Immunogenicity and reactogenicity following MMR vaccination in 5–7-month-old infants: a double-blind placebo-controlled randomized clinical trial in 6540 Danish infants

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Summary

Background Measles is a highly contagious viral disease. Vaccinated mothers transfer fewer antibodies during pregnancy, resulting in shortened infant immunity. Earlier primary vaccination might avert the gap in protection.

Methods Healthy 5–7-month-old Danish infants were assigned in a 1:1 ratio to M-M-RVaxPro or placebo (solvent) in a double-blind, randomized trial between April 15, 2019 and November 1, 2021 (ClinicalTrials.gov NCT03780179, EudraCT 2016-001901-18). Eligibility criteria were birth weight >1000 g and gestational age ≥32 weeks. Immunogenicity was measured by plaque reduction neutralization test (PRNT) and IgG ELISA before intervention, four weeks after intervention and routine MMR. Reactogenicity data were collected for six weeks and measured by hazard ratios (HR).

Findings 647 and 6540 infants participated in the immunogenicity and reactogenicity study, respectively; 87% and 99% completed follow-up. After early MMR, seroprotection rates (SPRs) were 47% (13%) in measles PRNT; 28% (2%), 57% (8%) in mumps and rubella IgG (placebo). For measles PRNT, geometric mean ratio was 4.3 (95% CI: 3.4–5.3) between randomization groups after intervention and 1.5 (95% CI: 1.3–1.9) after routine MMR. Reactogenicity was independent of randomization (HR, 1.0; 95% CI: 0.9–1.1). Severe adverse events occurred in 25 infants (HR, 1.8; 95% CI: 0.8–4.0); none deemed vaccine related.

Interpretation Early MMR elicited low SPRs but did not negatively impact short-term responses to a subsequent MMR. MMR at 5–7 months was safe and not associated with higher rates of reactogenicity than placebo.

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Keywords: MMR; Measles vaccination; Early immunization; Immunogenicity; Reactogenicity

Introduction

Measles is a highly contagious disease, which can be effectively controlled by vaccination; however, only if the majority of the population is immunized.[1] Lower levels at birth and earlier waning of maternal antibodies has been observed in the post-vaccine era.[2] Earlier primary measles vaccination (MCV) may provide protection to young infants, who have lost their innate protection and

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Research in context

Evidence before this study

Compared to infants born by previously measles-infected mothers, infants born by mothers with vaccine-induced antibodies are susceptible to measles from a younger age. However, lower age and presence of even low levels of maternal antibodies has been found to decrease immunogenicity of measles-containing vaccines (MCV). Only one prior observational study has investigated immunogenicity of a trivalent measles-mumps-rubella vaccine (MMR) at 6–8 months of age, and found reduced immunogenicity, a negative impact on the subsequent vaccine response, and a faster decay of antibodies compared to vaccination at a higher age.

MCV has been shown to be as safe in infants <9 months of age as in older age groups, however, no prior placebo-controlled trials have been performed regarding safety of MMR in infants <12 months of age.

Added value of this study

This first double-blind, placebo-controlled randomized clinical trial in 6540 infants allowed us to get closer to the true rate of adverse reactions following MMR at 5–7 months of age. No significant differences were found between the MMR and the placebo group (composite reactogenicity outcome, Hazard ratio 1.00 (0.94–1.07)). This is the first trial to investigate immunogenicity following MMR across all three pathogens in infants around 6 months of age. In the immunogenicity subpopulation (N = 647), the proportion achieving seroprotection following early MMR was 47%, 33%, and 61% against measles, mumps, and rubella, respectively, and a positive impact on short-term immunogenicity following routine MMR at 15 months of age was found.

Implications of all the available evidence

WHO recommendations include measles immunization from 6 months of age in high-risk settings, during outbreaks, and prior to traveling with infants to high-risk settings. The results from this trial show that MMR can be safely used in infants at 5–7 months of age but induces lower seroresponses across pathogens than administration at an older age.

are at the highest risk of severe measles infection,[3] thereby contributing to herd immunity in a high-risk setting given the increasing immunity gap in infants.[2]

Since 1987, measles-mumps-rubella vaccines (MMRs) have been offered as part of the Danish National Immunization Program (NIP) as a two-dose schedule from 15 months of age. MMRs are well-tolerated and routinely administered from 12 months of age and in endemic settings from nine months of age.[3] In high-risk settings MCVs are recommended from 6 months of age.[3] Immunogenicity and safety of MMR when administered in 5–7-month-old infants is not well described, but non-placebo-controlled studies have documented safety of vaccinating 5–7-month-old infants with standard titer MCV.[4] The reactogenicity burden after MMR has been limited or absent in placebo-controlled randomized controlled trials (RCTs) in older children.[5,6]

Presence of maternal antibodies may blunt immunogenicity of MCV in infants <9 months of age by neutralizing the live-attenuated virus.[2,4] However, lower immunogenicity has been observed in both the presence and absence of maternal antibodies, suggesting that the low maturation state of the immune system plays a separate role.[7] To our knowledge, only one study has reported on measles immunogenicity following MMR in 6–8-month-old infants.[8] No previous studies reported on mumps and rubella immunogenicity following a trivalent MMR vaccine in this age group. An impaired primary vaccine response in infants might lead to a faster decay of antibodies and might have consequences for the duration of clinical protection, however, the evidence is sparse.[8,9] Evaluation of measles immunogenicity is most precisely done by the gold standard plaque reduction neutralization test, but other high throughput, less labor-intensive methods like enzyme immunoassays are needed for serosurveillance of population immunity, thus, both methods are applied in the current trial.[10]

Given the limited evidence on MMR in early infancy and increasing measles susceptibility in infants in the post-vaccine era, we conducted this double-blind, placebo-controlled RCT in 6540 healthy Danish infants to assess seroprotection and absolute levels of neutralizing antibodies after early MMR.[11] This paper presents findings regarding humoral immunogenicity following M-M-RVaxPro[12] or placebo in 5–7-month-old infants in a measles elimination setting. Finally, reactogenicity was evaluated.

