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# The association between BCG scars and self-reported chronic diseases: A cross-sectional observational study within an RCT of Danish health care workers

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ABSTRACT

*Introduction:* The live-attenuated vaccines *Bacillus Calmette-Guérin* (BCG) and *Vaccinia* have been associated with beneficial non-specific effects. We assessed the prevalence of BCG and *Vaccinia* vaccine scars in a cohort of Danish health care workers and investigated the association between the presence of vaccine scars and self-reported chronic diseases.

*Methods:* Cross-sectional study utilizing baseline data collected during 2020–2021 at enrollment in a BCG trial aiming to assess the effect of BCG vaccination on absenteeism and infectious disease morbidity during the SARS-COV-2 pandemic. In Denmark, *Vaccinia* was discontinued in 1977, and BCG was phased out in the early 1980s. We used logistic regression analysis (adjusted for sex, birth year, and smoking status) to estimate the association between scar status and chronic diseases, providing adjusted Odds Ratios (aORs) with 95 % Confidence Intervals, for participants born before 1977, and born from 1965 to 1976.

*Results:* The cohort consisted of 1218 participants (206 males; 1012 females) with a median age of 47 years (Q1–Q3: 36–56). Among participants born 1965–1976 (n = 403), who experienced the phase-outs, having BCG and/or *Vaccinia* scar(s) vs. having no vaccine scars yielded an aOR of 0.51 (0.29–0.90) of self-reported chronic disease; an effect primarily driven by BCG. In the same birth cohort, having vaccine scar(s) was most strongly associated with a lower prevalence of chronic respiratory and allergic diseases; the aORs being 0.39 (0.16–0.97) and 0.39 (0.16–0.91), respectively.

*Conclusion:* Having a BCG scar was associated with a lower prevalence of self-reported chronic disease.

## 1. Introduction

The live-attenuated vaccines *Bacillus Calmette-Guérin* (BCG) (against tuberculosis) and *Vaccinia* (against smallpox) have been associated with a reduction in all-cause mortality risk that cannot be explained by the protection against the target diseases [1–7]. The proposed explanation is that some vaccines have non-specific effects (NSEs) on the immune system, which modulate the capacity to deal with other infections [8]. Most observations come from studies involving newborns and children, but reduced mortality risks have also been seen over longer time periods and in adults. In a register study of Danish schoolchildren born from 1965 to 1976, who experienced the phase-outs of BCG and *Vaccinia*, those who had received both BCG and *Vaccinia* had an adjusted hazard ratio for mortality due to natural causes of 0.54 (95 % Confidence Interval (CI) 0.36–0.81), when compared with unvaccinated controls [9]. It is possible that the vaccines, through their effect on the immune system and the associated protection against untargeted infections, or through other mechanisms, also have protective effects against chronic diseases.

In Denmark, *Vaccinia* was recommended for children aged 0–7 years

*Abbreviations:* aOR, adjusted Odds Ratio; BCG, *Bacillus Calmette-Guérin*; CI, Confidence Interval; HCW, health care worker; NSE, non-specific effect.

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until 1977, when smallpox vaccination was discontinued [10]; three years later, the WHO declared smallpox eradicated worldwide [11]. Regarding BCG, Danish first grade children (6–7 years of age) were tested using the tuberculin skin test, with children testing negative then being offered BCG vaccination. The BCG vaccination program was gradually phased out in the early 1980s [12]. Vaccination is now only recommended for children at high risk of tuberculosis exposure, while BCG for adults is considered when there is a risk of exposure to multi-drug- and extensively drug-resistant tuberculosis [13]. Both vaccines are administered intradermally. Normally, a characteristic scar develops at the site of injection [14,15].

It is well known that BCG has antitumor effects [16]; intravesical immunotherapy with BCG is used in the treatment of non-muscle invasive bladder cancer [17,18]. Results from a 60-year follow-up study of a randomized controlled trial (RCT) of BCG recently indicated that childhood BCG vaccination reduces the risk both of developing lung cancer and death due to lung cancer [19]. Other studies suggest that BCG might reduce the risk of other chronic diseases as well. For example, BCG vaccination may reduce the risk of developing eczema [20] and multiple sclerosis [21]. Further, a significantly reduced risk of developing Alzheimer's disease was seen for bladder cancer patients treated with intravesical BCG immunotherapy compared with patients who were not treated with BCG [22]. Given the significant burden of chronic diseases on health care systems, it is of interest to further investigate if live-attenuated vaccines influence the incidence of chronic diseases.

In this study, we assessed the prevalence of BCG and *Vaccinia* vaccine scars in a cohort of health care workers enrolled in an RCT in Denmark investigating the effect of BCG on absenteeism and infectious disease morbidity during the SARS-COV-2 pandemic. We hypothesized that having vaccine scars was associated with a reduced prevalence of self-reported chronic diseases.

## 2. Methods

### 2.1. Participants and setting

Cross-sectional study utilizing baseline data collected on the day of inclusion in an RCT among health care workers (HCWs) in Denmark [23]. Enrollment started in May 2020 and ended in January 2021. The aim of the trial was to assess the effect of BCG vaccination on absenteeism and infectious disease morbidity during the SARS-COV-2 pandemic. Participant recruitment and eligibility criteria are detailed elsewhere; trial exclusion criteria included contraindications to BCG vaccination [24]. Participants with missing information concerning presence of scars (n = 9) and chronic disease (n = 3) were excluded from this study, resulting in a sample size of 1218 HCWs.

### 2.2. Definition of cohorts

The participants' birth year ranged from 1948 to 1997. To fulfill our aim of investigating the association between presence of vaccine scar(s) and self-reported chronic diseases, we limited our sample to participants born up to 1976 in the main analyses since few individuals born hereafter could have received the *Vaccinia* vaccine. In addition to the sub-cohort of participants born 1948–1976, we conducted analyses for participants born 1965–1976. Individuals born between 1965 and 1976 experienced a natural experiment created by the phase-outs of BCG and *Vaccinia*, and this birth year range has been used in previous studies [9,25,26].

