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Research article

Determinants of Bacille Calmette-Guérin scarification in Danish children

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ABSTRACT

Background: Studies in low-income countries have shown that among Bacille Calmette-Guérin (BCG) vaccinated children, those who develop a BCG-scar have significantly better survival than those who do not develop a scar. In a Danish multicenter randomized clinical trial we assessed determinants for developing a BCG-scar and for BCG scar size following neonatal BCG vaccination.

Methods: At three Danish hospitals, newborns were randomized 1:1 to BCG vaccination or no BCG vaccination. The infants were invited for a clinical examination at the ages of 3 and 13 months. At 13 months, the scar site was inspected and scar size measured. We investigated three groups of determinants; external, parental, and individual-level determinants on relative scar prevalence and differences in median scar sizes.

Results: Among 2118 BCG vaccinated infants, 2039 (96 %) were examined at 13 months; 1857 of these (91 %) had developed a BCG-scar. Compared with Copenhagen University Hospital, Hvidovre (85 %), Copenhagen University Hospital, Rigshospitalet had a scar prevalence of 95 % (adjusted Prevalence ratio (aPR) = 1.24 [CI 95 %: 1.18 to 1.30]); it was 93 % at Kolding Hospital (aPR 1.27 [CI 95 %: 1.19 to 1.35]). Increasing vaccine experience was positively associated with developing a scar and with scar size.

Conclusion: Across multiple potential determinants of BCG scarring and size, logistical factors dominated. The results support that injection technique is an important determinant of developing a scar. Given the strong link between having a BCG scar and subsequent health, improved BCG vaccination technique could play a major role for child health.

1. Introduction

The Bacille Calmette-Guérin (BCG) vaccine provides protection against tuberculosis (TB) and other mycobacterial infections, and is one of the most used vaccines globally [1]. The vaccine is given routinely in more than 100 countries to newborns as part of the childhood vaccination program. The BCG vaccine contains live attenuated *Mycobacterium bovis* and following intradermal injection the BCG vaccine elicits a local immune response. This response most often results in an ulcer that heals over weeks and leaves a flat permanent scar at the injection site [2]. The skin lesion has been shown to follow simple dose-response functions; if the dose of the vaccine is halved, the size of the scar will decrease by

approximately 1 mm [3]. Also, vaccines with low viability result in smaller scars compared to vaccines with high viability [3].

Whether the development of a BCG scar is associated with TB efficacy is debated [2, 4, 5], but BCG scar size and tuberculin skin test (TST) response seem to correlate [3, 4, 6, 7, 8].

Observational data from a growing number of studies conducted in low-income countries have suggested that the BCG vaccine has strong beneficial non-specific effects (NSEs) beyond the protection against tuberculosis [9, 10, 11, 12, 13]. Recently, these findings were supported in three randomized trials from Guinea-Bissau in West Africa, showing that BCG vaccination at birth to low-birth-weight neonates reduced neonatal mortality by 38 % (95 % CI: 17 %–54 %) [14]. The reduction in

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neonatal mortality was due to fewer cases of septicemia and pneumonia [14, 15, 16]. Furthermore, among BCG-vaccinated children, those who develop a scar, have 39 % (26–49 %) lower mortality compared with those who do not develop a scar [17].

Understanding the factors, which determine whether BCG vaccination leads to development of a scar, is important for understanding the link between BCG vaccination and specific effects as well as NSEs. We know from observational data from Guinea-Bissau that scar prevalence may differ by vaccinator, whether the vaccine is injected correctly, the fieldworker who conducted the scar reading, sex, season, co-administration of other vaccinations, place of delivery, region, and maternal ethnicity [18], but this has not been tested in a high-income setting.

Within The Danish Calmette Study, a randomized clinical trial, we investigated determinants for developing a BCG scar and for the scar size at 13 months of age among the children allocated to neonatal BCG. We analyzed three groups of determinants: *External determinants* such as study site, vaccine batch and season of the year [3, 19]. *Parental determinants* such as maternal BCG vaccination, parental smoking, and younger children at home and finally, *individual factors* such as gestational age and anthropometric measures.

2. Methods

2.1. Study design

The Danish Calmette Study was a randomized, prospective, single-blinded, clinical trial conducted at three Danish hospitals; Copenhagen University Hospital, Hvidovre, Copenhagen University Hospital, Rigshospitalet, and Kolding Hospital as described in detail elsewhere [20]. A total of 4262 infants born at the three Danish hospitals were randomized after birth with 1:1 allocation to BCG vaccination or no BCG vaccination. The primary outcomes were hospitalization and development of atopic dermatitis during the first year of life. Secondary outcomes were morbidity including infectious and allergic disease [21, 22, 23, 24, 25]. Children randomized to BCG vaccination received an intradermal BCG vaccine (Danish strain 1331, Statens Serum Institut) in the standard dose of 0.05 ml in the left deltoid region. Two different vaccine batches were used; *batch 112032A* and *batch 111046B*. Batch 112032A was used from October 2012 until April 2013. Batch 111046B was used from March 2013 until November 2013. The ratios between Batch 112032A and Batch 111046B were overall equal between the three sites. On Copenhagen University Hospital, Hvidovre the ratio was 1.21. On Copenhagen University Hospital, Rigshospitalet it was 1.36 and on Kolding Hospital it was 1.32. Chi-2 test for difference; $p = 0.52$.

All study staff involved in randomization were specially trained to administer the vaccine in accordance with the guidelines (Figure 1). In The Danish Calmette Study, 34 trained staff members were administering the BCG vaccine, but two staff members were responsible for most of the vaccinations.

2.2. Scar prevalence and size

At 3 and 13 month of age, the parents were invited for a clinical examination of their child. Prior to the clinical examinations the parents were asked to cover the vaccination site in both vaccinated and non-vaccinated children, thereby keeping the examiners blinded to the randomization during the examination. At the end of the 13-month clinical examination, the vaccination site was inspected, and it was registered whether the child had developed a visible BCG scar (yes/no). In case of a scar, the scar size was measured as two perpendicular diameters (mm) with a transparent ruler (Tuberculin PPD RT 21 SSI), and a mean value was calculated. BCG scars were measured with a precision of 0.1 cm (Figure 1).

In a previous paper based on the Calmette trial [26], we reported that 1794 children developed a scar after BCG vaccination and a scar



Figure 1. The skin reaction after BCG vaccination. Upper: Following a correctly applied intradermal BCG vaccination a visible white wheal appears at the injection site. Lower: Measuring a BCG scar (mm) at the end of the 13-month clinical examination.

frequency of 84.7 %. It was subsequently discovered that a proportion of the scar assessment data had not been entered, and figured as a “0” in the database, which was erroneously interpreted as “No scar”. The database was updated with the missing scar assessment data for the present study.