Methods

Trial summary

The Danish MMR trial, conducted in Denmark from April 2019 through January 2022 (last randomization November 1, 2021, trial termination due to sufficient power).[11] The trial had two co-primary outcomes[11]: measles humoral immunogenicity and non-specific effects (non-targeted infectious disease hospitalizations before 12 months of age).[13] Except from merging the two trial sites after two weeks of inclusion due to feasibility, no substantial protocol changes were made. All participants were included in the non-specific effects

and reactogenicity study. A subpopulation contributed with samples for immunogenicity evaluation.

Participants

Healthy 5–7-month-old infants born in the Capital Region of Denmark with birth weight ≥1000 g and gestational age ≥32 weeks were eligible for enrollment. Exclusion criteria overlapped with contraindications for routine use of M-M-RVaxPro. See the supplement for a complete list of inclusion and exclusion criteria. The immunogenicity subpopulation was identified by parents accepting to be part of this at the inclusion phone call. No selection was performed by staff.

Antibodies present in infant samples at baseline were assumed to be maternal as the trial was performed in an elimination setting. During the study period four measles cases and ten mumps cases were confirmed in Denmark. The last rubella case was verified in 2008 according to public surveillance data from the Danish Serum Institute. The infants were required to be MMR naïve.[11] No reimbursement was offered. All participants were recommended to adhere to the NIP including MMR at 15 months and 4 years of age.

Randomization and masking

Infants were randomly assigned (1:1) to receive intramuscular injection in the anterolateral region of the thigh with either one dose of M-M-RVaxPro[12] or placebo (vaccine solvent: sterile water, thus, same handling, packaging, and delivery as the vaccine). Randomization was performed in REDCap stratified by sex, study site and prematurity (gestational age <37 weeks) in permuted blocks of 2–4–6 participants by a staff member without interaction with participants, who also prepared the intervention. The syringe was blinded by colored tape to the specially trained staff member administering the injection and the parents. The allocation was encrypted in REDCap until unblinding (last randomization or the participant turning one year old, whichever came last). Randomization was preceded by a child examination.

Trial procedures

Blood sampling was performed as cubital venipuncture preceded by local anesthetic band aids. Mother and infant sampling was performed immediately before receiving the intervention, and 3–5 weeks after both intervention and routine MMR at 15 months of age (see published study protocol).[11]

Serum samples were analyzed in duplicate using an established protocol for measles plaque reduction neutralization test (PRNT)[14] (laboratory protocol in Supplement) with serial 4-fold dilutions to estimate the dilution at which the sample prevented 50% of the measles plaque formation in a Vero cell monolayer, and by commercial ELISA kits in unicate (Creative Diagnostics kit numbers: DEIA359, DEIA363, and DEIA011). The WHO 3rd International Standard for measles antibody (3 IU/mL; NIBSC code 97/648) was included in all PRNT runs. Concentrations were calculated using the Kärber formula and a WHO-conversion factor (see protocol in Supplement).

Parents were informed about reactogenicity of MMR according to the package leaflet[12] and given an adverse events diary card (Supplementary Figure S1). They were asked to register any untoward event occurring within six weeks following intervention. Reactogenicity information was collected by phone 35–49 days after intervention.

Reactogenicity data were collected in two categories: reactogenicity and adverse events.

- Reactogenicity: predefined symptoms from the package leaflet and reactogenicity symptoms reported in other trials[4,12] (Supplementary Table S1, Supplementary Figure S1)
- Adverse events: any symptom not predefined under reactogenicity (Supplementary Table S2)

Outcomes

The primary outcome was humoral immunogenicity four weeks after randomization. Measles neutralizing antibodies were measured by PRNT. The geometric mean ratio (GMR) between randomization groups was considered the main result. Secondary outcomes included post-routine MMR GMR, GMRs adjusted for baseline levels, effect modifications by sex, age at randomization and prematurity (all prespecified, see statistical analysis plans (SAPs)), seroconversion rates (SCRs), arithmetic (AMCs) and geometric (GMCs) mean concentrations and humoral immunogenicity results from ELISA (see SAPs for elaborations). Seroprotection rates (SPRs) were reported based on WHO conventions (see Supplement. For mumps, SPR means seropositivity rate as a protective threshold cannot be established).

Every symptom was registered with onset (time from intervention to event) and pooled into a composite reactogenicity outcome (within each individual). For adverse events, a description of the course of disease was registered. The severity according to GCP criteria (see SAEs in Supplement), and the extent to which the symptom was suspected to be related to intervention, was assessed. Adverse events were categorized by organ system based on search terms (Supplementary Table S2). Severe adverse events (SAEs) were handled separately. Systematic collection of data only included up to day 42, and registrations later than that were censored (not SAEs). Recurrent events were not registered.

Statistics

For the primary outcome, the measles neutralizing antibodies in the post intervention samples, the a priori sample size calculation was based on 90% power and a

minimal detectable difference of ≥0.3 standard deviations between randomization groups at the 5% significance level. These assumptions led to the required sample size of 500 infant-mother pairs. To adjust for drop-out, a sample size of 600 was targeted, however, the final sample size was 647 for the main analysis. The reactogenicity population was defined by the power calculation for the non-specific effects co-primary outcome.[13] A post-hoc power calculation for reactogenicity was performed: Given the sample size of 6540 infants and an event rate of 60%, we were able to detect a HR of at least 1.09 between randomization groups.

All analyses were based on the per-protocol principle, i.e., adherence to allocation meaning that only infants receiving the allocated intervention were included. All analyses were adjusted for sex and prematurity according to the randomization procedure. Results are considered statistically significant in all analyses presented in this manuscript if 95% confidence intervals for GMRs and HRs do not include 1. The primary outcome, post intervention levels of antibodies measured by measles PRNT was compared between randomization groups using GMR based on Tobit regression[15] to accommodate censored values due to detection limits in all laboratory analyses. The same procedure was applied for all timepoints and laboratory methods (MMR ELISA IgG). The specific implementation of detection limits is described below.

For PRNT, lower limit of detection corresponded to a titer of 1:8 and upper limit to a titer of 1:8192.[14]

For ELISA, optical density results were converted to titers using 4 parameter logistics based on control values for each run. Interpretation of results depended on pathogen (Supplementary Tables S4 and S5). Lower and upper limits corresponded to lowest and highest controls (Supplementary Table S4).

