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Smallpox and BCG vaccination in childhood and cutaneous malignant melanoma in Danish adults followed from 18 to 49 years

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ABSTRACT

Background: Early smallpox and Bacillus Calmette-Guérin (BCG) vaccinations have been associated with reduced risk of cutaneous malignant melanoma (CMM). We assessed the association between pre-school smallpox vaccination and early-school BCG vaccination and CMM in a young Danish population.
Methods: We conducted a register-based case-cohort study of individuals growing up during the phase-out period of smallpox and BCG vaccination in Denmark (born 1965–1976) utilising the decrease in vaccination during this period. Information on childhood vaccinations and potential confounders from Copenhagen school health records were linked with nationwide registers on cancer (CMM diagnoses), migrations and deaths by personal identification numbers.
Results: The individuals were followed from age 18 until 31/12/2014 (maximum age at end of follow-up, 49 years). 188 cases of CMM occurred in the background population of 46,239 individuals; 172 CMM cases (91%) had full information and were analysed. The adjusted hazard ratio (HR) for CMM by BCG and/or smallpox vaccination compared with neither vaccine was 1.29 (95% confidence interval (CI) 0.72–2.31). For smallpox vaccination only, HR = 1.23 (95% CI 0.53–2.86) for BCG vaccination only, HR = 1.13 (95% CI 0.61–2.09) and for both smallpox and BCG vaccination, HR = 1.75 (95% CI 0.87–3.48) compared with none of these. Vaccination below the age of one year gave similar results.
Conclusions: We found no strong beneficial effect of smallpox and BCG vaccination against CMM among young adult Danes and with broad confidence intervals our data alone could be compatible with both modest preventive effects, no effects, and modest harmful effects. Our estimates do not contradict a potential modest beneficial effect of neonatal vaccination.

1. Introduction

Evidence suggest that live vaccines protect against non-targeted diseases [1]. For instance, the Bacillus Calmette-Guérin (BCG) vaccine is used as a standard treatment in intermediate to high-risk non-muscle-invasive bladder cancer [2], even though the BCG vaccine was developed to protect against tuberculosis. During the 1970–80s, the use of the BCG vaccine prompted great enthusiasm as an immunotherapy against cutaneous malignant melanoma (CMM) [3,4]. However, in 1993, a meta-analysis of the BCG vaccine and its impact on CMM provided inconclusive results [5], and the research subsequently diminished.

In 2002, it was hypothesized that the smallpox and BCG vaccines may have *prophylactic* effects against CMM [5]. Pfahlberg et al. noted that early timing of smallpox and BCG vaccination could be important for the immune reactions, and they suggested that this could be a reason why previous immunotherapeutic studies [3,4,7,8] failed to show an effect [6]. One mechanistic explanation was that the peptide, HERV-K-MEL, expressed by most melanomas, has homologous epitope sequences with the smallpox vaccine and the BCG vaccine, hence, cross-reactivity could play a role in the protective effect [9]. It was also suggested that the vaccines

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replicated natural infections inducing regulatory mechanisms for the immune system [10].

We utilized the phase out period of smallpox and BCG vaccination in Denmark to investigate whether pre-school smallpox vaccination and early-school BCG vaccinations are associated with a lower risk of developing CMM among young Danes.

2. Methods

This study is a retrospective register-based case-cohort study among Danes, who attended school in Copenhagen and were followed from age 18–49 years. We used information from all cases in the full background population and information from a subcohort the full background population. Case-cohort studies are typically performed when retrieving information on exposure and confounder information is costly and timing consuming.

2.1. Setting and study population

In Denmark, smallpox vaccination was phased out in 1977 and phasing out the BCG vaccination was suggested in 1980, but recommendations were officially changed in 1987, which restricted BCG vaccination to "high risk" children [11]. Smallpox vaccination used to be compulsory [12] and children had to be smallpox vaccinated before entering school [13]. BCG vaccination was voluntary (children were tuberculosis skin tested [Mantoux or Moro test] and negative responders were referred for vaccination free of charge) [13–15]. BCG was typically given during the first years of school, at the ages of 5–7 [16]. Due to the difference in age at vaccination, the birth cohorts 1965–76 were affected by phase outs of both vaccines – the birth cohort 1965 had almost 100% vaccination coverage; the birth cohort 1976 had almost none (Supplementary Fig. 1). More information about the vaccination programme can be found in the supplementary material to Rieckmann et al. [11].