### 2.3. Data collection

On the day of inclusion, participants attended a scheduled interview with study personnel trained in distinguishing BCG and *Vaccinia* scars by visual inspection of the deltoid region on both arms. For each scar, the interviewer determined the type (BCG or *Vaccinia*) based on its characteristics. Usually, BCG scars are 0.5–1.0 cm and flat [13], while *Vaccinia* scars are pitted and considerably larger [27].

Background information used for this study included, apart from scar presence, self-reported vaccination status, chronic diseases, sex, birth year and smoking status (current smoker or non-smoker). To assess chronic disease prevalence, participants were asked: "*Do you have a chronic disease?*". If the participant answered yes, a follow-up question regarding which disease(s) was asked. Responses were categorized as heart disease, lung disease, kidney disease, diabetes, metabolic disorder, or other chronic disease.

### 2.4. Statistical analysis

We grouped participants with one, two, or three scars from the respective vaccines together, as very few participants had multiple scars from the same vaccine. In the assessment of scar prevalence, participants were grouped based on their birth year in intervals of five years. Associations between vaccine scars and the prevalence of chronic disease were analyzed using a logistic regression model providing adjusted Odds Ratios (aORs) with 95 % CIs.

In the regression analyses, participants having one or more vaccine scars were compared with those with no vaccine scars (neither BCG nor *Vaccinia*). First, we compared those with only BCG, or only *Vaccinia* scars, to those with no scars. Second, we conducted the analyses using a combined group of participants having both BCG and *Vaccinia* scars. Third, we compared having BCG and/or *Vaccinia* with no scars. We investigated the association with major categories of chronic disease among participants born 1965–1976. Diseases were grouped into cardiovascular diseases (such as hypertension and arrhythmias), respiratory diseases (such as asthma and COPD), and allergic diseases (such as allergies and eczema). The remaining smaller categories, such as metabolic diseases (n = 47) and diabetes mellitus (n = 17), were grouped with "other chronic diseases". In the Appendix, we present separate analyses for females, but not for males given the low number of male participants. Additionally, we present analyses investigating the association between having BCG scar(s) and chronic disease for the entire cohort.

For the BCG scar prevalence estimates, participants born after 1976 were excluded if they were recorded as having only *Vaccinia* scars (n = 3) as such recordings were considered unreliable. Analyses were adjusted for sex, birth year and smoking status [28,29]. In the regression analyses, participants with missing information concerning smoking status were excluded (sub-cohort born <1977 (n = 5); sub-cohort born 1965–1976 (n = 1)). Further, birth year was treated as single years, and smoking status was dichotomized. Statistical analysis was performed using Stata version 17 (StataCorp LP, College Station, TX) [30]. The STROBE checklist was used to guide reporting of the study results [31].

### 2.5. Ethical approval

The original RCT was approved by the Ethics Committee of the Region of Southern Denmark (approval number: S-20200062C) and the Danish Medicines Agency (approval number: 2020041936). It was registered at ClinicalTrials.gov (NCT04373291) and at the EU Clinical Trials Register (2020-001888-90). The trial was monitored by the Good Clinical Practice unit at Odense University Hospital. All trial participants provided written informed consent.

## 3. Results

### 3.1. Participant characteristics

The cohort consisted of 1218 participants at baseline; 206 males and 1012 females, with a median age of 47 years (Q1–Q3: 36–56). The median birth year was 1973 and 34 % (416/1218) reported having one

or more chronic diseases. Among the 1218 participants, 61 % (737) were born before 1977 and 33 % (403) were born between 1965 and 1976 (Fig. 1). For the sub-cohorts born <1977 and 1965–1976, the prevalences of chronic disease were 40 % (297/737) and 36 % (144/403), respectively (Table 1).

### 3.2. Prevalence of vaccine scars

Among participants born before 1977, the scar prevalence was 74 % for BCG scar(s) and 69 % for *Vaccinia* scar(s) (Table 1). The prevalence of BCG and *Vaccinia* scars varied depending on birth year, reflecting the changes in the vaccination policies. For the five-year birth cohorts in the 1948–1969-range, both BCG and *Vaccinia* scar prevalences were consistently high, ranging from 82 to 93 %. As expected, a decrease in scar prevalence was observed in the 1970–1974 birth cohort with BCG and *Vaccinia* prevalences of 59 % and 40 %, respectively. For subsequent birth cohorts, the decrease in BCG scar prevalence continued, with 5 % of participants born 1975–1976 having *Vaccinia* scar(s) (Fig. 2; Appendix Table 1). In the sub-cohort born <1977, the proportion recorded with BCG scar(s) among those who reported previous BCG vaccination was 89 % (527/593). The corresponding proportion for *Vaccinia* was 93 % (485/521).

### 3.3. Vaccine scars and chronic diseases

Having only BCG scar(s) was associated with lower prevalence of self-reported chronic disease when compared with no vaccine scars: For the cohort born <1977 and for the cohort born 1965–1976, the BCG scar (s) vs. no scars aORs were 0.46 (95 % CI: 0.26–0.81) and 0.39 (0.21–0.75), respectively. In the corresponding *Vaccinia* scar analyses the estimates were 1.10 (0.58–2.05) and 0.80 (0.35–1.84), respectively. In analyses comparing having both BCG and *Vaccinia* scars with no scars, the aORs were 0.63 (0.38–1.05) and 0.64 (0.32–1.27), respectively. When combining the groups and comparing having BCG and/or *Vaccinia* scars with no scars, the aOR in the 1965–1976 cohort was 0.51 (0.29–0.90) (Table 2). When conducting the same analyses in females only, all estimates of the relative effect tended to be stronger, compared with the combined estimates for females and males. For example, the aOR for presence of chronic disease when comparing BCG and/or *Vaccinia* scars vs. no scars in the 1965–1976 cohort was 0.41 (0.22–0.76) in females (Appendix Table 2) and 0.51 (0.29–0.90) for both sexes (Table 2). Lastly, for the entire cohort (any birth year), having only BCG or BCG and *Vaccinia* scar(s) vs. no scars yielded an aOR of 0.69 (0.47–1.01) of having self-reported chronic disease (Appendix Table 3).

