

Study title: Measles-mumps-rubella vaccine at 6 months of age, immunology, and childhood morbidity in a high-income setting

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Abstract

Measles is a highly contagious, serious disease, and a recent study found that long-term measles-induced immunomodulation may increase overall childhood infectious disease mortality. Due to sub-optimal routine vaccine coverage and probably also declining level and duration of passively acquired maternal antibodies, outbreaks of measles in high-income countries have been observed with increasing frequency; also in Denmark. It has high priority to optimise the timing of the primary measles vaccine and the vaccine coverage. Besides the disease-targeted effects, vaccines may affect morbidity and mortality to unrelated infections by changing the general level of resistance toward infections, the so-called non-specific effects of vaccines. Recently, we found MMR to reduce hospitalisation due to severe infection with respiratory syncytial virus, the single leading cause of hospitalisation in infancy and early childhood. Further, MMR may reduce the risk of asthma and allergy in childhood. The immunologic response towards vaccines may be blunted or enhanced by environmental stimuli such as pollution and maternal stressors. To tease out these confounders information regarding these are collected simultaneously. This large, double blind, placebo controlled, clinical trial aims to study the specific immunogenicity of an extra MMR-vaccine administered already at 6 months of age, and to study the potential of positive, non-specific effects of the MMR-vaccine. Thus, the trial has two co-primary aims:

Aim in relation to the specific effect of the MMR vaccine. Sub-group study among 600 children.

1. To measure the level of specific immunity, measured as level of measles neutralising antibodies by plaque-reduction neutralisation test at inclusion, 1 month after experimental MMR vaccination at 6 months of age, and again 1 month after the MMR booster scheduled at 15 months of age.

Aim in relation to the potential non-specific, heterologous effect of the MMR vaccine. 6426 children.

2. To test if MMR administered to healthy Danish children at 6 months of age decreases non-measles childhood morbidity defined as hospitalisation for infection between 6 and 12 months of age before the third DTaKPHib is scheduled according to the Danish child vaccination programme.

600 children will be followed for the specific immunogenicity of MMR using blood samples, urine, hair and saliva and all the children will be followed for potential non-specific effects of MMR using information collected independently of the study group in the public Danish health registries.

Implications

According to the Danish child vaccination programme, Danish children are MMR-vaccinated at 15 months of age because of theoretical considerations about suppression of the specific immune-response by maternal antibodies. If we find that children mount a sufficient protective level of specific immune response to MMR already at 6 months of age, the MMR vaccine can be brought forward to 6 months of age to decrease the risk of disease transmission from infants and to increase vaccine coverage.

The use of antibiotics is high and steadily increasing in Denmark, and atopic disease affects nearly 30% of our population. If early MMR has non-specific effects and reduces the risks of hospitalisation for infection, atopic disease and need of antibiotics, this is of clear public health interest and argues for earlier administration of MMR in the Danish child vaccination programme.

1. General information

Title (English)

Measles-mumps-rubella vaccine at 6 months of age, immunology, and childhood morbidity in a high-income setting

Non-technical language: Can the measles-mumps-rubella vaccine be given to children already at 6 months of age

Abbreviated title: The 6 months MMR Trial

Title (Danish)

Mæslinge-fåresyge-røde hunde-vaccine ved 6 måneders alderen, immunologi, og morbiditet blandt danske småbørn

Læg-mands titel: Er det bedre at vaccinere børn mod mæslinger, fåresyge og røde hunde allerede ved 6-måneders alderen

EudraCT and permission from the Danish Medicines Agency

2016-001901-18. J.no. 2016103428

Ethical Committee's permission

H-16041195

Sponsor

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Lone Graff Stensballe initiated the trial.

Lone Graff Stensballe has the overall responsibility for the trial and data.

Lone Graff Stensballe has the authorisation to sign the protocol and the protocol amendments.

The study will be carried out in accordance with this study protocol and with the current legislation regarding randomised, clinical trials.

The sponsor protocol number is: LGS.MMR.01.2016.2022.