The background population for this study was children born in1965–1976 and registered in the Copenhagen School Health Records Register, which comprises all children who went to school in the municipality of Copenhagen [17]. Children attended several school health examinations during their schooling, for which information about vaccination, infections, anthropometrics, and other social factors were noted on paper records [17]. A case-cohort design was applied to reduce costs for digitalising information from the physical school health records for the full register. Hence, information on vaccinations and potential confounders was digitalised for all CMM cases in the background population and for a sub-cohort. The sub-cohort was selected as a 10% random sample of children within strata of sex and year of birth and for all children born the first day of every month. The digitalisation was aimed at investigating several outcomes and the stratifications were made due to specific hypotheses of sex differential effects and to account for the gradual phase out period of smallpox and BCG vaccination affecting these birth cohorts.

We excluded children who had no information about any childhood vaccines to ensure especially weak children were not included among individuals categorised as not vaccinated with the smallpox and BCG vaccines.

2.2. Smallpox and BCG vaccinations and covariates

The Copenhagen School Health Records Register contains information about childhood vaccines and potential confounders from the first school health examination where most parents or guardians participated [17]. The school health records were updated at each subsequent school health examination.

Based on an assumed causal structure, we adjusted for sex, number of siblings (as a proxy of social class), and family social class at school entry. Contraindication against vaccination was approached by excluding children who did not have any registered childhood vaccinations on their health record. We did not have information on potentially important confounders as ethnicity and skin colour. Information on immigration (indicated by information from the school health record on birth place) and birth weight (grouped in accordance with Jensen et al. [18]) were only available for subgroups and were adjusted for in sensitivity analyses.

2.3. Cutaneous malignant melanoma

From 1968, all Danish citizens were assigned a unique personal identification number enabling linkage between the school health records and national health registers. The CMM cases were identified in the Danish Cancer Registry [19] with the international classification of disease codes version 10 (ICD-10), C43 and all subdiagnoses. A validation study of the Danish cancer registry showed that the positive prediction value (PPV) and sensitivity for CMM diagnoses were 97% and 90%, respectively. The PPV varied between 87 and 100% for histologic subtypes of CMM (excluding "Melanoma not otherwise specified") [20].

2.4. Statistical analyses

We excluded individuals without a unique personal identification number, no health record, no vaccines received, no information about sex, family social class, and number of siblings, or who were not alive at their 18th birthday. We investigated whether individuals excluded due to missing values were different with regard to covariates from the study population. Among the included individuals, descriptive statistics between the potential confounders and BCG and/or smallpox vaccination were assessed with a prevalence ratio using a Poisson regression with robust standard errors [21]. The association between co-variables and CMM was assessed using the Cox proportional hazards model. CMM morphology and tumour location were classified according to definitions by Bay et al. [22].

For the main analysis, hazard ratios (HRs) were estimated with the Cox proportional hazards model with robust variance estimation to account for the case-cohort design that would otherwise underestimate the variance [23,24]. Age was the underlying time scale and individuals entered the study at their 18th birthday (no CMM cases had occurred earlier than 18 years of age allowing us to fix baseline variables at this age). CMM cases who were not part of the sub-cohort were included in the analysis one day before their diagnosis date as described by Prentice [23,24]. This is done to ensure that cases outside the sub-cohort do not contribute to the exposure distribution among controls at earlier risk sets and thus bias the estimate by using knowledge of the exposure distribution of future cases. Individuals were followed until diagnosed with CMM or censoring (emigration, death, unknown whereabouts by the Danish authorities, or 31 December 2014 [last available update of the Danish Cancer Registry]), whichever occurred first. Information on deaths and migrations was obtained from the Danish Civil Registration System [25]. All analyses were stratified by birth year and sex due to the sampling procedure. The model assumption of proportional hazards was tested using the Schoenfeld residual test using event time, which is applicable for case-cohort designs [26]. Our main analysis compared all combinations of smallpox and BCG vaccination as well as smallpox and/or BCG vaccination with not having received smallpox and BCG vaccinations. Furthermore, we analysed the association between the age of smallpox vaccination [No smallpox vaccination, smallpox

vaccination given at <1 year, smallpox vaccination given at ≥1 year] and the age of BCG vaccination [No BCG vaccination, BCG vaccination given at <1 year, BCG vaccination given at ≥1 year] as well as of combinations of these classification.