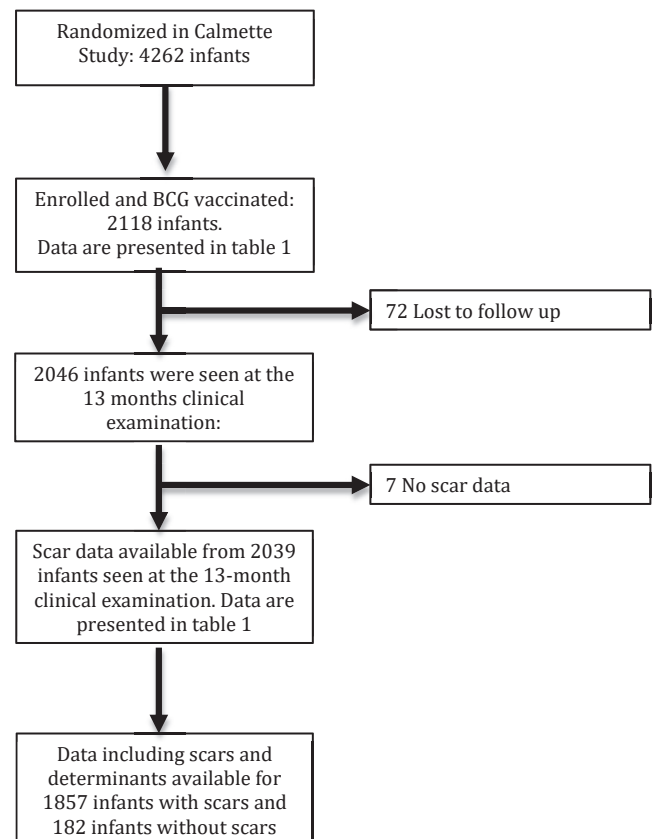


Figure 2. Trial profile of infants included into the study.

Table 1. Baseline measurements and scar size after neonatal BCG vaccination at 13 month among the 1857 BCG-vaccinated children in the Danish Calmette Study who developed a scar. Analysed by bootstrap.

Determinants			Median (IQR)	Crude			Adjusted*			
			P50 (P25–P75)	Difference in median/cm.	95 % CI	p-value	Difference in median/cm.	95 % CI	**p-value	
External determinants	Study site	Hvidovre hospital	0.45 (0.35–0.60)	0 (ref.)			0 (ref.)			
		Rigshospitalet	0.50 (0.40–0.60)	0.05	(0.01–0.09)	<0.001	0.01	(-0.12 to 0.14)	0.84	
		Kolding hospital	0.40 (0.33–0.55)	-0.05	(-0.09 to -0.01)		0.05	(-0.11 to -0.20)		
	Vaccine batch	1. "112032A"	0.45 (0.35–0.55)	0 (ref.)			0 (ref.)			
		2. "111046B"	0.50 (0.35–0.60)	0.05	(0.03–0.07)	<0.001	0.03	(-0.02 to 0.08)	0.20	
	Vaccinator experience	0 to 9	0.45 (0.35–0.58)	0 (ref.)			0 (ref.)			
		10 to 49	0.44 (0.35–0.55)	0.00	(-0.04 to 0.04)	<0.001	-0.02	(-0.06 to 0.02)	<0.001	
50 to 99		0.45 (0.35–0.55)	0.00	(-0.05 to 0.05)		0.01	(-0.04 to 0.07)			
≥ 100		0.50 (0.40–0.60)	0.05	(0.02–0.08)		0.06	(-0.02 to 0.14)			
Parental determinants	Maternal age at delivery	<27 years	0.45 (0.35–0.55)	0 (ref.)			0 (ref.)			
		27–32 years	0.45 (0.35–0.55)	0.00	(-0.04 to 0.04)	1.00	-0.02	(-0.04 to 0.02)	0.38	
		>32 years	0.45 (0.35–0.60)	0.00	(-0.05 to 0.05)		-0.00	(-0.03 to 0.03)		
	Maternal BCG	Yes	0.45 (0.35–0.59)	0 (ref.)			0 (ref.)			
		No	0.45 (0.35–0.55)	-0.03	(-0.07 to 0.02)	0.28	-0.01	(-0.04 to 0.02)	0.36	
	Parental smoking ^b	Yes	0.45 (0.35–0.58)	0 (ref.)			0 (ref.)			
		No	0.45 (0.35–0.55)	0.00	(-0.04 to 0.04)	1.00	0.01	(-0.02 to 0.04)	0.50	
	Atopic disposition ^c	Yes	0.45 (0.35–0.55)	0 (ref.)			0 (ref.)			
		No	0.45 (0.35–0.58)	0.00	(-0.03 to 0.03)	1.00	0.02	(-0.00 to 0.04)	0.12	
	Other children <4 years of age in the home ^d	Yes	0.45 (0.35–0.55)	0 (ref.)			0 (ref.)			
		No	0.45 (0.35–0.58)	-0.00	(-0.03 to 0.03)	1.00	-0.00	(-0.03 to 0.02)	0.78	
	Individual determinants	Sex	Boys	0.48 (0.35–0.60)	0 (ref.)			0 (ref.)		
			Girls	0.45 (0.35–0.55)	-0.03	(-0.07 to 0.02)	0.30	-0.01	(-0.03 to 0.01)	0.26
Prematurity		<37 weeks	0.48 (0.33–0.60)	0 (ref.)			0 (ref.)			
		≥37 weeks	0.45 (0.35–0.55)	-0.03	(-0.12 to 0.07)	0.61	-0.03	(-0.11 to 0.05)	0.44	
Birth weight		<2500 g	0.45 (0.31–0.60)	0 (ref.)			0 (ref.)			
		≥2500 g	0.45 (0.35–0.55)	0.00	(-0.09 to 0.09)	1.00	0.02	(-0.06 to 0.11)	0.58	
Caesarean section		Yes	0.45 (0.35–0.55)	0 (ref.)			0 (ref.)			
		No	0.45 (0.35–0.58)	0.00	(-0.04 to 0.04)	1.00	0.01	(-0.02 to 0.04)	0.42	
Child age at BCG vaccination		Day 0–1	0.45 (0.35–0.55)	0 (ref.)			0 (ref.)			
		Day 2–7	0.45 (0.35–0.60)	0.00	(-0.03 to 0.03)	1.00	-0.00	(-0.03 to 0.02)	0.63	
Ethnicity ^e		Yes	0.45 (0.35–0.55)	0 (ref.)			0 (ref.)			
		No	0.45 (0.35–0.58)	0.00	(-0.04 to 0.04)	1.00	0.01	(-0.01 to 0.03)	0.41	
Exclusively breastfeed ^a		Yes	0.45 (0.35–0.55)	0 (ref.)			0 (ref.)			
		No	0.48 (0.35–0.60)	0.03	(-0.01 to 0.06)	0.18	-0.00	(-0.02 to 0.02)	0.77	
Mid-upper arm circumference ^f		1	0.40 (0.30–0.55)	0 (ref.)			0 (ref.)			
		2	0.45 (0.35–0.55)	0.05	(0.02 to 0.08)	<0.001	0.03	(-0.00 to 0.06)	0.27	
	3	0.45 (0.35–0.60)	0.05	(0.00–1.00)		0.02	(-0.01 to 0.06)			
	4	0.50 (0.38–0.60)	0.1	(0.07–0.13)		0.03	(-0.01 to 0.07)			
Δweight ^g	1	0.43 (0.33–0.55)	0 (ref.)			0 (ref.)				
	2	0.45 (0.35–0.55)	0.03	(-0.02 to 0.07)	<0.001	0.01	(-0.01 to 0.04)	0.15		
	3	0.48 (0.35–0.60)	0.05	(-0.02 to 0.11)		0.03	(0.00–0.06)			
	4	0.50 (0.38–0.60)	0.08	(0.03–0.12)		0.04	(0.01–0.08)			