All participants were included in the reactogenicity study.[11,13] Infants were followed from date of injection, and infants not adhering to allocation (N = 5) were excluded. The analyses were based on Cox regression with time to symptom as the outcome and randomization group as the exposure (stratified by sex, prematurity, and study site in accordance with the randomization procedure) to accommodate the timing of the events. All HRs were reported with placebo as the reference group. Crude rates were also reported.

SAPs were deposited with the DSMB prior to unblinding. Data were analyzed using Stata version 17.0.

Ethics

The protocol was approved by the Capital Region biomedical research ethics committee (H-16041195), the Danish Medicines Agency, and the Danish Data Protection Agency (J.no. 2015-41-4508). The trial was monitored by a steering committee, the Capital Region Good Clinical Practice Unit, and a data safety monitoring board (DSMB). All legal guardians signed informed consent forms prior to participation. The trial was performed in accordance with the principles of the Declaration of Helsinki and reported in accordance with the CONSORT guidelines.

Role of the funding source

The funder of the study had no role in designing the study, patient recruitment, data collection, analysis, and interpretation, writing of manuscripts, the decision to submit for publication, or any aspect pertinent to the study.

Results

Design and demographics

Among 6540 randomized infants (Fig. 1), 6535 received their allocated intervention. Participants' baseline characteristics were equally distributed across randomization groups. The only significant differences (X^2-test at the 5% level) were detected for family income in the overall trial population and prematurity in the immunogenicity population (Table 1). The immunogenicity subpopulation was defined by participation in the main outcome analysis with a PRNT result after intervention (N = 647). Mean time from intervention to post-randomization sampling was 27 days (Table 1). The immunogenicity subpopulation and the overall trial population had similar baseline characteristics except from prematurity status and an insignificant age difference, as the interest in the extended version (immunogenicity) of the trial exceeded capacity. The follow-up phone call was performed in 99% of participants (N = 6473, Fig. 1). The 66 non-responders were equally distributed between randomization groups (34 MMR and 32 placebo). Mean time from intervention to follow-up phone call was 45 days. In a post-hoc analysis, maternal birth year before and after the introduction of MMR vaccination in Denmark in 1987 was used as a proxy for previous wild-type measles infection (Supplementary Figures S10 and S11). Based on this assumption, it is likely that a much higher proportion of mothers in the trial have been previously infected (PIM) with measles than the self-reported rate around 10% (Table 1).

Immunogenicity

For the main immunogenicity outcome, measles neutralizing antibodies by PRNT after intervention, geometric mean concentration (GMC) was 120 mIU/mL for the MMR group (25 mIU/mL for placebo), GMR 4.3 (95% CI: 3.4–5.3) (Tables 2 and 3, Supplementary Figure S4). SPR was 47% after early MMR (13% for placebo).

At baseline, SPR was 88% in mothers and 15% in infants for measles neutralizing antibodies (data by randomization group, Table 2), i.e., 85% of infants

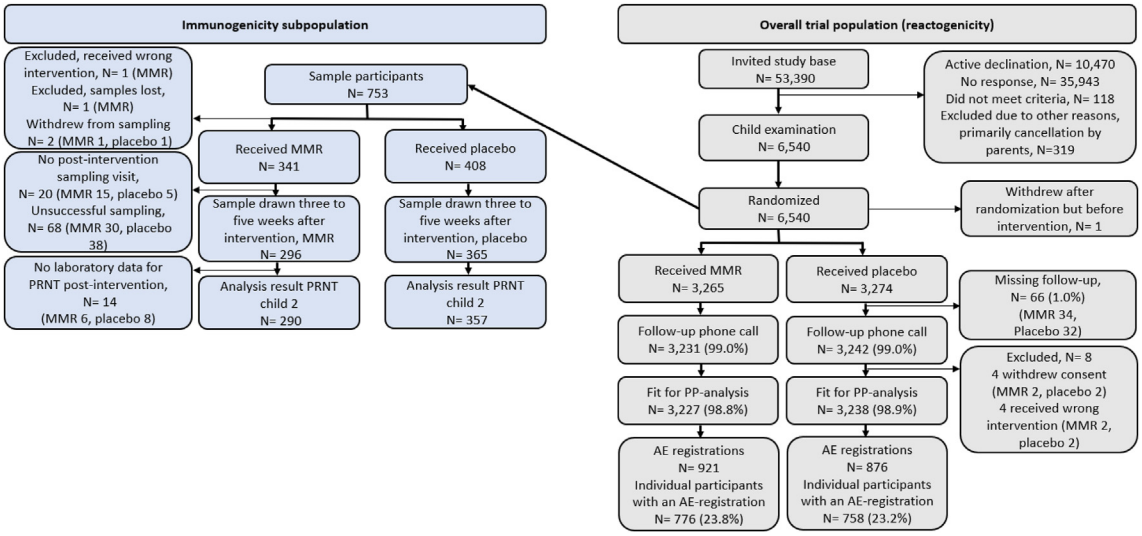

Fig. 1: Participant flow chart. Percentages are based on N = 6539 children receiving an intervention. The immunogenicity subpopulation flow chart presented in blue is embedded in the overall trial population presented in grey.

were susceptible to measles (mean age at baseline was 6.4 months). Maternal antibodies above 40 mIU/mL at baseline notably decreased the proportion of infants responding with seroprotective levels to early vaccination (Supplementary Figure S5). Prematurity was associated with increased response to early MMR (GMR 13.4 (95% CI: 4.9–36.1) vs. 4.0 (95% CI: 3.2–4.9) for term infants, Table 3). Increasing age at intervention was positively associated with vaccine response (GMR 2.5 (95% CI: 1.3–4.6) in infants <6 months vs. 4.6 (95% CI: 3.7–5.7) in infants ≥6 months, Table 3). Immunogenicity was comparable between sexes, but females tended to have higher responses (Tables 2 and 3). Early MMR elicited an increase in the level of antibodies at short-term follow-up 3–5 weeks after routine MMR at 15 months from GMC 1174–1804 mIU/mL GMR 1.5 (95% CI: 1.3–1.9). In a post-hoc sub-analysis (Supplementary Figures S10 and S11), the mother GMC was much higher in the PIM group compared to the previously vaccinated mothers (PVM) group (906 mIU/mL (95% CI: 664–1238) (placebo: 922 (95% CI: 715–1188) vs. 452 mIU/mL (95% CI: 347–588) (placebo: 526 (95% CI: 436–633), Supplementary Table S10). More infants born by PVM were susceptible to measles at baseline (baseline SPR for infants born by PVM is 9% (placebo: 13%) compared to SPR for infants born by PIM, which was 23% (placebo: 22%). The PIM maternal antibodies resulted in lower seroconversion following early MMR (PIM SCR 34% vs. PVM SCR 59%, Supplementary Table S10). Consequently, a significant difference in GMR post-intervention between infants born by PIM and PVM was found (PIM GMR 2.9 (2.1–3.9) vs. PVM GMR 6.0 (4.3–8.4), Supplementary Table S11)). Maternal birth year did not affect post-routine MMR at 15 months GMCs or SCRs. All SPRs post-routine MMR were 94% or higher (Supplementary Table S10).