Investigators

The study is a two-center study. Participants will be recruited among all children born and alive at three months of age at two study sites in Region Hovedstaden, Denmark.

1. The Danish National University Hospital "Rigshospitalet". Lone Graff Stensballe is sponsor and investigator.

2. Herlev Hospital. Investigator: Jannet Svensson, Paediatrician, PhD. The Paediatric Department, The University Hospital of Herlev, Herlev Ringvej 75, 2730 Herlev. Tel.: +45 38681089. Email: Jannet.Svensson@regionh.dk.

Other important parties

Regarding data

The screening population will be identified by Dataformidling og Forskerservice, Sundhedsdatastyrelsen, Ørestads Boulevard 5, 2300 København S. Data collected from the trial population will be linked to the public Danish health registries via “forskermaskinen” at Dataformidling og Forskerservice. The contact person at Dataformidling og Forskerservice is Ane Dahl Jørgensen, email: ADJ@sundhedsdata.dk.

Regarding blood samples

Blood samples collected by the study will be analysed for measles, mumps and rubella antibodies by professor of paediatrics Eric Simoes, The Childrens Hospital Colorado, 13123 E. 16th Avenue, B055, Aurora CO 80045-7106, Tel.: 001 720 777 6981. Email: ERIC.SIMOES@CUANSCHUTZ.EDU.

Assays to detect neutralising antibodies are not laboratory standards, however, to detect protective immunity against measles it is the best function assay (1). Thus, we collaborate with professor Eric Simoes, who has the necessary equipment, trained lab technicians and experience with this assay essential to the trial (2). In addition, Eric Simoes has the facilities and expertise to carry out the test for IgM and IgG against measles, mumps and rubella. In addition to neutralisation antibody assay for measles, commercial ELISAs will be carried out for measles, mumps and rubella antibodies on paired sera to look for a 4-fold rise in titre. A correlation with a panel from the CDC will be set up once funding is assured. Measles and mumps IgG antibody avidity might be useful to measure at 1 1/2 years or even 2 years, however this is not planned as part of the present protocol (3).

Assays for the T-cellular immune response analyses will be carried out at the University of Copenhagen by professor Søren Buus, who is expert in this field (4).

Perfluoroalkyl substances (PFAs) detection in serum is a specialised task which will be executed in collaboration with professor Tina Kold Jensen and senior researcher PhD Flemming Nielsen from University of Southern Denmark. This research group has previously been involved in investigation of PFAs in children and are skilled in both methods and analysis (5).

Pro-inflammatory cytokines in dried blood spots are analyzed in collaboration with Manager of Section of Immuneassays PhD Kristin Skogstrand at Statens Serum Institute (SSI).

Analysis for COVID-19 will be carried out at Rigshospitalet, Blegdamsvej.

Analysis for common child infections will be carried out based on the VirScan technique (6).

Regarding hair, urine and saliva samples

Cortisol and oxytocin in hair and saliva are analyzed using standardized methods in collaboration with the Section of Clinical Biochemistry at Glostrup Hospital led by Dr. Niklas Rye Jørgensen.

Salivary alpha-amylase (sAA) is analyzed in collaboration with Manager of Section of Immuneassays PhD Kristin Skogstrand at Statens Serum Institute (SSI)(7-9).

DNA is collected using buccal swaps and genotyped using the Illumina or Affymetrix human whole-genome array. These array genotype 500-800,000 common genetic variants and will allow optimal genetic differentiation between high and low stress responders. GWAS detects only variance with minor allele frequency > 4-5%, which means that rare variants are not detected. In addition, detailed HLA genotypes for the following HLA loci (HLA-A, -B, -C, -DPA1, -DPB1, -DQA1, -DQB1, -DRB1, -DRB3, -DRB4, and -DRB5) can be imputed from these arrays. The genetic analysis is done in collaboration with professor Flemming Pociot and Simranjet Kaur at Steno Diabetes Center.