* Analysis adjusted for study site, vaccine batch, vaccine experience, maternal age at delivery, maternal BCG vaccination, parental smoking, atopic disposition, other children <4 years of age in the home, sex, prematurity, birth weight, caesarean section, child age at BCG vaccination, ethnicity, exclusively breastfeed, mid-upper arm circumference and Δweight.

** Wald test.

^a Exclusively breastfeed: breastfeeding in the first 3 months of life.

^b Smoking: all types of smoking by parents included as well as inside and outside smoking in the first 12 month of life.

^c Atopic disposition: at least one first degree relative with atopic disease (physician-diagnosed atopic eczema, asthma or asthmatic bronchitis, allergic rhino conjunctivitis or food allergy).

^d Any other children <4 years of age in the house at least 5 days a week.

^e Danish ethnicity or other ethnicity.

^f Mid upper arm circumference (MUAC) is divided in quartiles: 1) 12.1 cm–14.9 cm, 2) 14.9 cm–15.6 cm, 3) 15.6 cm–16.5 cm and 4) 16.5 cm–20 cm.

^g Δweight: gain in weight from birth to the 13-month clinical examination; divided in quantiles: 1) 3415 g–6042 g, 2) 6044 g–6688 g, 3) 6689 g–7460 g, 4) 7464 g–10910 g.

Table 2. The association between month of vaccination and BCG-scar prevalence 13 month after neonatal BCG vaccination among 2039 BCG-vaccinated in the Danish Calmette Study.

Determinants		(+) BCG, N = 2118	Data for scars		Crude (N = 2039)		Adjusted* (N = 1968)		
		Number of Infants (%)	Number of infants with scar information (2039), (% with information)	No scar	Scar (%)	Prevalence ratio (95% CI)	p-value	Prevalence ratio (95% CI)	p-value
Month of vaccination	January	190 (9)	188 (99)	13	175 (93)	1 (ref.)		1 (ref.)	
	February	166 (8)	162 (98)	11	151 (93)	1.00 (0.95–1.06)	0.96	1.01 (0.95–1.08)	0.68
	March	179 (8)	174 (97)	5	169 (97)	1.04 (1.00–1.09)	0.07	1.02 (0.97–1.07)	0.43
	April	158 (7)	152 (96)	7	145 (95)	1.02 (0.97–1.08)	0.92	0.94 (0.87–1.00)	0.07
	May	158 (7)	156 (99)	5	151 (97)	1.04 (0.99–1.09)	0.11	0.91 (0.84–0.98)	0.01
	June	165 (8)	162 (98)	7	155 (96)	1.03 (0.98–1.08)	0.29	0.91 (0.84–0.99)	0.02
	July	177 (8)	171 (97)	25	146 (85)	0.92 (0.85–0.99)	0.02	0.81 (0.74–0.90)	0.00
	August	178 (8)	170 (96)	15	155 (91)	0.98 (0.92–1.04)	0.51	0.87 (0.80–0.94)	0.00
	September	158 (7)	156 (99)	7	149 (96)	1.03 (0.97–1.08)	0.33	0.89 (0.82–0.96)	0.01
	October	196 (9)	186 (95)	31	155 (83)	0.90 (0.83–0.97)	0.00	0.82 (0.75–0.90)	0.00
	November	227 (11)	206 (91)	41	165 (80)	0.86 (0.80–0.93)	0.00	0.87 (0.81–0.95)	0.00
	December	166 (8)	156 (94)	15	141 (90)	0.97 (0.91–1.04)	0.37	0.98 (0.92–1.04)	0.44

* Analysis adjusted for study site, vaccine batch, vaccine experience, maternal age at delivery, maternal BCG vaccination, parental smoking, atopic disposition, other children <4 years of age in the home, sex, prematurity, birth weight, caesarean section, child age at BCG vaccination, ethnicity, exclusively breastfed, mid-upper arm circumference and Δweight.

2.3. Classification of determinants

The following determinants were included in the analyses: External determinants were *study site* (Hvidovre hospital, Rigshospitalet, Kolding hospital), *vaccine batch* (112032A, 111046B), *vaccinator* (1–34), *month of BCG vaccination*, *vaccinators' BCG vaccinator experience* (0–9; 10–49; 50–99; ≥100 BCG vaccinations given during the duration of the trial). Parental determinants were *maternal age at delivery* (<27 years, 27–32 years, >32 years), *maternal BCG vaccination*, *parental smoking*, *atopic disposition* and *other children < 4 years of age in the home*. Individual determinants were *sex*, *prematurity* (<37 weeks, ≥37 weeks), *birth weight* (<2500 g, ≥ 2500 g), *caesarean section*, *child age at BCG vaccination* (day 0–1, day 2–7), *ethnicity* (Danish ethnicity, other ethnicity), *exclusively breastfed in the first 3 months of life*, *mid-upper arm circumference of left arm (MUAC)* (measured at the 13-month clinical examination; quartiles), *weight gain from birth to the 13-month clinical examination* (quartiles).