Following early MMR, SPR against measles, mumps, and rubella was 33%, 28%, and 57% measured by IgG ELISA (placebo 1%, 2%, and 8%, Table 2). SPR after routine MMR in all participating children at 15 months of age was comparable between randomization groups regarding measles, but higher in the MMR group for mumps (91% vs. 47%) and rubella (92% vs. 68%). Early MMR was positively associated with the level of antibodies at short-term follow-up 3–5 weeks after routine MMR with GMRs ranging from 1.4 to 10.6 depending on the pathogen (Table 3).

Mothers showed high SPRs against measles (60%), mumps (83%), and rubella (74%) (data by randomization group, Table 2). Infant SPR at baseline (mean age 6.4 months, Table 1) was low for measles (3%), mumps (1%), and rubella (17%) (data by randomization group, Table 2). Lower SPRs (difference 7–22 percentage points) across sampling time points was shown for measles IgG compared to measles PRNT.

Reactogenicity and adverse events

Around 60% of the study population experienced at least one predefined symptom (Table 4) during the follow-up (Table 1). The predominant symptoms were cold (34%), fever defined as temperature >37.5 °C (24%), isolated rhinorrhea (21%), and vomiting/diarrhea (17%). The timing of symptoms after intervention showed the same pattern in the two randomization groups (Supplementary Figure S2). The proportion of infants experiencing temperatures ≥39.0 °C was similar across randomization groups (MMR 10.0% vs. placebo 9.3%).

	Participants in the overall trial			Participants in immunogenicity study		
	Total N	MMR N (%)	Placebo N (%)	Total N	MMR N (%)	Placebo N (%)
Baseline characteristics	6465	3227 (49.9)	3238 (50.1)	647	290 (44.8)	357 (55.2)
Study site	6465			647		
Rigshospitalet		3189 (98.8)	3199 (98.8)		290 (100)	357 (100)
Herlev Hospital		38 (1.2)	39 (1.2)		0 (0.0)	0 (0)
Sex boys	6465	1675 (51.9)	1673 (51.7)	647	158 (54.5)	187 (52.4)
Mean infant age months[c]	6465	6.2 (6.1–6.2)	6.2 (6.1–6.2)	647	6.4 (5.2–7.0)	6.4 (5.1–7.1)
Age at randomization <6 months	6465	1236 (38.3)	1274 (39.4)	647	38 (13.1)	33 (9.2)
Mean time to follow-up call in days[a]**/mean sampling interval**[b]	6465	41.3[a]	41.3[a]	647	26.8 (18.5–41.5)[b]	26.9 (19.6–42.5)[b]
Premature (GA <37 weeks)	6415	211 (6.6)	203 (6.3)	633	23 (8.0)	12 (3.5)
Number of siblings	6411			645		
0		1568 (49.0)	1601 (49.9)		140 (48.4)	185 (52.0)
1		1169 (36.5)	1121 (34.9)		98 (33.9)	113 (31.7)
2 or more		466 (14.6)	486 (15.1)		51 (17.7)	58 (16.3)
Mean maternal age years[c]	6405	33.1 (32.9–33.2)	33.1 (33.0–33.3)	643	33.3 (32.8–33.8)	32.8 (32.4–33.3)
Household income per year (USD)	6331			640		
Less than 27,000		72 (2.3)	68 (2.1)		7 (2.4)	11 (3.1)
Between 27,000–54,000		485 (15.4)	402 (12.7)		33 (11.5)	53 (15.0)
More than 54,000		2598 (82.4)	2706 (85.2)		247 (86.1)	289 (81.9)
Parents living together	6366	3003 (94.5)	3032 (94.8)	635	265 (93.0)	330 (94.3)
Mother's educational level	6399			644		
≤High-school education		153 (4.8)	138 (4.3)		15 (5.2)	24 (6.8)
Vocational education-bachelor's degree		1207 (37.8)	1169 (36.5)		118 (40.8)	137 (38.6)
≥Master's degree		1832 (57.3)	1894 (59.2)		156 (54.0)	194 (54.7)
Maternal measles immunization status[d]	5839			592		
Previously infected		126 (4.3)	119 (4.1)		15 (5.7)	10 (3.1)
Vaccinated		2618 (89.6)	2621 (89.7)		242 (91.7)	299 (91.2)
Both previously infected and vaccinated		158 (5.4)	166 (5.7)		7 (2.7)	18 (5.5)
Not immunized		17 (0.6)	14 (0.5)		0 (0.0)	1 (0.3)

N (%) within population for non-missing data. GA: gestational age; USD: US Dollars. [a]Follow-up time was defined as time from intervention until follow-up phone call or censoring on day 42 after vaccination whichever came first. [b]Sampling interval defined as time from intervention until follow-up blood sampling. [c]Ages were reported as means with 95% confidence intervals in parenthesis. [d]Self-reported maternal immunization status.

Table 1: **Demographics based on randomization group.**

Skin symptoms were presented in five categories: rash defined as a generalized rash (4.6% in MMR vs. placebo 4.0%), injection site reactions included redness (MMR 3.4% vs. placebo 3.4%), bruising (MMR 1.4% vs. placebo 1.9%) and itching (MMR 0.1% vs. placebo 0.2%) and any other localized skin phenomenon not affecting the injection site (MMR 15% vs. placebo 14%).

Severe adverse events (SAEs)

SAEs occurred in 25 individuals (27 events in total). All SAEs were deemed unrelated or unlikely to be related to intervention. Most of the SAEs (N = 22) were related to infectious disease admissions (14 airway-related, of which six occurred within the first 21 days after intervention). One participant experienced febrile seizures on day 33 after randomization. Except from the airway infection admissions, the SAEs were equally distributed between the randomization groups (Supplementary Table S3). No deaths were reported.