Data collection and storage system

All trial data will be entered directly in electronic case report forms (e-crf) via the safe, password protected data portal REDCap administered by Region Hovedstaden.

In case of suspected unexpected serious adverse reactions, the allocation code of the relevant study participant can be revealed.

Time plan

- Permissions, logistics, funding April 2016 – December 2018.
- Study start (staff) February 2019.
- Invitation letters February 2019 – March 2021.
- Consultation with inclusion, informed consent, interview, randomisation, vaccination/placebo March 2019 - April 2021.
- Start of register-based follow-up when all children included are 12 months old October 2021.
- Follow-up until 16 months of age February 2020 - February 2022.
- End-of-Trial defined as “last-patient-last-visit” (last date for patient visit at the clinic) February 2022.
- Data management and analyses October 2021 - December 2025.
- Results 2023 – 2025.
- Register-based follow-up is planned until the children are two years old. Dependent on the results, the register-based research may be continued after the age of two years.

Abbreviations

CPR	Central Person Registry
e-crf	Electronic case report form
IgG	Immune globulin G
IgM	Immune globulin M
MMR	Measles, mumps, rubella

SOP Standard operation procedures

2. Background information

Intervention

The live measles, mumps, and rubella vaccine, MMR VaxPro (10;11) used routinely in the Danish child vaccination programme at 15 months and 4 years of age (12), will be used as intervention. One standard dose (0.5 ml) will be injected intramuscularly at the anterolateral area of the thigh shortly after randomisation by specially trained trial staff (nurse, physician, medical student). It is not difficult to apply the injection correctly. Intervention/placebo is administered once. No other treatment than intervention/placebo is administered. The manufacturer of MMR VaxPro, Sanofi Pasteur MSD (13) manufactures the intervention-injection, which is the same as used commercially (MMR VaxPro). Thus, package and labelling are the same as used commercially.

The trial aims to study the efficacy, effectiveness, and non-specific effect of an extra MMR VaxPro vaccine when administered already at 6 months of age.

Summary of results from prior studies

MMR-VaxPro is a widely used, safe, and effective vaccine indicated for simultaneous vaccination against measles, mumps, and rubella in individuals from 12 months of age. One injection in children \geq 12 months of age provided seroconversion and protective immunity against measles in 100% of recipients, 100% seroconversion against rubella, and 84% seroconversion against mumps (14).

Posology

Individuals 12 months of age or older: individuals 12 months or older should receive one dose at an elected date. A second dose may be administered at least 4 weeks after the first dose in accordance with official recommendation. The second dose is intended for individuals who did not respond to the first dose for any reason (10).

Infants between 9 and 12 months of age: immunogenicity and safety data show that MMR VaxPro can be administered to infants between 9 and 12 months of age, in accordance with official recommendations or when an early protection is considered necessary (e.g., day-care, outbreak situations, or travel to a region with high prevalence of measles). Such infants should be revaccinated at 12 to 15 months. An additional dose with a measles-containing vaccine should be considered according to official recommendations (10).

Infants below 9 months of age: no data on the efficacy and safety of MMR VaxPro for use in children below 9 months of age are currently available (10). However Statens Serum Institut recommends vaccination as early as 6 months of age during outbreaks (15). All study participants will be recommended to follow the Danish child vaccination programme as usual.

MMR VaxPro powder and solvent for suspension for injection

In the present trial, one standard dose (0.5 ml) will be injected intramuscularly at the anterolateral area of the thigh shortly after randomisation. After reconstitution, one dose (0.5 ml) contains:

Measles virus1 Enders' Edmonston strain (live, attenuated).....not less than 1x10³ CCID50*

Mumps virus1 Jeryl Lynn™ [Level B] strain (live, attenuated).....not less than 12.5x10³ CCID50*

Rubella virus2 Wistar RA 27/3 strain (live, attenuated)not less than 1x10³ CCID50*

*50% cell culture infectious dose 1 produced in chick embryo cells.