2.4. Statistical analysis

The study population consisted of infants vaccinated as part of The Danish Calmette Study who had been allocated to BCG vaccine at birth and who participated in the 13-month clinical examination (Figure 2). Prevalence ratios (PR) of BCG-scar development were estimated using Poisson regression with robust standard errors providing prevalence rate ratios [27]. Differences in BCG-scar size were estimated by median-based quantile regression (95 % confidence intervals are shown bootstrapped (Table 1) among children who had developed a BCG scar [28]. Estimates are presented crude and mutually adjusted. All adjusted analyses were also adjusted for month of vaccination (Tables 2 and 3). BCG-scar size by vaccinator and study site were visualised in a dot plot.

The statistical analyses were conducted using Stata 13 (StataCorp, Texas, USA) and the visualisation was made using R (Foundation for Statistical Computing, Vienna, Austria).

2.5. Ethics

The Danish Data Protection Board (J.nr. 2009-41-4141), The Danish and the European Medicines Agencies (ref. no. EuDract 2010-021979-85), and The National Committee on Health Research Ethics in Denmark (H-3-2010-087) approved The Danish Calmette Study. The trial was registered at clinicaltrials.org (ref. no. EudraCT2010-021979-85).

3. Results

Between September 2012 and November 2015, 4262 infants were included in The Danish Calmette Study. Of these, 2118 (49.7 %) were BCG vaccinated and 2039 (96 %) were seen at the 13-months examination [20]. Scars were observed in 1857 infants, leading to an overall BCG vaccination scar prevalence of 91 % among children examined at 13 months of age (Figure 2).

3.1. Determinants of BCG scar prevalence

3.1.1. External determinants

Scar prevalence after BCG vaccination varied by study site. The prevalence was 85 % at Copenhagen University Hospital, Hvidovre, compared with 95 % at Copenhagen University Hospital, Rigshospitalet, (crude PR (cPR) 1.11 [95 % CI: 1.08 to 1.16]; adjusted PR (aPR) 1.24 [95 % CI: 1.18 to 1.30]) and 93 % at Kolding hospital (cPR 1.08 [95 % CI: 1.04 to 1.13]; aPR 1.27 [95 % CI: 1.19 to 1.35]). Vaccine batch 2 "111046B" resulted in a lower scar prevalence compared to vaccine batch 1 "112032A" (cPR 0.95 [95 % CI 0.92 to 0.98]; aPR 0.90 [95 % CI: 0.85 to 0.96]). Vaccinator experience with more than 100 administrated doses was associated with a higher prevalence (cPR 1.06 [95 % CI 1.01 to 1.10]; aPR 1.16 [95 % CI: 1.09 to 1.22]). At the same time vaccinator experience with 10–49 doses and 50 to 99 doses were associated with a lower prevalence, but only in the adjusted analyses (Table 4). Month of vaccination was also associated with the development of a scar; infants vaccinated from May until November were less likely to develop a scar compared with infants vaccinated in the remaining of the year (Table 2).

3.1.2. Parental related determinants

Not having an atopic disposition was associated with increased scar prevalence, but only in the crude analysis (cPR 1.03 [95 % CI: 1.00 to 1.06]; aPR 1.02 [95 % CI: 0.99 to 1.05]) (Table 4). None of the other parental related factors were associated with scar prevalence.

3.1.3. Individual related determinants

Among individual determinants on scar prevalence, term born infants and children with birth weight ≥2500 g were less likely to develop a scar in the crude analysis (cPR 0.94 [95 % CI: 0.90 to 0.98]; cPR 0.92 [95 % CI: 0.89 to 0.96]) but the associations were weakened slightly when adjusted for other co-variables (aPR 0.96 [95 % CI: 0.91 to 1.01]; aPR 0.98

Table 3. The association between month of vaccination and BCG-scar size 13 month after neonatal BCG vaccination among the 1857 children in the Danish Calmette Study who developed a scar.

Determinants		Median (IQR)	Crude (N = 2039)			Adjusted* (N = 1793)		
		P50 (P25–P75)	Difference in median/cm.	95 % CI	p-value	Difference in median/cm.	95 % CI	p-value
Month of vaccination	January	0.50 (0.40–0.60)	0 (ref.)			0 (ref.)		
	February	0.50 (0.38–0.63)	0	(-0.05 to 0.05)	1.00	0.01	(-0.04 to 0.05)	0.71
	March	0.45 (0.35–0.55)	-0.05	(-0.09 to -0.01)	0.03	-0.06	(-0.10 to -0.01)	0.01
	April	0.45 (0.35–0.55)	-0.05	(-0.10 to 0.00)	0.04	-0.06	(-0.11 to 0.00)	0.04
	May	0.45 (0.35–0.58)	-0.05	(-0.10 to 0.00)	0.03	-0.03	(-0.09 to 0.02)	0.26
	June	0.45 (0.35–0.55)	-0.05	(-0.10 to 0.00)	0.03	-0.04	(-0.10 to 0.01)	0.13
	July	0.41 (0.33–0.55)	-0.08	(-0.12 to -0.03)	0.00	-0.06	(-0.12 to 0.00)	0.06
	August	0.45 (0.35–0.55)	-0.05	(-0.10 to 0.00)	0.03	-0.04	(-0.10 to 0.01)	0.13
	September	0.45 (0.35–0.55)	-0.05	(-0.10 to 0.00)	0.03	-0.06	(-0.11 to 0.00)	0.05
	October	0.45 (0.35–0.55)	-0.05	(-0.10 to 0.00)	0.03	-0.05	(-0.11 to 0.00)	0.04
	November	0.40 (0.30–0.55)	-0.1	(-0.14 to -0.06)	0.00	-0.09	(-0.13 to -0.04)	0.00
	December	0.50 (0.40–0.63)	0	(-0.05 to 0.05)	1.00	-0.01	(-0.05 to 0.04)	0.70

* Analysis adjusted for study site, vaccine batch, vaccine experience, maternal age at delivery, maternal BCG vaccination, parental smoking, atopic disposition, other children <4 years of age in the home, sex, prematurity, birth weight, caesarean section, child age at BCG vaccination, ethnicity, exclusively breastfed, mid-upper arm circumference and Δweight.

[95 % CI: 0.94 to 1.03]) (Table 4). None of the other individual level factors were associated with scar prevalence.