### 3.4. Vaccine scars and major categories of chronic disease

In analyses investigating the association between having vaccine scar (s) and major categories of chronic disease, having only BCG scar(s) yielded lower aORs in all analyses when compared with having no vaccine scars. The analysis concerning chronic respiratory diseases was statistically significant with an aOR of 0.12 (0.03–0.59). For those having only *Vaccinia* scar(s) compared with no vaccine scars, none of the estimates were statistically significant. When comparing participants with BCG and/or *Vaccinia* scar(s) with no vaccine scars, the estimates were significantly reduced for chronic respiratory and allergic diseases, but not for cardiovascular and other diseases. The aOR for respiratory diseases was 0.39 (0.16–0.97), and the aOR for allergic diseases was 0.39 (0.16–0.91) (Table 3).

## 4. Discussion

### 4.1. Main findings

Having BCG scar(s) only or both BCG and *Vaccinia* scars was associated with a lower prevalence of self-reported chronic disease, when compared with having no vaccine scars. Specifically, for participants born 1965–1976 who experienced the “natural experiment” created by the discontinuation of vaccination, the BCG and/or *Vaccinia* scar aOR was 0.51 (95 % CI: 0.29–0.90). Regarding major categories of chronic disease, having BCG and/or *Vaccinia* scars was associated with a lower prevalence of having chronic respiratory and allergic diseases. When compared with having no scars, having *only* BCG scar(s) was consistently associated with reduced odds across our analyses, a pattern not seen for *Vaccinia* scar(s), suggesting that a possible protective effect against chronic disease was primarily driven by BCG. Noteworthy, few participants had only *Vaccinia* scar(s). The study was cross-sectional but as vaccines were given in childhood and the chronic diseases largely start later in life, the findings indicate that BCG, but not *Vaccinia*, may confer substantial long-term protection against chronic diseases.

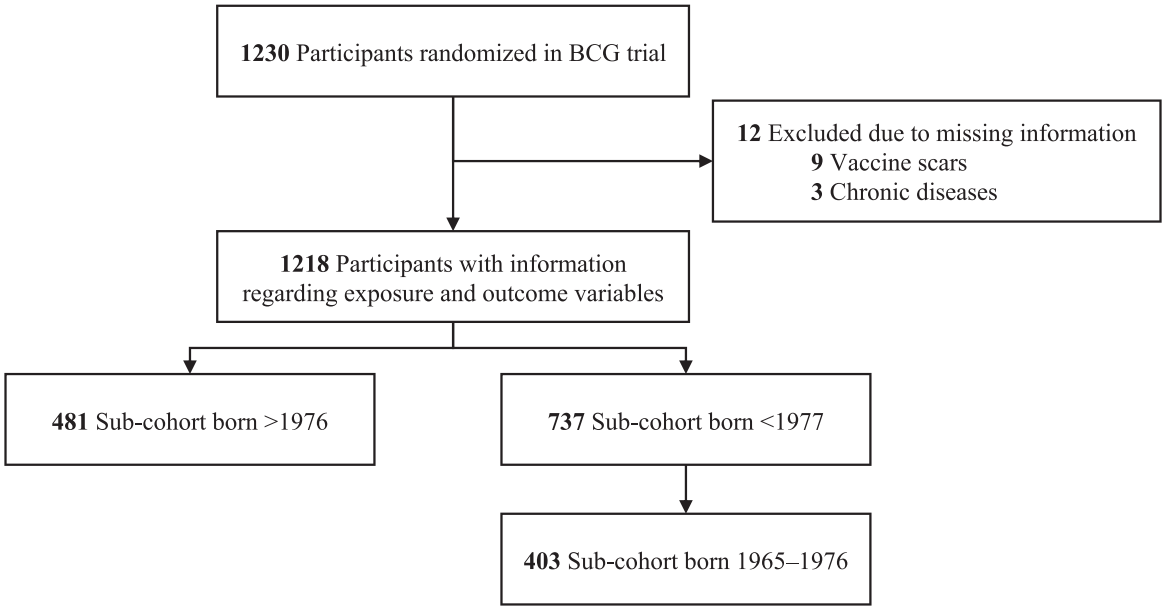


**Fig. 1.** Flowchart of the study cohort. Abbreviation: BCG, *Bacillus Calmette-Guérin*.

**Table 1**
Baseline characteristics of the entire cohort and the sub-cohorts born before 1977 and from 1965 to 1976, collected at enrollment (May 2020 to January 2021).

| Characteristic | Entire cohort | Sub-cohort born <1977 | Sub-cohort born 1965–1976 |
|---|---|---|---|
| | N = 1218 | N = 737 | N = 403 |
| **Female sex – % (no.)** | 83 (1012) | 87 (641) | 87 (352) |
| **Age, years*** | | | |
| Median (Q1–Q3) | 47.2 (36.2–56.5) | 54.5 (48.5–59.8) | 49.0 (46.3–52.7) |
| **Birth year** | | | |
| Median (Q1–Q3) | 1973 (1964–1984) | 1966 (1960–1972) | 1971 (1967–1974) |
| **Self-reported BCG vaccination – % (no.)** | | | |
| Yes | 54 (652) | 80 (593) | 68 (276) |
| Do not know | 2.6 (31) | 3.5 (26) | 6.0 (24) |
| **BCG scar – % (no.)** | | | |
| 0 | 51 (618) | 26 (189) | 34 (138) |
| 1 | 46 (563) | 70 (513) | 63 (254) |
| 2 | 2.9 (35) | 4.6 (34) | 2.5 (10) |
| 3 | 0.2 (2) | 0.1 (1) | 0.2 (1) |
| ≥1 | 49 (600) | 74 (548) | 66 (265) |
| **Self-reported *Vaccinia* vaccination – % (no.)** | | | |
| Yes | 44 (533) | 71 (521) | 52 (209) |
| Do not know | 4.4 (53) | 6.1 (45) | 7.4 (30) |
| Missing information | 0.2 (2) | 0 (0) | 0 (0) |
| ***Vaccinia* scar – % (no.)** | | | |
| 0 | 58 (712) † | 31 (231) | 49 (197) |
| 1 | 22 (266) † | 36 (266) | 28 (113) |
| 2 | 19 (235) † | 32 (235) | 23 (91) |
| 3 | 0.4 (5) | 0.7 (5) | 0.5 (2) |
| ≥1 | 42 (506) † | 69 (506) | 51 (206) |
| **Chronic disease – % (no.)** | | | |
| Yes (≥1 chronic disease) | 34 (416) | 40 (297) | 36 (144) |
| Category **of chronic disease – % (no.)** | | | |
| Cardiovascular | 9.4 (115) | 15 (108) | 10 (41) |
| Respiratory | 9.2 (112) | 10 (74) | 9.7 (39) |
| Allergic | 8.2 (100) | 9.2 (68) | 10 (41) |
| Other | 16 (195) | 18 (136) | 17 (69) |
| **Smoking – % (no.)** | | | |
| Non-smoker | 90 (1095) | 91.3 (673) | 90.8 (365) |
| Current smoker | 9.5 (116) | 8.0 (59) | 9.2 (37) |
| Missing information | 0.6 (7) | 0.7 (5) | 0.2 (1) |