Discussion

The 47% measles SPR in early vaccinated infants (13% in the placebo group) was low compared to vaccination at 15 months of age (Supplementary Figure S4). This is in accordance with prior studies, albeit lower than expected.[4,8,16] To our knowledge, Brinkman et al.,[8] is the only prior study on immunogenicity of MMR at 6–8 months of age; however, the sample size was small, infant age not entirely comparable, and the study only reported on measles immunogenicity showing an 80% SPR 6–8 months after first immunization.[8]

Effective inhibition of MCV response by maternal antibodies is a well-known phenomenon in infants born by both previously vaccinated and naturally immunized mothers.[4] In a measles-elimination setting, the high seroprotection rate of maternal measles antibodies in 5–7-month-old infants born by predominantly vaccinated mothers was surprising, although a recent observational study showed similar SPR around 6 months of age.[2]

	MMR				Placebo			
	Mother	Baseline	Post interv.	Post routine	Mother	Baseline	Post interv.	Post routine
PRNT								
Measles	N = 285	N = 264	N = 290	N = 247	N = 352	N = 327	N = 357	N = 316
GMC	640 (531–773)	25 (20–30)	120 (102–141)	1804 (1555–2094)	670 (581–772)	29 (25–34)	25 (22–29)	1174 (1030–1339)
AMC	2771 (3–119,513)	82 (1–1829)	455 (2–37,295)	3400 (14–87,948)	1606 (4–51,366)	74 (2–1731)	67 (2–1429)	1912 (3–13,407)
SCR (%)	–	–	47	84	–	–	7	95
SPR (%)	86	16	47	98	90	14	13	96
Sex GMC	N = (155,130)	N = (146,118)	N = (158,132)	N = (139,108)	N = (186,166)	N = (172,155)	N = (187,170)	N = (162,154)
Male	633 (486–825)	23 (18–30)	116 (94–144)	1630 (1348–1970)	707 (582–858)	34 (27–42)	28 (22–35)	1214 (1015–1453)
Female	649 (496–850)	26 (20–35)	124 (96–159)	2057 (1620–2611)	631 (511–778)	25 (20–31)	22 (18–27)	1134 (934–1376)
Prematurity GMC	N = (21,262)	N = (22,241)	N = (23,265)	N = (18,227)	N = (11,329)	N = (10,306)	N = (12,333)	N = (10,295)
GA <37 weeks	419 (182–966)	16 (9–30)	188 (94–375)	1452 (651–3241)	421 (213–833)	10 (4–27)	12 (6–27)	1523 (701–3305)
GA ≥37 weeks	657 (543–794)	26 (21–31)	114 (96–135)	1828 (1572–2126)	685 (592–792)	30 (25–35)	26 (22–30)	1167 (1017–1339)
Age at intervention GMC	N = (38,247)	N = (36,228)	N = (38,252)	N = (36,211)	N = (32,320)	N = (32,295)	N = (33,324)	N = (30,286)
<6 months	535 (298–960)	31 (16–59)	92 (61–139)	1538 (1143–2069)	800 (507–1262)	44 (26–73)	34 (20–58)	1373 (971–1942)
≥6 months	658 (539–803)	24 (19–29)	124 (104–149)	1854 (1568–2192)	658 (566–764)	28 (24–33)	24 (21–28)	1155 (1004–1330)
ELISA IgG								
Measles	N = 278	N = 255	N = 281	N = 256	N = 342	N = 314	N = 343	N = 328
Titre	38 (1–221)	2 (1–27)	12 (1–71)	60 (1–204)	38 (1–237)	2 (1–27)	2 (1–25)	41 (1–249)
SPR (%)	69	2	33	91	68	3	1	89
Mumps	N = 269	N = 247	N = 273	N = 239	N = 329	N = 301	N = 330	N = 302
Titre	53 (1–240)	2 (1–39)	12 (1–155)	118 (1–287)	55 (1–194)	3 (1–31)	3 (1–129)	25 (1–188)
SPR (%)	83	0	28	91	82	1	2	47
Rubella	N = 287	N = 264	N = 290	N = 255	N = 355	N = 327	N = 356	N = 323
AMC	41 (0–157)	4 (0–63)	24 (0–101)	72 (1–174)	43 (0–152)	4 (0–83)	4 (0–66)	35 (0–298)
SPR 10 IU/mL (%)	74	18	57	92	75	17	8	68
SPR 4 IU/mL (%)	83	27	74	97	83	27	20	76

GMC, geometric mean concentration was presented as mIU/mL (95% CI). **AMC**, arithmetic mean concentration was presented as miliInternational Units (mIU)/mL (range). Observations for effect modification analysis was shown as N = (XX, YY), in which XX refers to the first group presented, and YY represents the second group. **Titer** was presented as Nova Tec Units (NTU) for measles and mumps IgG, and as AMC in International Units (IU)/mL for rubella IgG (range). Seroprotection rate (**SPR**): PRNT: defined as level ≥120 mIU/mL. ELISA: defined by the manufacturer as ≥11 NTU for measles and as ≥10 IU/mL for rubella. Post-vaccine era rubella seroprotective threshold ≥4 IU/mL is also presented. **For mumps, SPR means seropositivity rate**. Seroconversion rate (**SCR**): Only calculated for PRNT: More than a 4-fold increase or change of status from seronegative to seropositive from the former sample to the next. GA: gestational age.

Table 2: **Descriptive immunogenicity results by randomization group.**

Information on mother immunization status was self-reported. Before the introduction of MMR in 1987, most individuals were assumed infected by measles during childhood. It is likely that a significantly larger proportion of the mothers born before 1987 have been WT measles infected than the self-reported data indicated (Table 1) and that this affected immunogenicity following early MMR (Supplementary Tables S10 and S11) due to higher maternal antibodies than what would be expected in a population of primarily PVMs. Even though this was analyzed post-hoc, our findings agree with previous studies on maternal antibodies in PIMs and PVMs.