3.2. Determinants of scar size

3.2.1. External determinants

The median BCG scar size was different among infants vaccinated at the three hospitals. At Kolding hospital scar size was on average 0.05 cm smaller in crude analysis (cPR -0.05 [95 % CI: -0.07 to -0.03]), and 0.02 cm smaller in adjusted analysis (aPR -0.02 [95 % CI: -0.05 to 0.02]), compared with Copenhagen University Hospital, Hvidovre (p-value <0.001). BCG batch was associated with difference in scar size in the crude analysis, but the differences did not remain in the adjusted analysis (Table 5). Month of vaccination was associated with difference in scar size; being vaccinated in March, April and from September until November decreased the average scar size between 0.06 cm and 0.09 cm compared with the rest of the year (p-value <0.001) (Table 3).

Among vaccinators, the median BCG vaccination scar size varied from 0.28 cm to 0.62 cm (Figure 3). Vaccinators who had vaccinated more than 100 children during the trial had a larger median BCG scar size than those who just vaccinated a few children (Crude median difference 0.05 cm [95 % CI: 0.02 to 0.08], Adjusted median difference 0.05 cm [95 % CI: 0.01 to 0.09]) (Table 5).

3.2.2. Parental related determinants

Not having an atopic disposition tended to be associated with an increase in the median BCG scar with a crude median difference 0.03 cm [95 % CI: 0.00 to 0.05], and 0.02 cm and an adjusted median difference of 0.02 cm [95 % CI: 0.00 to 0.04]. The remaining parental related factors were not associated with median BCG scar size (Table 5).

3.2.3. Individual related determinants

The median BCG scar size difference was smaller for girls compared with boys, the crude median difference 0.03 cm [95 % CI: -0.04 to -0.01], but this estimate was weakened in the adjusted analysis (crude median difference 0.01 cm [95 % CI: -0.03 to 0.01]). MUAC and weight gain from birth to 13 months of age were associated with BCG-scar size in the crude, but not in the adjusted analysis. The remaining factors were not associated with median BCG scar size (Table 5).

4. Discussion

To our knowledge, this is the first study to investigate possible determinants of developing a scar and scar size after BCG vaccination in a high-income country. Development of a scar was significantly associated with study site and vaccinator experience. Furthermore, we found that vaccinator experience was positively associated with scar size. Among the many individual and parental-related factors studied, only very few were associated with differences in scar prevalence and scar size.

4.1. External determinants

It is well known from low-income settings that experience and training of the staff who injected the vaccine as well as BCG vaccination technique influenced the scar formation and the scar size [2, 3, 12, 19]. Certain factors should be considered when vaccinating, such as the specific dose of the vaccine injected, the volume leaking after vaccination, and whether the vaccine is injected intradermally or subcutaneously [13, 29]. Staff conducting vaccination on a regular basis would be expected to achieve higher vaccination skills. In the Calmette study all staff member received the same training at baseline. Predominantly one person were vaccinating at Copenhagen University Hospital, Hvidovre, compared with Copenhagen University Hospital, Rigshospitalet, and Kolding hospital, that had different staff members vaccinating the participants. However, Copenhagen University Hospital, Hvidovre, tended to have a lower scar-prevalence than the other two hospitals. It is possible that the staff conducting vaccinations at Copenhagen University Hospital, Hvidovre, was using a less optimal vaccination technique. It has previously been shown that the size of a wheal after vaccination is associated with the formation of a scar [30]. This supports the fact that vaccination technique is an important predictor for BCG scar size.

Two different vaccine batches were used in the study. Even though both of them are the Danish strain 1331, Statens Serum Institut (SSI), there may still be variation between batches [31]. We found a small but significant difference in scar size between the batches used in the study.

Also month of vaccination seemed to be associated with BCG scar formation and BCG scar size [18]. Though we did control for vaccinator

Table 4. Baseline characteristics and BCG-scarification 13 months after neonatal BCG vaccination among the 2118 BCG-vaccinated children in the Danish Calmette Study.