As in previous studies of the phase-out cohort [11,27], we explored the HRs by sex and birth cohorts [1965–68, 1969–72, 1973–76]. To describe the risk of CMM across birth cohorts, we standardised the follow up time (38 years of age across all birth cohorts) and reported the proportion of the full eligible background population diagnosed with CMM before the age of 38 years. In sensitivity analyses, we adjusted for immigration and birth weight in the subgroups with this information available.

3. Results

From the eligible background population of 46,239 individuals, 172 out of 188 CMM cases (91%) were analysed and 5090 out of 6015 individuals (85%) in the sub-cohort were analysed (Fig. 1). Among the sub-cohort, 411 were censored during follow-up (127 deaths, 273 emigrations, 11 unknown whereabouts by the Danish authorities). Individuals excluded due to missing information on vaccines differed compared with the study population (supplementary Table 1). The median age at end of follow-up was 44 years (maximum age was 49 years).

The smallpox and BCG vaccination coverage decreased over the birth years (Supplementary Fig. 1). Among the sub-cohort, 16.7% neither receive smallpox nor BCG vaccination; the proportion was 1.9%, 11.3% and 43.8% for the birth cohorts 1965–1968. 1969–1972, and 1973–1976, respectively. For the birth year 1965–68 (the early phase out period), the median age of smallpox vaccination was 4.3 years (The 25–75% distribution: 2.6–6.3 years) and the median age of BCG vaccination was 7.5 years (The 25–75% distribution: 6.5–8.1). Sex, number of siblings and family social class were not associated with having smallpox and/or BCG vaccination (Table 1).

Tumour characteristics of the 172 CMM cases are shown in Supplementary Table 2. The risk of CMM by the age of 38 years was 0.11% for the birth cohort 1965–68, 0.22% for the birth cohort 1969–72, and 0.27% for the birth cohort 1973–76 (Supplementary Table 3). Women compared with men had a higher risk of CMM (Table 1).

Smallpox and/or BCG vaccination was associated with an adjusted hazard ratio (aHR) of 1.29 (95% CI 0.72–2.31) for CMM compared with individuals who had not received these vaccines (Table 2).

Smallpox and BCG vaccination given at less than 1 year were respectively associated with aHR of 1.57 (95% CI 0.55–4.52) and 1.51 (95% CI 0.74–3.07) for CMM compared with having neither of the vaccines (Table 3).

The main estimates were similar between men and women (test of homogeneity by sex for smallpox and/or BCG vs. none of these vaccines, p-value = 0.42) (Table 2). Across the birth cohorts [1965–68, 1969–72, 1973–76], the aHR point estimate for BCG and/or smallpox for CMM increased from aHR of 0.54 (95% CI 0.13–2.28) to 1.12 (95% CI 0.48–2.66) and to 1.67 (95% CI 0.07–3.70) (test for trend, p-value = 0.25) (Supplementary Table 4). Adjusting for immigration (3.4% of the analysed individuals [cases and sub-cohort]) and birth weight had little impact on the estimates (Supplementary Tables 5 and 6).

4. Discussion

We found no protective effect of pre-school smallpox vaccination and early-school BCG vaccination against CMM among young Danes. Numbers were small, but the analysis of the subgroup of individuals vaccinated below 1 year of age did not indicate any protective effect either – however, due to uncertainty, the estimates may be compatible with a modest protective effect.

4.1. Strengths and limitations

Information about vaccination was collected at school health examinations and the outcome was registered prospectively using Danish registers, which has almost complete follow-up.

We adjusted for measured potential confounders and restricted the cohort to individuals who received one or more childhood vaccines, thus preventing us from including children in the smallpox and BCG unvaccinated group, who would have had no chance of receiving smallpox and BCG vaccination. Eczema was a contraindication for smallpox vaccination [11], but a meta-analysis of the evidence did not suggest that eczema is associated with an increased risk of CMM [28].