Although evaluated in a small sample size, early MMR seemed more immunogenic in premature infants, which may be explained by the transferal of antibodies primarily occurring late in pregnancy with fetal IgG reaching maternal levels around gestational week 32 and peak around week 40 and are absent before 6 months of age.[2] The premature infant immune system maturation may facilitate a response at an earlier age than previously assumed.[17,18]

Effects of age on waning of maternal antibodies and maturation status are greatly intertwined. Higher GMR between the two age groups (≥6 months vs. <6 months (Table 3)) was explained by a lower baseline level and higher response in the ≥6 month-group (Table 2). The effect of age analysis is provided in the Supplement. Our findings support that maternal measles antibodies are long-lived and exert efficient inhibitory effects at a much lower level than the protective threshold at 120 mIU/mL (inhibitory effects appear from 40 to 60 mIU/mL, Supplementary Figure S5).[2,19]

Older studies found impaired MCV efficacy in infants vaccinated <12 months of age. In these studies infants were primarily born by PIMs[16] and the MCV efficacy was found to be negatively associated with decreasing age.[16] For infants born by PVMs, this relationship is unknown. However, efficacy is associated with immunogenicity measures of seroprotection also

	Post intervention (MMR/placebo)				Post routine vaccine (MMR/placebo)	
	N	GMR	N	Adjusted GMR	N	GMR
Measles PRNT						
GMR	647	4.3 (3.4–5.3)	591	4.2 (3.5–5.1)	563	1.5 (1.3–1.9)
Effect modification						
Sex		P = 0.181		P = 0.626		P = 0.161
Male	345	3.7 (2.8–5.0)	318	4.0 (3.1–5.3)	301	1.3 (1.0–1.8)
Female	302	5.0 (3.6–6.8)	273	4.4 (3.4–5.9)	262	1.8 (1.3–2.4)
Prematurity		P = 0.038		P = 0.047		P = 0.509
GA <37 weeks	35	13.4 (4.9–36.1)	32	11.0 (4.4–28.0)	28	1.0 (0.4–2.4)
GA ≥37 weeks	598	4.0 (3.2–4.9)	547	4.0 (3.3–4.9)	522	1.6 (1.3–1.9)
Age at intervention		P = 0.071		P = 0.092		P = 0.249
<6 months	71	2.5 (1.3–4.6)	68	2.7 (1.6–4.7)	66	1.1 (0.6–2.0)
≥6 months	576	4.6 (3.7–5.7)	523	4.5 (3.7–5.5)	497	1.6 (1.3–2.0)
ELISA IgG						
Measles GMR	646	3.7 (3.2–4.3)	584	3.7 (3.2–4.3)	584	1.4 (1.2–1.6)
Mumps GMR	603	3.2 (2.8–3.6)	548	3.3 (2.9–3.8)	541	10.6 (8.2–13.8)
Rubella GMR	646	13.2 (9.6–18.1)	591	14.0 (11.0–17.9)	578	5.7 (4.2–7.9)

Measles PRNT: Plaque reduction neutralization test. GMR: Geometric mean concentration ratio provided as estimate (95% CI). P-values refer to significance testing of the effect modification comparing the estimate within the modifier (sex, prematurity status and age at intervention). Adjusted GMR: Adjusted for baseline level antibodies. All analyses adjusted for sex and prematurity. GA: gestational age.

Table 3: **Analytical immunogenicity results by randomization group.**

in <9 months old infants.[4] Efficacy could not be evaluated in the present trial (no disease transmission) and the measurement of immunogenicity was only done shortly after each of the vaccinations (allocated intervention and routine MMR), which leaves an important question unanswered: The duration of serological protection from an early two-dose MMR schedule. Thus, long-term follow-up of the cohort is planned. The impact of an impaired primary response on long-term protection and vaccine effectiveness was questioned in a recent meta-analysis comparing MCV before and after 9 months of age, however, it is recommended that MCV administered before 9 months of age should be considered an MCV0 dose (i.e., an additional early dose).[20] In the present trial, early MMR did not impair short-term immunogenicity of the subsequent vaccination.[8] However, the effect on long-term protection is not clear and a long-term impairment cannot be ruled out.[8,9]

In the present study, an increase of the early MMR response was found across all pathogens 3–5 weeks after MMR at 15 months, both explained by an increase of responses achieved after early MMR and higher responses in the children in the MMR group who were seroprotected at baseline (Supplementary Figures S7 and S8). Monovalent MCV immunogenicity at 6 months of age has been shown to be impaired in both the presence and absence of maternal antibodies compared to administration at 9 and 12 months of age but also to enhance the response to a subsequent dose of MCV in an older study.[7] The clinical implications of this finding remain unclear,[7] but in a small cohort the long-term follow-up showed reduced seroprotection 7–10 years later, when infants were MCV vaccinated in both presence and absence of maternal antibodies at 6 months of age compared to infants ≥9 months of age.[9]

It is reassuring and in accordance with a recent review that the majority of mothers in this trial, who received MMR during childhood have persisting seroprotective antibodies in adulthood.[21] Even though timely MCV-induced serological protection wanes over time, it has been shown to primarily affect quantitative antibody levels and neither seroprotection rates[21] nor T-cell immunity in 20 year follow-up studies in elimination settings.[9] T-cell responses in infants are independent of presence of maternal antibodies and are key in eliminating viral infections.[19] Studies on measles-specific T-cell responses have been planned by the author group.

The inconsistency of SPR between measles PRNT and measles IgG ELISA is a well described phenomenon.[10,14] Although PRNT is the gold standard for measuring seroprotection, it is labor-intensive, why other ways to measure responses are useful. Measles IgG ELISAs have lower sensitivity (underestimating SPR by around 10 percentage points), which causes false negative results.[10] Samples in the ELISAs were run in unicate causing uncertainty for the estimated level of antibodies in each individual sample, however, this was mitigated by a high number of samples, and reading of controls being very similar across plates underlining the uniform performance of the assay and the laboratory work.