Abbreviation: BCG, *Bacillus Calmette-Guérin*.
* Age at day of enrollment.
† Participants recorded with *Vaccinia* scar(s) born after 1976 (one scar, n = 7; two scars, n = 2) were recategorized as having no *Vaccinia* scars, as few could have received the vaccine after this year.

### 4.2. Strengths and limitations

We chose to investigate the association between vaccine scars and chronic disease rather than vaccination. With this approach, we avoided recall bias regarding vaccination status. For BCG, scar formation after vaccination is associated with the BCG strain administered and with vaccination technique [32,33]. Our data was obtained from a trial with a rigorous methodology, with study personnel (MDs and medical students) who had been trained to accurately classify vaccine scars. Moreover, few participants were excluded from analyses due to missing data.

The study has some limitations. First, there are no official vaccination records going back in time to the study cohorts. In the choice between recalled BCG vaccination and BCG vaccination scar, we chose BCG scar, as a more objective marker of a correctly applied vaccine [34]. The coverage by age was quite similar to that seen in other Danish cohorts [9]. Second, the data on chronic diseases was self-reported, which introduces the possibility of misclassification. However, this is supposedly non-differential by vaccine scar status, as there has been little attention to the proposed hypothesis regarding BCG and *Vaccinia* and chronic diseases. Furthermore, the RCT was focused on a new BCG vaccine's potential effects against infectious diseases. Thus, even though participants would be aware of their vaccination status, there is little reason to believe that this influenced their reporting of chronic diseases. Since our data was collected before the conceptualization of this study, we did not collect very detailed information on the specific nature, duration, and treatment of the chronic diseases, so we could merely define chronic diseases by broad categories. Third, eczema was a contraindication to smallpox vaccination, and this could create a false association between *Vaccinia* scars and lower prevalence of allergic disease. However, the association with allergic disease was also present among participants having only BCG scar(s) (Table 3). Fourth, the original RCT recruited HCWs that had not yet been infected with COVID-19 and who did not fulfill the exclusion criteria for entering the trial such as having had cancer during the previous two years. Furthermore, the participants were mainly female. The specific effectiveness of BCG against tuberculosis varies considerably depending on the population and outcome measure. Some degree of variation in the magnitude of non-specific effects may also be anticipated. All this could affect the generalizability of the results. Lastly, it would have been optimal to have a control outcome, but given the non-specific nature of the vaccine effects, it is challenging to contemplate a control outcome that would not be affected by the vaccines.

We adjusted for birth year and smoking status, but there could be unmeasured confounders affecting both the probability of the outcome and of childhood vaccination, such as parental socio-economic status. If this was the case, however, then similar patterns would be expected for BCG and *Vaccinia*. Also, previous studies have not indicated a large variation in BCG and *Vaccinia* coverage by socio-economic class [9], and given that all participants were HCWs, we consider it unlikely that confounding by socio-economic class could explain the results.

### 4.3. Comparison with other studies

BCG and *Vaccinia* have been used against tuberculosis and smallpox for one and two centuries, respectively. Regarding BCG, studies with long follow-up indicate that a specific protective effect could last for as long as 20–60 years, with waning of vaccine efficacy over time [35–37], while *Vaccinia* vaccination has been reported to provide >50 % protection against severe disease 50 years after successful primary vaccination [38].

BCG and *Vaccinia* may provide long-term protection against other diseases as well. A Danish register study investigated the association between BCG and/or *Vaccinia* vaccination and the risk of death from natural causes in a cohort of Copenhagen schoolchildren born 1965–1976. The children grew up while the vaccines were phased out. The children were followed from their first school health examination until 2010. Having received BCG and *Vaccinia* compared with no vaccination yielded an adjusted hazard ratio of death from natural causes of 0.54 (0.36–0.81). No reduction in the risk of death due to accidents, murders, and suicides (control outcome) was observed. For specific disease categories, having received BCG and/or *Vaccinia* were associated with reduced risks of death due to infectious, cardiovascular, neurological, major autoimmune, and other diseases, but not cancers [9].