The main limitation of our study relates to potential unmeasured confounding (affecting the chance of being smallpox and BCG vaccinated and the risk of developing CMM). Exposures such as UV, skin type, and number of nevi are strong predictors of CMM [29], but are unlikely to affect vaccination status and thereby cannot be regarded as confounders. One's country of origin, which may affect the likelihood of vaccination and affect CMM through skin type, was not adjusted for in our main analysis. However, the sensitivity analysis of a proxy of skin type, immigration based on birth place, did not affect the results of the analysis. The risk of CMM increased by each birth cohort, but since we stratified for each birth year in our analyses, our results should not be confounded by general time trends such as changes in registration and diagnostics, sunscreen use, fashion changes, sun bathing habits and vacation destinations [29]. However, our analysis across birth cohorts had a high level of uncertainty but may suggest different associations for individuals born 1965–68 and 1973–76, with a more beneficial association of vaccination for the birth cohort born 1965–68. If true, this could be due to differential unmeasured confounding and/or differential effect modification.

We did not address potential effect modification by other vaccines or major childhood infections, which has been suggested to influence the development of CMM [30,31]. CMM subtypes develop through various distinct stages of transformations [32]. If smallpox and BCG vaccination only prevents some subtypes, including all subtypes of CMM could blur the association. We had limited statistical power due to the few CMM cases in our population and the wide confidence intervals are compatible with both preventive and harmful effects of smallpox and/or BCG vaccinations on CMM at any age.

4.2. Comparison with other studies

The evidence suggesting that early smallpox and BCG vaccination prevents CMM comes from a multi-centre case-control study by the Febrile Infections and Melanoma (FEBIM) working group in six European countries and Israel, with 603 CMM patients and 627 controls. This study showed that being smallpox and BCG vaccinate was associated with an OR of 0.44 (95% CI 0.26–0.72) for CMM [6]. Based on an hypothesis from 1986 by Rosenthal [33], neonatal BCG vaccination destroys embryonic remnants and prevents leukaemia and other cancers throughout life. Most individuals in the FEBIM study were BCG vaccinated before the age of 1 year, and interestingly data from centres in France and Italy, where BCG vaccination supposedly was given after 1 year of age, showed no beneficial association of BCG vaccination on CMM [30]. In our study, most children were BCG vaccinated at early-school ages (5–7 years). However, those few who had been BCG vaccinated below 1 year did not tend to have a lower risk of