Determinants		(+) BCG, N = 2118 Numbers of Infants (%) [Missing]	Data for scars			Crude		Adjusted*		
			Number of infants with scar information (2039), (% with information) [Missing]	No scar (182)	Scar prevalence (1857) (%)	Prevalence ratio (95 % CI) N = 2039	p-value**	Prevalence ratio (95% CI) N = 1968	p-value**	
External determinants	Study site	Hvidovre hospital	734 (35)	688 (94)	101	587 (85)	1 (ref.)		1 (ref.)	
		Rigshospitalet	761 (36)	741 (97)	36	705 (95)	1.11 (1.08–1.16)	<0.001	1.24 (1.18–1.30)	<0.001
		Kolding hospital	623 (29)	610 (98)	45	565 (93)	1.08 (1.04–1.13)		1.27 (1.19–1.35)	
	Vaccine batch	1. "112032A"	1195 (56)	1159 (97)	81	1078 (93)	1 (ref.)		1 (ref.)	
		2. "111046B"	923 (44)	880 (95)	101	779 (89)	0.95 (0.92–0.98)	<0.001	0.90 (0.85–0.96)	<0.001
	Vaccinator Experience (number of children vaccinated)	0 to 9	328 (16)	310 (95)	31	279 (90)	1 (ref.)		1 (ref.)	
		10 to 49	816 (40)	790 (97)	78	712 (90)	1.00 (0.96–1.05)	<0.001	0.94 (0.89–0.98)	<0.001
		50 to 99	262 (13)	259 (99)	37	218 (85)	0.95 (0.89–1.01)		0.93 (0.87–0.99)	
		≥100	643 (31)	641 (100)	31	589 (95)	1.06 (1.01–1.10)		1.16 (1.09–1.22)	
			[69]	[39]						
Parental determinants	Maternal age at delivery	<27 years	287 (14)	278 (97)	24	254 (91)	1 (ref.)		1 (ref.)	
		27-32 years	799 (38)	764 (96)	67	697 (91)	1.00 (0.96–1.04)	0.95	1.00 (0.96–1.04)	
		>32 years	1032 (49)	997 (97)	91	906 (91)	0.99 (0.95–1.04)		0.99 (0.95–1.03)	0.93
	Maternal BCG	Yes	356 (18)	357 (96)	39	318 (89)	1 (ref.)		1 (ref.)	
		No	1638 (82)	1661 (96)	141	1520 (92)	1.03 (0.99–1.07)	0.18	1.03 (0.98–1.07)	0.17
			[124]	[19]						
	Parental smoking ^b	Yes	313 (15)	301 (96)	29	272 (90)	1 (ref.)		1 (ref.)	
		No	1790 (85)	1738 (97)	153	1585 (91)	1.01 (0.97–1.05)	0.61	1.00 (0.96–1.03)	0.85
			[15]							
	Atopic disposition ^c	Yes	1349 (64)	1297 (97)	128	1169 (90)	1 (ref.)		1 (ref.)	
		No	769 (36)	742 (97)	54	688 (93)	1.03 (1.00–1.06)	0.03	1.02 (0.99–1.05)	0.13
	Other children <4 years of age in the home ^d	Yes	445 (21)	429 (96)	39	390 (91)	1 (ref.)		1 (ref.)	
		No	1656 (79)	1608 (97)	142	1446 (91)	1.00 (0.96–1.03)	0.81	0.99 (0.96–1.03)	0.66
		[17]	[2]							
Individual determinants	Sex	Boys	1096 (52)	1060 (97)	87	973 (92)	1 (ref.)		1 (ref.)	
		Girls	1022 (48)	979 (96)	95	884 (90)	0.98 (0.96–1.01)	0.23	1.00 (0.97–1.02)	0.73
	Prematurity	<37 weeks	71 (3)	63 (89)	2	61 (97)	1 (ref.)		1 (ref.)	
		≥37 weeks	2047 (97)	1976 (97)	180	1796 (91)	0.94 (0.90–0.98)	0.01	0.96 (0.91–1.01)	0.11
	Birth weight	<2500 g	61 (3)	53 (87)	1	52 (98)	1 (ref.)		1 (ref.)	
		≥2500 g	2057 (97)	1986 (97)	181	1805 (91)	0.92 (0.89–0.96)	<0.001	0.98 (0.94–1.03)	0.52
	Caesarean section	No	1694 (80)	1629 (96)	141	1488 (91)	1 (ref.)		1 (ref.)	
		Yes	424 (20)	410 (97)	41	369 (90)	0.99 (0.95–1.02)	0.41	0.98 (0.95–1.02)	0.38
	Child age at BCG vaccination	Day 2-7	554 (26)	528 (95)	49	479 (91)	1 (ref.)		1 (ref.)	
		Day 0-1	1564 (74)	1511 (97)	133	1378 (91)	1.00 (0.96–1.02)	0.74	0.99 (0.95–1.02)	0.42
	Danish ethnicity ^e	Yes	379 (18)	357 (94)	42	315 (88)	1 (ref.)		1 (ref.)	
		No	1725 (82)	1671 (97)	139	1532 (92)	1.04 (1.00–1.09)	0.15	1.02 (0.98–1.06)	0.60
			[14]	[11]						
	Exclusively breastfeed ^b	Yes	1210 (57)	1168 (96)	102	1066 (91)	1 (ref.)		1 (ref.)	
		No	899 (43)	869 (97)	80	789 (91)	1.00 (0.97–1.02)	0.79	0.99 (0.96–1.02)	0.71
			[9]	[2]						
	Mid-upper arm circumference ^f	1	519 (25)	517 (100)	43	474 (92)	1 (ref.)		1 (ref.)	
		2	521 (26)	520 (100)	46	474 (91)	1.00 (0.96–1.03)	0.88	0.98 (0.94–1.02)	0.51
		3	524 (26)	523 (100)	51	472 (90)	0.98 (0.95–1.02)		0.98 (0.92–1.01)	
		4	479 (23)	476 (100)	42	434 (91)	1.00 (0.96–1.04)		0.98 (0.93–1.03)	
		[75]	[3]							
ΔWeight ^g	1	536 (26)	533 (100)	49	484 (91)	1 (ref.)		1 (ref.)		
	2	495 (24)	495 (100)	48	447 (90)	0.99 (0.95–1.03)	0.72	0.99 (0.95–1.03)	0.66	
	3	529 (26)	526 (100)	41	485 (92)	1.01 (0.98–1.05)		1.01 (0.97–1.06)		

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Table 4 (continued)

Determinants	(+) BCG, N = 2118 Numbers of Infants (%) [Missing]	Data for scars			Crude		Adjusted*	
		Number of infants with scar information (2039), (% with information) [Missing]	No scar (182)	Scar prevalence (1857) (%)	Prevalence ratio (95 % CI) N = 2039	p-value**	Prevalence ratio (95% CI) N = 1968	p-value**
	4	486 (24) [72]	484 (100) [1]	44	440 (91)	1.00 (0.96–1.04)	1.01 (0.96–1.07)	

* Analysis adjusted for study site, vaccine batch, vaccine experience, maternal age at delivery, maternal BCG vaccination, parental smoking, atopic disposition, other children <4 years of age in the home, sex, prematurity, birth weight, caesarean section, child age at BCG vaccination, ethnicity, exclusively breastfed, mid-upper arm circumference and Δweight.

** Wald test.

^a Exclusively breastfed: breastfeeding in the first 3 months of life.

^b Smoking: all types of smoking by parents included as well as inside and outside smoking in the first 12 month of life.

^c Atopic disposition: at least one first degree relative with atopic disease (physician-diagnosed atopic eczema, asthma or asthmatic bronchitis, allergic rhino conjunctivitis or food allergy).

^d Any other children <4 years of age in the house at least 5 days a week.

^e Danish ethnicity or other ethnicity.

^f Mid upper arm circumference (MUAC) is divided in quartiles: 1) 12.1 cm–14.9 cm, 2) 14.9 cm–15.6 cm, 3) 15.6 cm–16.5 cm and 4) 16.5 cm–20 cm.

^g Δweight: gain in weight from birth to the 13-month clinical examination; divided in quantiles: 1) 3415 g–6042 g, 2) 6044 g–6688 g, 3) 6689 g–7460 g, 4) 7464 g–10910 g.

Table 5. Baseline characteristics and size of BCG-scar 13 month after neonatal BCG vaccination among the 1857 BCG-vaccinated children in the Danish Calmette Study who developed a scar.