The prevalence of asthma in children and adolescents (7–17 years old) increased 2.2-fold, from 5.3 % in 1986 to 11.7 % in 2001 across two cross-sectional studies with population samples from Copenhagen, matching the period when BCG was phased out [39]. Numerous studies using murine models have been conducted to examine the potential benefit of BCG against asthma. These studies have provided insights into several mechanisms that could protect against asthma [40]. However, the findings from studies in humans have been conflicting. In a recent review no significant association between BCG vaccination and the risk

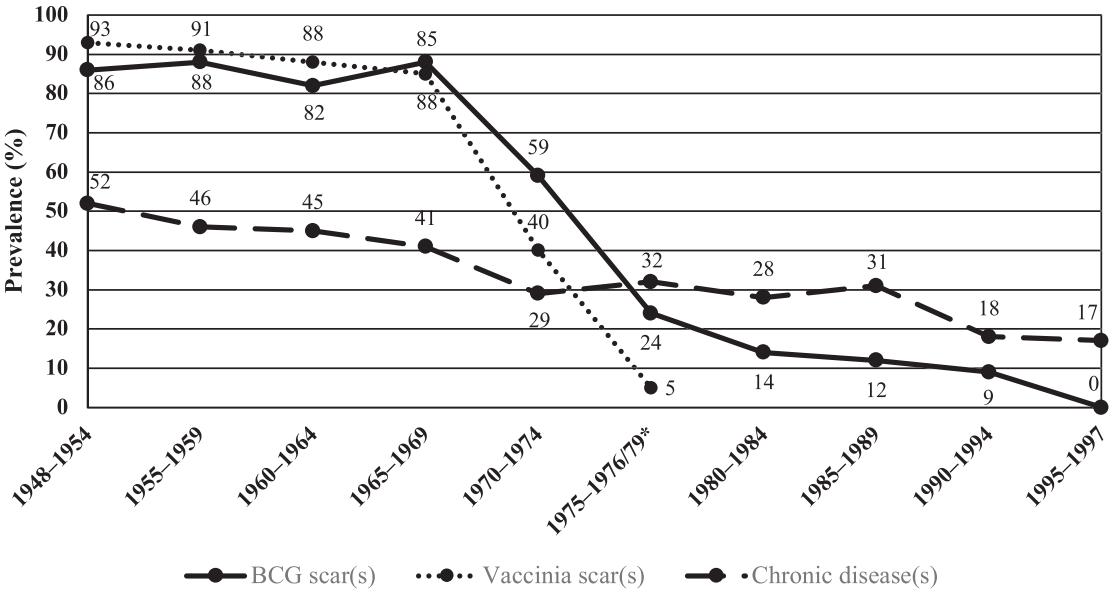


**Fig. 2.** Prevalence of BCG and *Vaccinia* scar(s) and self-reported chronic disease(s) by ~5-year birth year cohorts. Abbreviation: BCG, *Bacillus Calmette-Guérin*. * The prevalence of having one or more *Vaccinia* scars 1975–1976, and the prevalence of having one or more BCG scars 1975–1979. Notes: Scar status is not presented for *Vaccinia* after 1976, as few could have received the vaccine after this year. Participants recorded with only *Vaccinia* scar(s) born after 1976 (n=3) were excluded from BCG scar prevalence, as the recordings were considered unreliable. See Appendix Table 1 for number of participants in each group.

**Table 2**
The association between having vaccine scar(s) and self-reported chronic disease(s): Sub-cohorts born before 1977, and from 1965 to 1976. Bold font in effect estimates indicates p<0.05.

| Scar inspection | Has chronic disease % (n/N) | Adjusted odds ratio[1] (95 % CI) |
|---|---|---|
| **BCG and/or *Vaccinia* scar born <1977** | | |
| No vaccine scars | 42 (47/112) | Ref. |
| Only BCG scar(s) | 28 (33/118) | **0.46 (0.26–0.81)** |
| Only *Vaccinia* scar(s) | 53 (40/76) | 1.10 (0.58–2.05) |
| BCG and *Vaccinia* scars | 41 (175/426) | 0.63 (0.38–1.05) |
| BCG and/or *Vaccinia* scar(s) | 40 (248/620) | **0.61 (0.38–0.98)** |
| **BCG and/or *Vaccinia* scar born 1965–1976** | | |
| No vaccine scars | 42 (43/102) | Ref. |
| Only BCG scar(s) | 23 (22/94) | **0.39 (0.21–0.75)** |
| Only *Vaccinia* scar(s) | 42 (15/36) | 0.80 (0.35–1.84) |
| BCG and *Vaccinia* scars | 37 (63/170) | 0.64 (0.32–1.27) |
| BCG and/or *Vaccinia* scar(s) | 33 (100/300) | **0.51 (0.29–0.90)** |

Abbreviations: BCG, *Bacillus Calmette-Guérin*; CI, Confidence Interval.
Note: Participants with missing information regarding smoking status were excluded: sub-cohort born <1977 (n = 5); sub-cohort born 1965–1976 (n = 1).
[1] Adjusted for sex, birth year and smoking status.

**Table 3**
The association between having vaccine scar(s) and major categories of chronic disease: Sub-cohort born from 1965 to 1976. Bold font in effect estimates indicates p<0.05.

| Scar inspection | Category of chronic disease present% (n/N) | Adjusted odds ratio[1] (95 % CI) |
|---|---|---|
| **Cardiovascular disease** | | |
| No vaccine scars | 9.8 (10/102) | Ref. |
| Only BCG scar(s) | 5.3 (5/94) | 0.50 (0.16–1.57) |
| Only *Vaccinia* scar(s) | 17 (6/36) | 1.53 (0.47–5.06) |
| BCG and *Vaccinia* scars | 12 (20/170) | 1.02 (0.35–3.02) |
| BCG and/or *Vaccinia* scar (s) | 10 (31/300) | 0.80 (0.33–1.99) |
| **Respiratory disease** | | |
| No vaccine scars | 13 (13/102) | Ref. |
| Only BCG scar(s) | 2.1 (2/94) | **0.12 (0.03–0.59)** |
| Only *Vaccinia* scar(s) | 22 (8/36) | 1.39 (0.46–4.17) |
| BCG and *Vaccinia* scars | 8.9 (15/170) | 0.40 (0.13–1.22) |
| BCG and/or *Vaccinia* scar (s) | 8.3 (25/300) | **0.39 (0.16–0.97)** |
| **Allergic disease** | | |
| No vaccine scars | 15 (15/102) | Ref. |
| Only BCG scar(s) | 7.4 (7/94) | 0.40 (0.15–1.08) |
| Only *Vaccinia* scar(s) | 5.6 (2/36) | 0.26 (0.05–1.28) |
| BCG and *Vaccinia* scars | 10 (17/170) | 0.42 (0.15–1.20) |
| BCG and/or *Vaccinia* scar (s) | 8.7 (26/300) | **0.39 (0.16–0.91)** |
| **Other disease** | | |
| No vaccine scars | 19 (19/102) | Ref. |
| Only BCG scar(s) | 13 (12/94) | 0.66 (0.29–1.48) |
| Only *Vaccinia* scar(s) | 19 (7/36) | 1.01 (0.36–2.87) |
| BCG and *Vaccinia* scars | 18 (31/170) | 1.01 (0.43–2.37) |
| BCG and/or *Vaccinia* scar (s) | 17 (50/300) | 0.81 (0.40–1.63) |