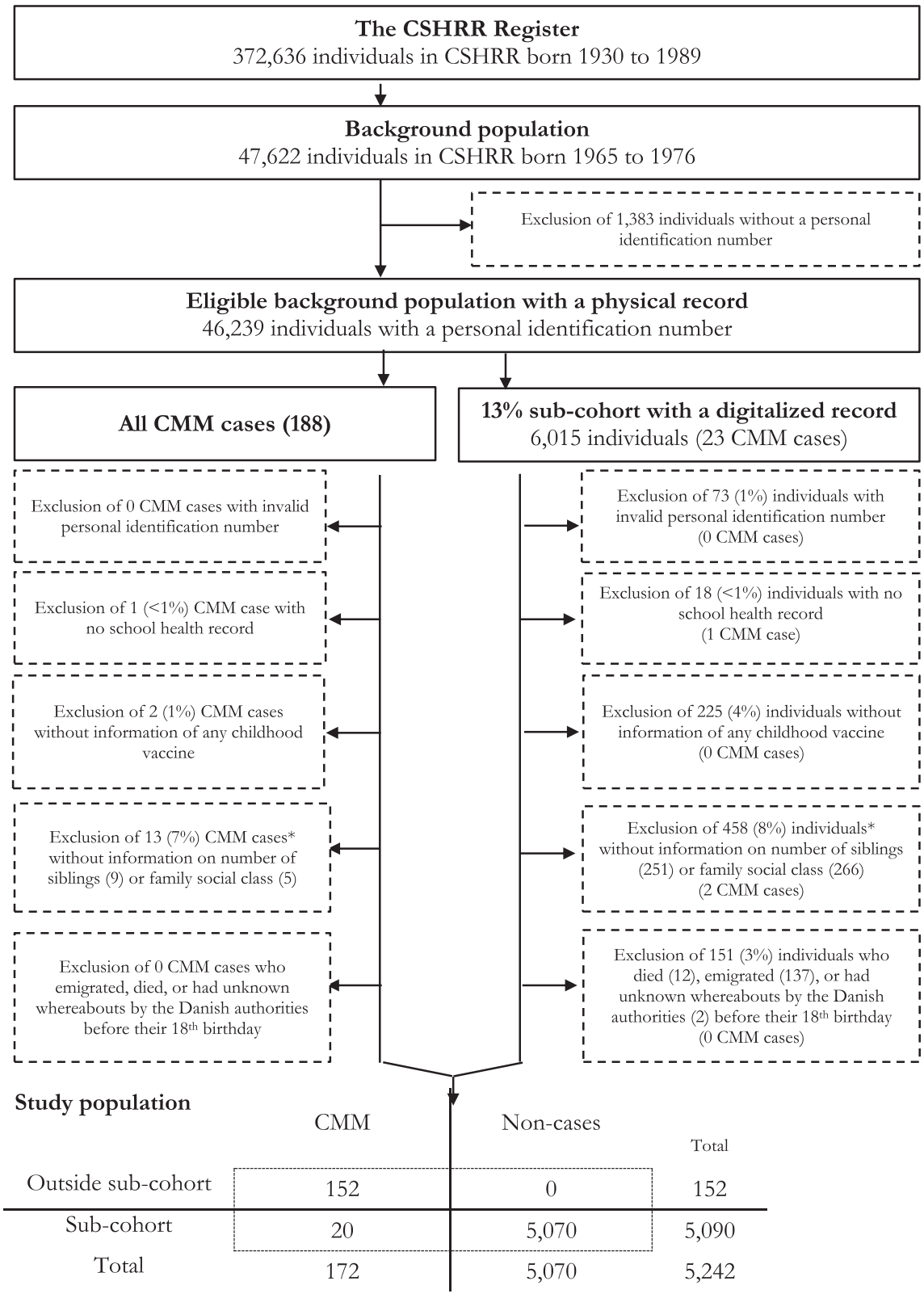

Fig. 1. Flow chart of the study population.

developing CMM compared with individuals who did not receive the BCG or smallpox vaccinations.

Other potential reasons for a difference between the FEBIM study and ours could relate to site and population differences. It has previously been argued that an effect of BCG vaccination on childhood cancers seemed to correlate with the efficacy of BCG vaccination on tuberculosis due to differences in environmental exposure to mycobacteria [34]. However, this would be unlikely to explain the difference in findings between our study and the FEBIM study in which most sites were also in Europe [30]. Our

Table 1
The association between background factors and vaccination status and the association between background factors and cutaneous malignant melanoma.

	Associations with BCG and smallpox vaccination			Associations with CMM		
	Sub-cohort (n = 5090)			Sub-cohort and cases (n = 5242)		
	BCG and Smallpox vaccination at last school health examination		Prevalence ratio (95% CI) for vaccinated with either BCG and/or smallpox*	All CMM	Sub-cohort person years	Crude hazard ratio (95% CI) for CMM**
	None 17% (n = 862)	Either or 83% (n = 4228)		n = 172	130,003	
Sex						
Women	17% (423)	83% (2119)	1 (ref)	119	64,846	1 (ref)
Men	17% (439)	83% (2109)	0.99 (0.97–1.01)	53	65,157	0.45 (0.32–0.62)
Number of siblings						
None	19% (148)	81% (618)	1 (ref)	25	19,280	1 (ref)
One	16% (420)	84% (2178)	1.01 (0.98–1.04)	97	66,032	1.12 (0.71–1.75)
Two or more	17% (294)	83% (1432)	0.98 (0.95–1.01)	50	44,690	0.88 (0.54–1.43)
Family occupational social class						
I	24% (87)	76% (280)	1 (ref)	9	8784	1 (ref)
II	15% (96)	85% (530)	1.05 (1.00–1.11)	19	15,375	1.26 (0.57–2.80)
III	16% (140)	84% (713)	1.02 (0.96–1.07)	33	21,832	1.54 (0.73–3.26)
IV	16% (283)	84% (1509)	1.02 (0.97–1.07)	75	46,351	1.64 (0.81–3.32)
V	16% (186)	84% (949)	1.00 (0.94–1.05)	30	29,746	1.07 (0.50–2.28)
Unclassified	22% (70)	78% (247)	0.96 (0.90–1.03)	6	7915	0.76 (0.27–2.17)