Determinants	Median (IQR)	Crude (N = 2039)			Adjusted* (N = 1793)				
		P50 (P25–P75)	Difference in median/cm.	95 % CI	p-value	Difference in median/cm.	95 % CI	p-value**	
External determinants	Study site	Hvidovre hospital	0.45 (0.35–0.60)	0 (ref.)			0 (ref.)		
		Rigshospitalet	0.50 (0.40–0.60)	0.05	(0.03–0.07)	<0.001	0.04	(0.01–0.07)	<0.001
		Kolding hospital	0.40 (0.33–0.55)	-0.05	(-0.07 to -0.03)		-0.02	(-0.05 to 0.02)	
	Vaccine batch	1. "112032A"	0.45 (0.35–0.55)	0 (ref.)			0 (ref.)		
		2. "111046B"	0.50 (0.35–0.60)	0.05	(0.03–0.07)	<0.001	0.03	(-0.01 to 0.08)	0.21
	Vaccinator experience	0 to 9	0.45 (0.35–0.58)	0 (ref.)			0 (ref.)		
		10 to 49	0.44 (0.35–0.55)	0.00	(-0.03 to 0.03)	<0.001	-0.01	(-0.04 to 0.02)	<0.001
50 to 99		0.45 (0.35–0.55)	0.00	(-0.04 to 0.04)		0.02	(-0.02 to 0.05)		
≥ 100		0.50 (0.40–0.60)	0.05	(0.02–0.08)		0.05	(0.01–0.09)		
Parental determinants	Maternal age at delivery	<27 years	0.45 (0.35–0.55)	0 (ref.)			0 (ref.)		
		27-32 years	0.45 (0.35–0.55)	0.00	(-0.04 to 0.04)	1.00	-0.01	(-0.04 to 0.02)	0.74
		>32 years	0.45 (0.35–0.60)	0.00	(-0.04 to 0.04)		-0.00	(-0.03 to 0.03)	
	Maternal BCG	Yes	0.45 (0.35–0.58)	0 (ref.)			0 (ref.)		
		No	0.45 (0.35–0.55)	0.00	(-0.03 to 0.03)	0.77	-0.01	(-0.03 to 0.02)	0.64
	Parental smoking ^b	Yes	0.45 (0.35–0.58)	0 (ref.)			0 (ref.)		
		No	0.45 (0.35–0.55)	0.00	(-0.04 to 0.04)	1.00	0.00	(-0.02 to 0.03)	0.86
	Atopic disposition ^c	Yes	0.45 (0.35–0.55)	0 (ref.)			0 (ref.)		
		No	0.48 (0.35–0.58)	0.03	(0.00–0.05)	0.04	0.02	(0.00–0.04)	0.02
	Other children <4 years of age in the home ^d	Yes	0.45 (0.35–0.55)	0 (ref.)			0 (ref.)		
		No	0.45 (0.35–0.58)	0.00	(-0.03 to 0.03)	1.00	0.01	(-0.02 to 0.03)	0.53
Individual determinants	Sex	Boys	0.48 (0.35–0.60)	0 (ref.)			0 (ref.)		
		Girls	0.45 (0.35–0.55)	-0.03	(-0.04 to -0.01)	0.01	-0.01	(-0.03 to 0.01)	0.28
	Prematurity	<37 weeks	0.48 (0.33–0.60)	0 (ref.)			0 (ref.)		
		≥37 weeks	0.45 (0.35–0.55)	-0.03	(-0.11 to 0.06)	0.55	-0.03	(-0.08 to 0.03)	0.37
	Birth weight	<2500 g	0.45 (0.31–0.60)	0 (ref.)			0 (ref.)		
		≥2500 g	0.45 (0.35–0.55)	0.00	(-0.09 to 0.09)	1.00	0.02	(-0.05 to 0.08)	0.61

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Table 5 (continued)

Determinants		Median (IQR)	Crude (N = 2039)			Adjusted* (N = 1793)		
		P50 (P25–P75)	Difference in median/cm.	95 % CI	p-value	Difference in median/cm.	95 % CI	p-value**
Caesarean section	Yes	0.45 (0.35–0.55)	0 (ref.)			0 (ref.)		
	No	0.45 (0.35–0.58)	0.00	(-0.04 to 0.04)	1.00	0.01	(-0.02 to 0.03)	0.61
Child age at BCG vaccination	Day 0-1	0.45 (0.35–0.55)	0 (ref.)			0 (ref.)		
	Day 2-7	0.45 (0.35–0.60)	0.00	(-0.03 to 0.03)	1.00	-0.02	(-0.04 to 0.01)	0.14
Danish ethnicity ^c	Yes	0.45 (0.35–0.55)	0 (ref.)			0 (ref.)		
	No	0.45 (0.35–0.58)	0.00	(-0.04 to 0.04)	0.86	0.01	(-0.01 to 0.04)	0.53
Exclusively breastfed ^a	Yes	0.45 (0.35–0.55)	0 (ref.)			0 (ref.)		
	No	0.45 (0.35–0.60)	0.00	(-0.03 to 0.03)	1.00	-0.00	(-0.02 to 0.02)	0.89
Mid-upper arm circumference ^f	1	0.40 (0.30–0.55)	0 (ref.)			0 (ref.)		
	2	0.45 (0.35–0.55)	0.05	0.03 to 0.07)	<0.001	0.03	(0.00–0.06)	0.09
	3	0.45 (0.35–0.60)	0.05	(0.03–0.07)		0.03	(0.00–0.06)	
	4	0.50 (0.38–0.60)	0.1	(0.08–0.12)		0.04	(0.00–0.07)	
Δ weight ^g	1	0.43 (0.33–0.55)	0 (ref.)			0 (ref.)		
	2	0.45 (0.35–0.55)	0.03	(0.00–0.05)	<0.001	-0.00	(-0.03 to 0.03)	0.20
	3	0.48 (0.35–0.60)	0.05	(0.03–0.07)		0.02	(-0.01 to 0.05)	
	4	0.50 (0.38–0.60)	0.08	(0.05–0.10)		0.03	(-0.00 to 0.07)	

* Analysis adjusted for study site, vaccine batch, vaccine experience, maternal age at delivery, maternal BCG vaccination, parental smoking, atopic disposition, other children <4 years of age in the home, sex, prematurity, birth weight, caesarean section, child age at BCG vaccination, ethnicity, exclusively breastfed, mid-upper arm circumference and Δ weight. N = 1793.

** Wald test.

^a Exclusively breastfed: breastfeeding in the first 3 months of life.

^b Smoking: all types of smoking by parents included as well as inside and outside smoking in the first 12 month of life.

^c Atopic disposition: at least one first degree relative with atopic disease (physician-diagnosed atopic eczema, asthma or asthmatic bronchitis, allergic rhinoconjunctivitis or food allergy).

^d Any other children <4 years of age in the house at least 5 days a week.

^e Danish ethnicity or other ethnicity.

^f Mid upper arm circumference (MUAC) is divided in quartiles: 1) 12.1 cm–14.9 cm, 2) 14.9 cm–15.6 cm, 3) 15.6 cm–16.5 cm and 4) 16.5 cm–20 cm.

^g Δ weight: gain in weight from birth to the 13-month clinical examination; divided in quantiles: 1) 3415 g–6042 g, 2) 6044 g–6688 g, 3) 6689 g–7460 g, 4) 7464 g–10910 g.

experience, the results may still be biased by the duration of the study as the inclusion started in September 2012 and ended in November 2014.