Abbreviations: BCG, *Bacillus Calmette-Guérin*; CI, Confidence Interval.
Note: One participant was excluded due to missing information regarding smoking status.
[1] Adjusted for sex, birth year and smoking status.

of asthma, hay fever, or food allergy was shown [41]. However, BCG given in infancy was associated with a reduced risk of childhood eczema at 13–18 months across three RCTs, the risk ratio being 0.89 (0.82–0.98) [20]. Our results support the hypothesis that BCG vaccination lowers the risk of developing allergic diseases including asthma.

BCG is also being investigated as a possible prophylactic or therapeutic tool for multiple sclerosis and type 1 diabetes mellitus [21]. Clinical trials suggest that BCG provided after disease-onset may reduce disease activity in patients with multiple sclerosis [42,43], and for patients who have had their first demyelinating event [44]. Regarding type 1 diabetes mellitus, previous RCTs did not support that BCG vaccination induces clinical remission or preserves β-cell function [45,46]. Yet, anti-diabetic effects have been observed in more recent studies, e.g., long-term lowering of blood sugars measured by HbA1c levels [47]. Moreover, in a large BCG trial with 60 years of follow-up, the prevalence of type 2 diabetes was significantly lower in the BCG group than in the placebo group [37].

Noteworthy, in a combined analysis of eight published RCTs testing BCG versus placebo against COVID-19 in HCWs and elderly, BCG was associated with 39 % (3–62 %) significantly lower all-cause mortality

[6,48].

### 4.4. Perspectives

Non-specific effects of vaccines represent an intriguing area of investigation. The potential benefits of live vaccines as a prophylactic or therapeutic intervention could be significant for patients, given the debilitating nature of chronic diseases and the rising disease prevalence in our aging societies. The low cost and great safety of BCG makes it a favorable intervention in this regard. The present study should be seen as hypothesis generating, and we hope other groups will test the findings. In recent years, the potential mechanisms behind the NSEs of vaccines have been uncovered [49,50]. Immunological studies should be continued to investigate which mechanisms may be at play regarding chronic diseases.

## 5. Conclusion

The presence of BCG scar(s) with or without *Vaccinia* scar(s) was associated with a substantially lower prevalence of self-reported chronic disease. This was not observed for *Vaccinia* scar(s) alone. This natural experiment thus supports that vaccination with BCG might reduce the risk of developing chronic disease. These findings could have a major impact on public health and should be explored further.

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## CRediT authorship contribution statement

**William Leander Mæland Søvik:** Writing – original draft, Visualization, Methodology, Formal analysis, Data curation. **Anne Marie Rosendahl Madsen:** Writing – review & editing, Investigation, Funding acquisition, Data curation. **Peter Aaby:** Writing – review & editing, Methodology. **Sebastian Nielsen:** Writing – review & editing, Methodology, Formal analysis. **Christine Stabell Benn:** Writing – review & editing, Methodology, Funding acquisition. **Frederik Schaltz-Buchholzer:** Writing – review & editing, Methodology, Investigation, Funding acquisition, Data curation, 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.

## Data availability

De-identified participant data with a data dictionary can be shared after approval of a data-sharing proposal sent to Prof. Christine S. Benn (cbenn@health.sdu.dk).

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## Appendix A. Supplementary material

In Appendix A, we provide three tables with main study outcomes provided by ~5-year birth year cohorts (Appendix Table 1), in females only (Appendix Table 2), and in relevant sub-cohorts (Appendix Table 3).

Supplementary material to this article can be found online at https://doi.org/10.1016/j.vaccine.2024.02.049.