	N (%) event	N (%, 95% CI) event–MMR	N (%, 95% CI) event–placebo	HR MMR (95% CI)	HR-effect modification, age[e]	HR-effect modification, sex[f]
Reactogenicity						
Composite[a]	3879 (60)	1935 (60, 58–62)	1944 (60, 58–62)	1.00 (0.94–1.07)	0.95 (0.86–1.05) 1.03 (0.95–1.12)	0.96 (0.88–1.05) 1.05 (0.96–1.15)
Cold	2225 (34)	1103 (34, 33–36)	1122 (35, 33–36)	0.99 (0.91–1.07)	1.02 (0.90–1.18) 0.96 (0.87–1.07)	0.94 (0.83–1.06) 1.04 (0.92–1.17)
Rhinorrhoea	1342 (21)	652 (20, 19–22)	690 (21, 20–23)	0.95 (0.85–1.05)	0.85 (0.71–1.02) 1.00 (0.87–1.14)	0.90 (0.77–1.04) 1.00 (0.85–1.16)
Diarrhoea or vomiting	1079 (17)	532 (17, 15–18)	547 (17, 16–18)	0.97 (0.86–1.10)	0.97 (0.80–1–18) 0.97 (0.84–1.13)	0.93 (0.78–1.10) 1.02 (0.86–1.21)
Generalized rash	278 (4.3)	149 (4.6, 3.9–5.4)	129 (4.0, 3.4–4.7)	1.16 (0.92–1.47)	1.05 (0.69–1.60) 1.21 (0.91–1.61)	1.13 (0.82–1.55) 1.20 (0.84–1.70)
Fever (>37.5 °C)	1575 (24)	784 (24, 23–26)	791 (24, 23–26)	1.00 (0.90–1.10)	0.93 (0.79–1.10) 1.03 (0.91–1.17)	0.99 (0.87–1.14) 1.00 (0.86–1.15)
Fever (≥39.0 °C)	630 (9.6)	327 (10.0, 9.0–11.1)	303 (9.3, 8.3–10.3)	[c]	–	–
Injection site redness	219 (3.4)	110 (3.4, 2.8–4.1)	109 (3.4, 2.8–4.0)	1.01 (0.77–1.31)	–	–
Injection site bruising	109 (1.7)	46 (1.4, 1.1–1.9)	63 (1.9, 1.5–2.5)	0.73 (0.50–1.07)	–	–
Injection site itching	10 (0.15)	3 (0.1, 0.0–0.3)	7 (0.2, 0.1–0.4)	0.43 (0.11–1.65)	–	–
Febrile seizure	1 (0.02)	1 (0.03, 0.01–0.18)	0 (0.00, 0.00–0.12)	[d]	–	–
Thrombocytopenia	0 (0.00)	0 (0.00, 0–0.12)	0 (0.00, 0–0.12)	[d]	–	–
Adverse events						
Ear-nose-throat	266 (4.1)	137 (4.2, 3.6–5.0)	129 (4.0, 3.4–4.7)	1.07 (0.84–1.36)	–	–
Lower airways	45 (0.70)	24 (0.74, 0.50–1.10)	21 (0.65, 0.42–0.99)	1.16 (0.65–2.09)	–	–
Gastro-intestinal	138 (2.1)	69 (2.1, 1.7–2.7)	69 (2.1, 1.7–2.7)	1.01 (0.72–1.41)	–	–
Skin	941 (15)	492 (15, 14–17)	449 (14, 13–15)	1.11 (0.98–1.26)	–	–
Eyes	51 (0.79)	20 (0.62, 0.40–0.95)	31 (0.96, 0.67–1.35)	0.65 (0.37–1.14)	–	–
General conditions	331 (5.12)	163 (5.05, 4.35–5.86)	168 (5.19, 4.48–6.01)	0.97 (0.78–1.20)	–	–
Severe[b]	25 (0.39)	16 (0.50, 0.31–0.80)	9 (0.28, 0.15–0.53)	1.77 (0.78–4.01)	–	–

The number of events is reported with proportion of affected individuals per group N (%, 95% CI). Hazard ratios are reported comparing MMR to placebo with 95% CIs. [a]The composite outcome contained all registrations in the reactogenicity category pooled within each participant. [b]Recurrent events had not been analyzed as only the date of first occurrence was registered, but two infants had a recurrent SAE, and thus the total number of SAEs in the trial is 27. [c]We could not report the HR, as we did not register the timing of the highest temperature. [d]Analyses would only be presented if the number of events in question was sufficient for the likelihood function to converge in the refining of the estimates based on the default options in the software used (stcox command in Stata). [e]Results are reported for the <6-month-group above and the ≥6-month-below. [f]Results are reported for males above and females below.

Table 4: **Frequency of reactogenicity and adverse events by randomization groups (total N = 6465) and Hazard Ratios.**

Maternal antibody presence is not an as impactful inhibitor of immunogenicity of mumps and rubella immunizations compared to maternal measles antibodies.[7,22] We tested two different protective thresholds for rubella IgG: the conservative and currently widely accepted 10 IU/mL and the post-vaccine era estimated protective threshold at 4 IU/mL (see Supplement for elaboration). We found rubella SPR at 57% or 74% depending on which protective threshold is applied, after early MMR, suggesting good immunogenicity in the 5–7 months old infants. With an SPR at 92% or 97%, depending on the protective cutoff, after two doses (6 months and 15 months in the MMR group), SPRs are comparable to the two-dose schedule in older children.[22] For mumps IgG, a clear cut protective threshold has not been established, but immunogenicity has been shown to be independent of maternal antibody presence and dependent of age.[7]

No significant difference in reactogenicity between MMR and placebo was found. Loss to follow-up was 1% and non-differential. MCVs in <9-month-old infants is as safe as in older children[4] and in a head to head observational comparison between age groups, MMR causes the fewest adverse reactions in the youngest (6 months old infants).[23] Our findings highlighted the improper labeling of symptoms as vaccine-related merely based on timing. During the last 30 years, only one double-blind RCT investigated standard-titer MCV without co-administrations and safety in infants <12 months.[24] All groups received an MCV. Reactogenicity varied by strain: fever (1.7–3.8%), rash (0–2.3%), diarrhea (0.8–13.3%) and rhinorrhea (8.4–14.4%) during 28 days of follow-up. No studies were placebo controlled. Other non-blind studies in the 5–7-month-old infants found fever in 7–30%[4] depending on valency and design. We recorded fever in 24% of infants in both groups indicating a high level of reporting. Subcutaneous administration results in more local adverse reactions compared to intramuscular administration.[12] We found similar levels of injection site reactions compared

to MCV studies in the 6 months age group.[4,23] Furthermore, local reactions were as frequent in the placebo group suggesting that the reactions were not caused by the vaccine content but by the injection of fluids. Sex-differential reactogenicity has been described,[25] but we did not observe notable differences.

