus, Samj, S 39 (1965) 847–850.
[13] H.J. Ustvedt, A. Aanonsen, B.C.G. Diagnostic, Test, Acta Tuberc. Scand. 23 (1949) 1–35.
[14] P. Villanueva, L.F. Pittet, C. Nourse, N. Curtis, What is the significance of an accelerated BCG reaction in children? Arch. Dis. Child. 107 (2022) 507–512.
[15] World Health Organization, WHO global lists of high burden countries for TB, multidrug/rifampicin-resistant TB (MDR/RR-TB) and TB/HIV, 2021–2025 [cited 2 August 2022]Available from: https://cdn.who.int/media/docs/default-source/hq-tuberculosis/who_globalhbcliststb_2021-2025_backgrounddocument.pdf?sfvrsn=f6b854c2_9, 2021.
[16] L.F. Pittet, et al., BCG vaccination to reduce the impact of COVID-19 in healthcare workers: protocol for a randomised controlled trial (BRACE trial), BMJ Open 11 (2021) e052101.
[17] A/S, A.V. BCG AJV Summary of, Product Characteristics, 2019 [cited] Available from: https://www.medicines.org.uk/emc/product/9890/smpc#gref.
[18] P.A. Harris, et al., Research electronic data capture (REDCap)—a metadata-driven methodology and workflow process for providing translational research informatics support, J. Biomed. Inf. 42 (2009) 377–381.
[19] US Food and Drug Administration, Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials, 2007.
[20] R. Mehta, L. Saini, S.K.A. Mittal, Critical evaluation of B.C.G. test applicability in pediatric practice, Indian Pediatr. 23 (6) (1986) 419–428.
[21] E.H. da Silva, E. Lima, T.R. Dos Santos, M.C. Padoveze, Prevalence and incidence of tuberculosis in health workers: a systematic review of the literature, American Journal of Infection Control 50 (7) (2022 Jul) 820–827.
[22] T.N.D. Prado, et al., Prevalence and risk factors for latent tuberculosis infection among primary health care workers in Brazil, Cad. Saúde Pública 33 (2017) e00154916.

[23] E. Caroli, L. Weber-Baghdiguian, Self-reported health and gender: the role of social norms, Social science & medicine 153 (1982) 220–229, 2016.
[24] O. Yousaf, E.A. Grunfeld, M.S. Hunter, A systematic review of the factors associated with delays in medical and psychological help-seeking among men, Health Psychol. Rev. 9 (2015) 264–276.
[25] P. Aaby, et al., The non-specific and sex-differential effects of vaccines, Nat. Rev. Immunol. 20 (2020) 464–470.
[26] B. Freyne, et al., Neonatal BCG vaccination influences Cytokine responses to Toll-like receptor ligands and heterologous antigens, J. Infect. Dis. 217 (2018) 1798–1808.
[27] S.L. Klein, K.L. Flanagan, Sex differences in immune responses, Nat. Rev. Immunol. 16 (2016) 626–638.
[28] P. Villanueva, et al., Revaccination with Bacille Calmette-Guérin (BCG) is associated with an increased risk of abscess and lymphadenopathy, NPJ vaccines 7 (2022) 6.