## References

[1] Schaltz-Buchholzer F, Biering-Sørensen S, Lund N, Monteiro I, Umbasse P, Fisker AB, et al. Early BCG vaccination, hospitalizations, and hospital deaths: analysis of a secondary outcome in 3 randomized trials from Guinea-Bissau. J Infect Dis 2019;219:624–32. https://doi.org/10.1093/infdis/jiy544.
[2] Higgins JPT, Soares-Weiser K, López-López JA, Kakourou A, Chaplin K, Christensen H, et al. Association of BCG, DTP, and measles containing vaccines with childhood mortality: systematic review. BMJ 2016;355:i5170. https://doi.org/10.1136/bmj.i5170.
[3] Aaby P, Gustafson P, Roth A, Rodrigues A, Fernandes M, Sodemann M, et al. Vaccinia scars associated with better survival for adults: an observational study from Guinea-Bissau. Vaccine 2006;24:5718–25. https://doi.org/10.1016/j.vaccine.2006.04.045.
[4] Jensen ML, Dave S, van der Loeff MS, da Costa C, Vincent T, Leligdowicz A, et al. Vaccinia scars associated with improved survival among adults in rural Guinea-Bissau. PLoS One 2006;1:e101. https://doi.org/10.1371/journal.pone.0000101.
[5] Benn CS, Fisker AB, Rieckmann A, Sørup S, Aaby P. Vaccinology: time to change the paradigm? Lancet Infect Dis 2020;20:e274–83. https://doi.org/10.1016/S1473-3099(19)30742-X.
[6] Aaby P, Netea MG, Benn CS. Beneficial non-specific effects of live vaccines against COVID-19 and other unrelated infections. Lancet Infect Dis 2023;23:e34–42. https://doi.org/10.1016/S1473-3099(22)00498-4.
[7] Costello VH, Raiciulescu S, Santosham M, Harrison L, Aronson NE. 1721. BCG vaccination impact on mortality: a 71 year follow-up of a US BCG controlled trial. Open Forum Infect Dis 2023;10. doi: 10.1093/ofid/ofad500.1553.
[8] Benn CS, Netea MG, Selin LK, Aaby P. A small jab - a big effect: nonspecific immunomodulation by vaccines. Trends Immunol 2013;34:431–9. https://doi.org/10.1016/j.it.2013.04.004.
[9] Rieckmann A, Villumsen M, Sørup S, Haugaard LK, Ravn H, Roth A, et al. Vaccinations against smallpox and tuberculosis are associated with better long-term survival: a Danish case-cohort study 1971–2010. Int J Epidemiol 2017;46:695–705. https://doi.org/10.1093/ije/dyw120.
[10] The Danish Parliament. Lov om tilbud om gratis vaccination mod visse sygdomme. 1976. https://www.folketingstidende.dk/samling/19761/lovforslag/L95/19761_L95_som_vedtaget.pdf (accessed January 10, 2024).
[11] Henderson DA. The eradication of smallpox - an overview of the past, present, and future. Vaccine 2011;29:D7–9. https://doi.org/10.1016/j.vaccine.2011.06.080.
[12] Rieckmann A, Villumsen M, Sørup S, Haugaard L, Ravn H, Roth A. Vaccinations against smallpox and tuberculosis are associated with better long-term survival: a Danish case-cohort study 1971–2010 (Supplementary data). Int J Epidemiol 2017;46:695–705. https://doi.org/10.1093/ije/dyw120.
[13] Anonymous. BCG vaccine (BCG Vaccine "AJ Vaccines"). Statens Serum Institut. 2023. https://www.ssi.dk/vaccinationer/vaccineleksikon/b/bcgvaccine-aj-vaccines (accessed January 10, 2024).
[14] Anonymous. BCG vaccines: WHO position paper – February 2018. Wkly Epidemiol Rec 2018;93:73–96.
[15] Belongia EA, Naleway AL. Smallpox vaccine: the good, the bad, and the ugly. Clin Med Res 2003;1:87–92. https://doi.org/10.3121/cmr.1.2.87.
[16] Singh AK, Netea MG, Bishai WR. BCG turns 100: its nontraditional uses against viruses, cancer, and immunologic diseases. J Clin Invest 2021;131:e148291. https://doi.org/10.1172/JCI148291.
[17] Herr HW, Morales A. History of bacillus Calmette-Guerin and bladder cancer: an immunotherapy success story. J Urol 2008;179:53–6. https://doi.org/10.1016/j.juro.2007.08.122.
[18] Woldu SL, Bagrodia A, Lotan Y. Guideline of guidelines: non-muscle-invasive bladder cancer. BJU Int 2017;119:371–80. https://doi.org/10.1111/bju.13760.
[19] Usher NT, Chang S, Howard RS, Martinez A, Harrison LH, Santosham M, et al. Association of BCG vaccination in childhood with subsequent cancer diagnoses. JAMA Netw Open 2019;2:e1912014. https://doi.org/10.1001/jamanetworkopen.2019.12014.
[20] Pittet LF, Thøstesen LM, Aaby P, Kofoed P-E, Curtis N, Benn CS. Neonatal bacillus Calmette-Guérin vaccination to prevent early-life eczema: a systematic review and meta-analysis. Dermatitis 2022;33:S3–16. https://doi.org/10.1097/DER.0000000000000945.
[21] Ristori G, Faustman D, Matarese G, Romano S, Salvetti M. Bridging the gap between vaccination with Bacille Calmette-Guérin (BCG) and immunological tolerance: the cases of type 1 diabetes and multiple sclerosis. Curr Opin Immunol 2018;55:89–96. https://doi.org/10.1016/j.coi.2018.09.016.
[22] Gofrit ON, Klein BY, Cohen IR, Ben-Hur T, Greenblatt CL, Bercovier H. Bacillus Calmette-Guérin (BCG) therapy lowers the incidence of Alzheimer's disease in bladder cancer patients. PLoS One 2019;14:e0224433. https://doi.org/10.1371/journal.pone.0224433.