The present two predominant MMR vaccines differ mainly in their standard titre measles component: M-M-RVaxPro contains the Enders' Edmonston strain whereas Priorix contains the Schwarz strain.[12,26] These two measles strains showed similar reactogenicity profiles when administered as monovalent MCVs in a prior study in 3–8 months old infants, but the trivalent counterparts have not been compared in this age group.[27] An international multicentre RCT (N = 4072) reported on MMR-II (comparable to M-M-RVaxPro) and Priorix in children 9–24 months of age and found similar reactogenicity between the two vaccines: fever in 39% in the second week following administration and rash in 8.5% of the vaccinees but differed in injection site redness (MMR-II 16.3% and Priorix 9.8%).[28] Also, an older double-blind, placebo-controlled RCT of a Schwarz strain-containing MMR in children above one year of age found no differences in rates of reactogenicity between the children receiving MMR and placebo.[5] Lower immunogenicity of a Schwarz-strain-containing MCV has been suggested in a recent review of primarily monovalent MCVs.[4]

The registration of predefined and non-predefined symptoms enabled information on both objective and subjective symptoms. Not surprisingly, the rate of predefined symptoms was higher.[29] Non-measurable symptoms are complicated by the reporting through the parents. Especially symptoms related to the specific infant, e.g., excessive crying, loss of appetite, disturbed sleeping pattern may be observer-dependent as illustrated in another double-blind RCT conducted in twins.[6] One twin received an MMR vaccine followed by a placebo injection 3 weeks later and vice versa. The twin trial showed that only few symptoms were reported slightly more following MMR (fever, drowsiness, irritability, conjunctivitis) compared to placebo. Another double-blind placebo-controlled RCT of MMR in >1-year-old infants did not find differences in reactogenicity between MMR and placebo.[5] This highlighted the importance of control groups to disentangle symptoms caused by vaccination.

The data collection was pragmatic without objectively measurable information (except fever). Other trials used more strict observation regimes: assessment by personnel or more clear case definitions, e.g., temperature measurement at an anatomical site, daily measurements, or graded adverse reactions. However, the parental observations were not affected by the staff reflecting what families experience after vaccination. Most registrations occurred within 21 days after randomization (Supplementary Figure S3) suggesting increased focus on child health complaints shortly after vaccination.

The placebo-controlled trial design is ever more important, since parents who refrain from vaccination express safety concerns as a main reason.[30] Additionally, as earlier vaccination has been suggested, high-level evidence of safety is crucial.

The SAEs in this trial were deemed unrelated to vaccination as they were equally distributed between the two randomization groups and due to the late onset of symptoms (Supplementary Table S2). The higher rate of SAEs in infants receiving early MMR was based on few events. One case of febrile seizure occurred in the MMR group in a highly predisposed individual. As the event occurred 33 days after vaccination it was deemed unrelated. The febrile seizure incidence was comparable to other studies in older children (1:3227).[31]

MMR has been widely used for decades without observing negative long-term effects,[31] however evaluation of rare long-term events must await pragmatic population-based observational studies. This paper focused on humoral immunogenicity and short-term consequences of early MMR vaccination, but other aspects should be considered regarding future vaccination strategies including effectiveness, long-term protection[8] and long-term safety of early MMR.

While explored in a small sample, premature infants, the most vulnerable, may be the population to gain the most from an additional early MMR. Given the high measles mortality rate in infants in low-income countries,[32] and the evidence of a growing population of infants susceptible to measles at a younger age (from around 2–3 months of age),[17] early measles immunization may be an effective protective strategy against measles during this vulnerable period of life in high-risk settings.[17] The absence of reactogenicity after early MMR and the overall achievement of measles seroprotection for one third of vaccinated participants, described in this trial, stresses the desirable situation of reducing the number of susceptible young individuals in high-risk settings and thereby decreasing the risk of measles in unprotected infants[33] and preventing the spread of measles by providing an additional early dose of MMR.

This first placebo-controlled, double-blind RCT of MMR at 5–7 months of age provided evidence of immunogenicity across pathogens without compromising safety. Although the seroprotection rate against measles was only increased from 13% to 47%, such an increase is of clear clinical relevance in high-risk settings in the post-vaccine era with shortened duration of maternal immunity. The early MMR did not impair the short-term response 3–5 weeks after a subsequent MMR vaccination, however, the duration of the protection provided by an early MMR regimen is yet to be investigated.

Contributors

DMV conceptualized the study, collected data, performed the data analysis and interpretation of results, and drafted the initial manuscript. AJ performed the data analysis and interpretation of results. MM, ACZ, JKS, MRJ, and SC collected the data and revised the manuscript. JS supervised data collection and critically revised the manuscript for intellectual content. SB, NSK, and EAFS performed interpretation of results and critically revised the manuscript for intellectual content. LGS conceptualized and designed the trial, supervised data collection, critically revised the manuscript for intellectual content and is the trial sponsor. All authors read and approved the final version of the manuscript. The underlying data has been verified by DMV, AJ, and LGS.

Data sharing statement

In 2025, a pseudonymized copy of the data will be stored in Dansk Data Arkiv (https://www.sa.dk/da/brug-arkivet/ddd/). The data can be accessed and used by other researchers.

Declaration of interests

The majority of authors have no conflicts of interest to declare (AJ, ACZ, JKS, NKS, LGS, MR, and SC). DMV has received payment for teaching activities supported by MSD. MM received grants from Helsefonden, The Beckett Fund, and the Rosalie Petersen's Fund. EAFS has received grants or contracts from AstraZeneca, Johnson and Johnson, Merck, Pfizer, and Roche; consulting fees from Adiago Therapeutics, Cidara Therapeutics, Merck, Nuance Pharmaceuticals, Pfizer, Sobi Inc., Icosavax, Johnson and Johnson, and Sanofi; payment or honoraria from AstraZeneca and Pfizer; support for meeting attendance and/or travel from AstraZeneca; and has participated in data safety monitoring boards or advisory boards for AbbVie, the Bill and Melinda Gates Foundation, and GSK. SB received a grant from the Innovation Fund Denmark. JS owns stocks in Novo Nordisk.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.eclinm.2023.102421.

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