Abbreviations: BCG = Bacillus Calmette Guérin.
CI = confidence interval.
CMM = cutaneous malignant melanoma.
* Prevalence ratios are calculated with Poisson regression and adjusted for birth year and sex.
** Hazard ratios are calculated using the Cox regression with age as underlying time variable, and delayed entrance at 18 years of age. The analyses were stratified for year of birth and sex.

Table 2
The association between BCG and smallpox vaccination status and cutaneous malignant melanoma, overall and stratified by sex.

Among all 5242 individuals (5090 in the sub-cohort)	CMM n = 172 (20 in sub-cohort)	Sub-cohort person years 130,003	Unadjusted hazard ratio* (95% CI)	Adjusted hazard ratio** (95% CI)
Smallpox/BCG vaccinated				
−/−	19 (3)	18,986	1 (ref)	1 (ref)
+/−	11 (2)	10,085	1.23 (0.53–2.83)	1.23 (0.53–2.86)
−/+	39 (3)	31,815	1.15 (0.62–2.14)	1.13 (0.61–2.09)
+/+	103 (12)	69,117	1.85 (0.93–3.66)	1.75 (0.87–3.48)
Either or	153 (17)	111,017	1.34 (0.75–2.38)	1.29 (0.72–2.31)
Test of proportional hazards (Either or vs none), p-value			0.94	0.17
Women, Smallpox/BCG vaccinated				
−/−	11 (1)	9295	1 (ref)	1 (ref)
+/−	8 (2)	5100	1.46 (0.50–4.24)	1.45 (0.49–4.28)
−/+	26 (1)	14,916	1.42 (0.63–3.19)	1.38 (0.61–3.11)
+/+	74 (6)	35,535	2.17 (0.86–5.43)	2.02 (0.79–5.13)
Either or	108 (9)	55,551	1.62 (0.75–3.51)	1.55 (0.71–3.39)
Men, Smallpox/BCG vaccinated				
−/−	8 (2)	9691	1 (ref)	1 (ref)
+/−	3 (0)	4985	0.92 (0.23–3.71)	0.93 (0.23–3.77)
−/+	13 (2)	16,899	0.82 (0.31–2.13)	0.81 (0.31–2.12)
+/+	29 (6)	33,582	1.45 (0.53–3.91)	1.40 (0.52–3.81)
Either or	53 (10)	55,466	0.98 (0.42–2.29)	0.96 (0.41–2.27)
Test of interaction for "Either or" vs none by sex, p-value			0.39	0.42

Abbreviations: BCG = Bacillus Calmette Guérin.
CI = Confidence interval.
CMM = cutaneous malignant melanoma.
* Hazard ratios are calculated using a Cox regression with age as underlying time variable, delayed entrance at age 18 years, and stratified for sex and year of birth.
** Hazard ratios are calculated using a Cox regression with age as underlying time variable, delayed entrance at age 18 years, adjusted for family social class and number of siblings and stratified for sex and year of birth.

study followed the study population until midlife whereas the median age of the study population reported in the FEBIM study was 57 years for cases and 55 years for controls [6] The FEBIM study reported the effect of both smallpox and BCG vaccination vs. none by age group. Contrary to our findings, they found that individuals younger than 50 years tended to have an even stronger effect compared with individuals 50 years or above (respective OR: 0.27 (0.09–0.80) and 0.48 (0.26–0.86)) [6].

One aspect for a potential differential effect by birth cohorts favouring the oldest birth cohorts relates to revaccination.

Table 3
The association between BCG and smallpox vaccination <1 year of age and cutaneous malignant melanoma.