[23] Madsen AMR, Schaltz-Buchholzer F, Nielsen S, Benfield T, Bjerregaard-Andersen M, Dalgaard LS, et al. Using BCG vaccine to enhance non-specific protection of health care workers during the COVID-19 pandemic: a randomized controlled trial. J Infect Dis 2023. https://doi.org/10.1093/infdis/jiad422.
[24] Madsen AMR, Schaltz-Buchholzer F, Benfield T, Bjerregaard-Andersen M, Dalgaard LS, Dam C, et al. Using BCG vaccine to enhance non-specific protection of health care workers during the COVID-19 pandemic: a structured summary of a study protocol for a randomised controlled trial in Denmark. Trials 2020;21:799. https://doi.org/10.1186/s13063-020-04714-3.
[25] Villumsen M, Sørup S, Jess T, Ravn H, Relander T, Baker JL, et al. Risk of lymphoma and leukaemia after bacille Calmette-Guérin and smallpox vaccination: a Danish case-cohort study. Vaccine 2009;27:6950–8. https://doi.org/10.1016/j.vaccine.2009.08.103.
[26] Rieckmann A, Villumsen M, Jensen ML, Ravn H, da Silva ZJ, Sørup S, et al. The effect of smallpox and bacillus Calmette-Guérin vaccination on the risk of human immunodeficiency virus-1 infection in Guinea-Bissau and Denmark. Open Forum Infect Dis 2017;4:ofx130. doi: 10.1093/ofid/ofx130.
[27] Cono J, Casey CG, Bell DM. Centers for Disease Control and Prevention. Smallpox vaccination and adverse reactions. Guidance for clinicians. MMWR Recomm Rep 2003;52:1–28.
[28] Flanagan KL, Fink AL, Plebanski M, Klein SL. Sex and gender differences in the outcomes of vaccination over the life course. Annu Rev Cell Dev Biol 2017;33:577–99. https://doi.org/10.1146/annurev-cellbio-100616-060718.
[29] U.S. Department of Health and Human Services. The health consequences of smoking: 50 years of progress: a report of the surgeon general. Atlanta, GA: Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health; 2014.
[30] StataCorp.. Stata statistical software: release 17. College Station, TX: StataCorp LLC; 2021.
[31] von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: Guidelines for Reporting Observational Studies. PLoS Med 2007;4:e296. https://doi.org/10.1371/journal.pmed.0040296.
[32] Roth A, Sodemann M, Jensen H, Poulsen A, Gustafson P, Gomes J, et al. Vaccination technique, PPD reaction and BCG scarring in a cohort of children born in Guinea-Bissau 2000–2002. Vaccine 2005;23:3991–8. https://doi.org/10.1016/j.vaccine.2004.10.022.
[33] Schaltz-Buchholzer F, Berendsen M, Roth A, Jensen KJ, Bjerregaard-Andersen M, Kjær Sørensen M, et al. BCG skin reactions by 2 months of age are associated with better survival in infancy: a prospective observational study from Guinea-Bissau. BMJ Glob Health 2020;5:e002993. https://doi.org/10.1136/bmjgh-2020-002993.
[34] Benn CS, Roth A, Garly M-L, Fisker AB, Schaltz-Buchholzer F, Timmermann A, et al. BCG scarring and improved child survival: a combined analysis of studies of BCG scarring. J Intern Med 2020;288:614–24. https://doi.org/10.1111/joim.13084.
[35] Mangtani P, Nguipdop-Djomo P, Keogh RH, Sterne JAC, Abubakar I, Smith PG, et al. The duration of protection of school-aged BCG vaccination in England: a population-based case-control study. Int J Epidemiol 2018;47:193–201. https://doi.org/10.1093/ije/dyx141.
[36] Nguipdop-Djomo P, Heldal E, Rodrigues LC, Abubakar I, Mangtani P. Duration of BCG protection against tuberculosis and change in effectiveness with time since vaccination in Norway: a retrospective population-based cohort study. Lancet Infect Dis 2016;16:219–26. https://doi.org/10.1016/S1473-3099(15)00400-4.
[37] Aronson NE, Santosham M, Comstock GW, Howard RS, Moulton LH, Rhoades ER, et al. Long-term efficacy of BCG vaccine in American Indians and Alaska natives: a 60-year follow-up study. JAMA 2004;291:2086–91. https://doi.org/10.1001/jama.291.17.2086.
[38] Nishiura H, Schwehm M, Eichner M. Still protected against smallpox? Estimation of the duration of vaccine-induced immunity against smallpox. Epidemiology 2006;17:576–81. https://doi.org/10.1097/01.ede.0000229196.41862.c2.
[39] Thomsen SF, Ulrik CS, Larsen K, Backer V. Change in prevalence of asthma in Danish children and adolescents. Ann Allergy Asthma Immunol 2004;92:506–11. https://doi.org/10.1016/S1081-1206(10)61757-7.
[40] Kowalewicz-Kulbat M, Locht C. BCG for the prevention and treatment of allergic asthma. Vaccine 2021;39:7341–52. https://doi.org/10.1016/j.vaccine.2021.07.092.
[41] Navaratna S, Estcourt MJ, Burgess J, Waidyatillake N, Enoh E, Lowe AJ, et al. Childhood vaccination and allergy: a systematic review and meta-analysis. Allergy 2021;76:2135–52. https://doi.org/10.1111/all.14771.
[42] Ristori G, Buzzi MG, Sabatini U, Giugni E, Bastianello S, Viselli F, et al. Use of Bacille Calmette-Guerin (BCG) in multiple sclerosis. Neurology 1999;53:1588. https://doi.org/10.1212/WNL.53.7.1588.
[43] Paolillo A, Buzzi MG, Giugni E, Sabatini U, Bastianello S, Pozzilli C, et al. The effect of Bacille Calmette-Guérin on the evolution of new enhancing lesions to hypointense T1 lesions in relapsing remitting MS. J Neurol 2003;250:247–8. https://doi.org/10.1007/s00415-003-0967-6.
[44] Ristori G, Romano S, Cannoni S, Visconti A, Tinelli E, Mendozzi L, et al. Effects of Bacille Calmette-Guerin after the first demyelinating event in the CNS. Neurology 2014;82:41–8. https://doi.org/10.1212/01.wnl.0000438216.93319.ab.
[45] Pozzilli P. BCG vaccine in insulin-dependent diabetes mellitus. IMDIAB Group Lancet 1997;349:1520–1. https://doi.org/10.1016/S0140-6736(05)62100-9.
[46] Allen HF, Klingensmith GJ, Jensen P, Simoes E, Hayward A, Chase HP. Effect of Bacillus Calmette-Guerin vaccination on new-onset type 1 diabetes. A randomized clinical study. Diabetes Care 1999;22:1703–7. https://doi.org/10.2337/diacare.22.10.1703.
[47] Kühtreiber WM, Tran L, Kim T, Dybala M, Nguyen B, Plager S, et al. Long-term reduction in hyperglycemia in advanced type 1 diabetes: the value of induced aerobic glycolysis with BCG vaccinations. npj Vaccines 2018;3:23. https://doi.org/10.1038/s41541-018-0062-8.
[48] Benn CS, Netea MG, Aaby P. BCG to Protect against Covid-19 in Health Care Workers. N Engl J Med 2023;389:191–2. https://doi.org/10.1056/NEJMc2306483.
[49] de Bree LCJ, Koeken VACM, Joosten LAB, Aaby P, Benn CS, van Crevel R, et al. Non-specific effects of vaccines: current evidence and potential implications. Semin Immunol 2018;39:35–43. https://doi.org/10.1016/j.smim.2018.06.002.
[50] Domínguez-Andrés J, dos Santos JC, Bekkering S, Mulder WJM, van der Meer JWM, Riksen NP, et al. Trained immunity: adaptation within innate immune mechanisms. Physiol Rev 2023;103:313–46. https://doi.org/10.1152/physrev.00031.2021.