4.2. Parental related determinants

In our study infants without atopic disposition tended to be more likely to develop a scar after BCG vaccination to have a larger scar compared to infants with atopic disposition. To our knowledge this has not been reported before. The Calmette study had a highly selected study population as 64 % of the children reported atopic disposition; thus, the external validity of this finding may be limited and more studies are warranted.

4.3. Individual related determinants

Earlier studies have investigated a potential sex-specific beneficial effect of BCG vaccination [11, 13, 29, 31, 32]. We did not see any difference in scar prevalence or scar size between boys and girls in the adjusted analyses.

Prematurity and low birth-weight was also analyzed as predictors as both are related to the maturity of the infant including maturity of the immune system. Earlier studies have shown that preterm and low-birth-weight infants have been less likely to develop a scar [19, 33, 34]. We found that preterm and low-birth-weight infants were more likely to develop a scar in the crude analysis, but this was not seen when taking all other covariates into account. In general the nutritional status in western infants is much better compared to infants from low-income countries. Maybe this could be an explanation for the difference in BCG scar

frequency seen in this study compared to others. Also, another study that evaluated the BCG vaccine efficacy in preterm infants compared to mature infants did not find a significant difference between the two groups [35]. We also speculated if low-birth-weight infants received a higher dose of vaccine pr. kg, which increased their response and resulted in a higher scar frequency. Although we cannot reject this as an explanatory factor it seems unlikely that this is the only explanation for observed results. We have not been able to find other studies that could provide more insight on this matter.

4.4. Strengths and limitations

It is a strength of the study that only trained staff conducted the BCG vaccinations, and only few were responsible for vaccinating the infants. The consistency in using one BCG strain and only two different batches increases the robustness of our findings.

There was an almost complete follow-up (79 infants included in the sub-study were lost during follow up, 3.7 % of all). It should be noted that precise measurement of BCG scar is difficult [36, 37], hence measuring errors may have occurred. We have attempted to control for confounding in our mutually adjusted estimates. This could lead to an unintended adjustments of effects mediated through some of the covariates, potentially causing our analysis to miss actual effects of determinants (type 2 error). The causal structure of the determinants may be discussed and thus we acknowledge the difficulties in a causal interpretation of some of the mutually adjusted estimates [38].

It is a limitation of the study that we did not use tuberculin skin test, TST, to assess the response to BCG vaccination. As BCG scar, TST

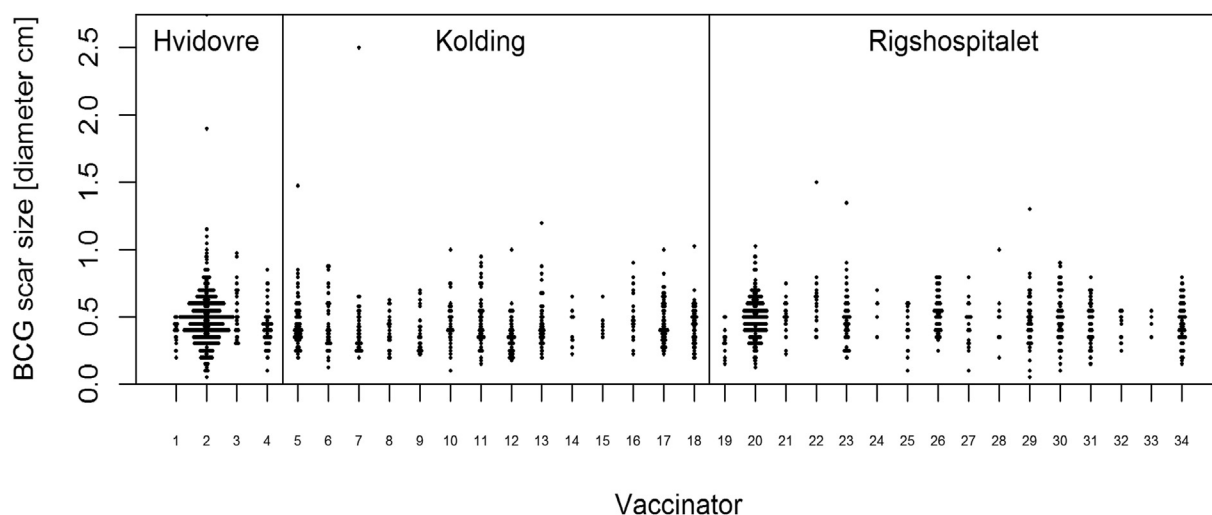


Figure 3. Difference in BCG vaccination scars by vaccinator and study site in the 1857 BCG-vaccinated children in the Danish Calmette Study who developed a scar.

response is considered a marker of BCG efficacy [19] and TST has also been shown to correlate with overall mortality [17]. However, TST is more difficult to assess and much fewer children develop a positive TST response than a scar; [39]. We therefore chose to focus on BCG scar in the present trial.

After BCG vaccination, a post injection wheal is supposed to be formed. Earlier work from the Calmette study group have shown that having a post injection wheal is highly correlated to a scar formation [30]. In this study we did not evaluate the post injection wheal.

5. Interpretation

It has been shown that among BCG vaccinated infants in low-income countries, those reacting to the BCG vaccine with a scar have significantly better survival rate [17]. It could therefore be considered that children that develop a scar after BCG vaccination react immunological stronger in general and therefore are more likely to survive; hence a scar is just a marker of a well functioning immune system [12, 31, 32, 33]. However, we know from studies in low-income settings that vaccination technique, type and strain of BCG vaccine, and dose of vaccine injected are important factors for the frequency and size of BCG scarring [13, 29]. Additionally, in the studies finding an association between scar and survival, the scar frequency varied between 52 % and 98 % even within the same country, and scar was still associated with survival, indicating that genetic factors alone cannot explain the association [18]. The present study supports that vaccinator experience rather than parental factors is the most important factor for developing a scar, also in a high-income setting.

6. Conclusion

We found that the probability of developing BCG-scars among Danish BCG vaccinated children in infancy depends on study site of vaccination and vaccinator experience. Like scar prevalence, scar size was influenced by vaccinator experience. Given the strong link between having a BCG scar and subsequent health, improved BCG vaccination technique could play a major role for child health.

Declarations

Author contribution statement

T. Jensen: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

S. Jensen: 2, N. Birk: Conceived and designed the experiments; Performed the experiments.

A. Rieckmann: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

T. Hoffmann, C. Benn, D. Jeppesen: Conceived and designed the experiments.

O. Pryds: Conceived and designed the experiments; Analyzed and interpreted the data.

T. Nissen: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data.

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Data availability statement

The authors do not have permission to share data.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

The clinical trial described in this paper was registered at The European Union Clinical Trials Register under the registration number 2010-021979-85.

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