Among all 5242 individuals (5090 in the sub-cohort)	Malignant melanoma n = 172 (20 in sub-cohort)	Sub-cohort person years 130,003	Unadjusted hazard ratio* (95% CI)	Adjusted hazard ratio** (95% CI)
Age at smallpox vaccination				
No smallpox vaccination	58 (6)	50,801	1 (ref)	1 (ref)
Smallpox vaccination <1 year	4 (0)	2367	1.68 (0.59–4.82)	1.57 (0.55–4.52)
Smallpox vaccination ≥1 year	110 (14)	76,835	1.55 (0.98–2.44)	1.47 (0.92–2.33)
Age at BCG vaccination				
No BCG vaccination	30 (5)	29,070	1 (ref)	1 (ref)
BCG vaccination < 1 year	12 (0)	7160	1.65 (0.84–3.34)	1.51 (0.74–3.07)
BCG vaccination ≥ 1 year	130 (15)	93,777	1.33 (0.86–2.07)	1.24 (0.80–1.94)
			Unadjusted hazard ratio* (95% CI)	Adjusted hazard ratio*** (95% CI)
Age at smallpox and BCG vaccination analysed together				
None	19 (3)	18,986	1 (ref)	1 (ref)
Both smallpox and BCG vaccination <1 year	2 (0)	587	3.76 (0.79–17.94)	3.70 (0.77–17.90)
Either smallpox or BCG vaccination <1 year	12 (0)	8350	1.41 (0.63–3.14)	1.36 (0.60–3.07)
Remaining individuals with either a smallpox or BCG vaccination ≥1 year	139 (17)	103,081	1.33 (0.74–2.37)	1.28 (0.71–2.29)

There were 178 individuals with smallpox vaccination for whom we did not have their age at the vaccination registered in the school health records. They were classified with smallpox vaccination at ≥1 year.
There were 109 individuals with BCG vaccination for whom we did not have their age at vaccination registered in the school health records. They were classified with BCG vaccination at ≥1 year.
Abbreviations: BCG = Bacillus Calmette-Guérin.
CI = Confidence interval.
CMM = cutaneous malignant melanoma.
* Hazard ratios are calculated using a Cox regression with age as underlying time variable, delayed entrance at age 18 years, and stratified for sex and year of birth.
** Hazard ratios are calculated using a Cox regression with age as underlying time variable, delayed entrance at age 18 years, adjusted for family social class and number of siblings, the respective age of the other vaccine (smallpox/BCG vaccination) and stratified for sex and year of birth.
*** Hazard ratios are calculated using a Cox regression with age as underlying time variable, delayed entrance at age 18 years, adjusted for family social class and number of siblings and stratified for sex and year of birth.

Revaccination with live vaccines, including smallpox and BCG vaccination, has been associated with additional beneficial non-specific effects, such as reducing morbidity and mortality [35]. WHO recommended re-vaccination with the smallpox vaccine every 5–10 years in non-endemic countries [36]; hence, in the previous study the smallpox vaccinated individuals may have benefitted from a boosting effect which the Danish study population did not because they received their vaccination just before smallpox vaccinations were stopped.

4.3. Conclusion and perspectives

We did not observe a strong preventive effect on the risk of CMM by smallpox and BCG vaccination among young Danish adults. Due to a limited number of CMM cases and the corresponding uncertainty, our results are compatible with hypotheses of a modest preventive effect, a null effect or a harmful effect. Though the smallpox vaccine is phased out globally and the BCG vaccine is phased out in many high income-countries, understanding if live vaccines confer additional protection against non-targeted diseases is of etiological interest and potentially of public health value. In addition to the live smallpox and BCG vaccines, the live yellow fewer vaccine has been suggested to reduce CMM [37]. If live vaccines alter the long-term risk for unrelated diseases, we may utilize existing and inexpensive vaccines for a much larger benefit. We therefore urge other research groups to investigate the association in other settings and possibly address the influence of age at vaccination, re-vaccination, and of other vaccines.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: At present, KDM works at Pfizer ApS (Denmark), however, her work in relation to this article was conducted while KDM worked at the Center for Clinical Research and Prevention.

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Author contributions

PA had the conception of the work; all authors contributed to the design of the work; AR, JLB, and SS acquired the data; AR analysed the data; all authors interpreted the data. All authors were involved in drafting and revising the manuscript. All authors have approved the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2019.09.023.

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