MMR VaxPro is produced in WI-38 human diploid lung fibroblasts. The vaccine may contain traces of recombinant human albumin (rHA). This vaccine contains a trace amount of neomycin. The vaccine contains 14.5 mg of sorbitol.

Adverse events

Table 1 presents known frequencies of adverse events after MMR VaxPro (10).

Table 1. Known frequencies of adverse events after MMR VaxPro	
Adverse reaction	Frequency
Nasopharyngitis, upper respiratory tract infection, viral infection	Uncommon: $\geq 1/1000$ to $\leq 1/100$
Rhinorrhoea	Uncommon: $\geq 1/1000$ to $\leq 1/100$
Diarrhoea or vomiting	Uncommon: $\geq 1/1000$ to $\leq 1/100$
Rash and urticaria	Common: $\geq 1/100$ to $< 1/10$
Fever (38.5°C or higher), injection site erythema, injection site pain, injection site swelling	Very common: $\geq 1/10$
Injection site bruising	Common: $\geq 1/100$ to $< 1/10$
Injection site rash	Uncommon: $\geq 1/1000$ to $\leq 1/100$

In children, MMR triples the risk of febrile seizures in the second week after vaccination, resulting in an estimated 3-16 additional cases per 10,000 vaccinated children (16;17). Other rare events of unknown frequency reported after MMR-VaxPro include: viral meningitis, atypical measles, epididymitis, orchitis, otitis media, parotitis, rhinitis, subacute sclerosing panencephalitis, regional lymphadenopathy, thrombocytopenia, anaphylactoid reaction, anaphylaxis and related phenomenon such as angioneurotic oedema, facial oedema, and peripheral oedema, irritability, afebrile convulsions or seizures, ataxia, dizziness, encephalitis, encephalopathy, guillain-barre syndrome, headache, measles inclusion body encephalitis, ocular palsies, optic neuritis, paraesthesia, polyneuritis, polyneuropathy, retrobulbar neuritis, syncope, conjunctivitis, retinitis, nerve deafness, bronchial spasm, cough, pneumonia, pneumonitis, sore throat, nausea, panniculitis, purpura, skin induration, stevens-johnson syndrome, pruritus, arthritis and/or arthralgia, myalgia, burning and/or stinging of short duration at the injection site, malaise, papillitis, peripheral oedema, vesicles at the injection site, vasculitis.

The parents will be informed about the risk of these side effects and asked to contact the study staff by phone or email if the children experience any of these known side effects, or if the children experience other reactions or diseases within 6 weeks after the injection. The parents will be instructed in how to handle adverse events. The rate of adverse events after MMR is lower if the children are vaccinated at 6-8 months of age (18).

Placebo

Control children receive 0.5ml placebo-injection, containing exactly the same substances as for MMR VaxPro but without any virus, injected intramuscularly at the anterolateral area of the thigh shortly after randomisation by specially trained trial staff (nurse, physician, medical student). The placebo-injection consists of vaccine solvent only, using the solvent from the MMR VaxPro vaccine.

Intervention/placebo is administered once. No other treatment than intervention/placebo is administered. The manufacturer of MMR VaxPro, MSD (13) manufactures the placebo-injection (which is the solvent used for the intervention-injection) using exactly the same substances as for MMR VaxPro but without any virus. Thus, the manufacturer MSD handles the identical package and labelling of intervention and placebo by the same procedures as used commercially. Package and labelling of intervention and placebo are identical. The differentiation takes place in the randomisation procedure described below. To keep the study staff person who is going to administer the intervention/placebo blinded, the randomisation procedure will take place in two steps:

1. One study staff carries out the randomisation electronically via the e-crf and receives information about whether the child is randomised to intervention or placebo. After electronic confirmation of receipt, the information about the allocation is blinded in the e-crf.

If the child is randomised to intervention, the solvent of the vaccine is reconstituted with the powder containing attenuated virus according to the reconstitution instruction. If the child is randomised to placebo, the solvent is *not* reconstituted. In both cases, the content of the syringe is covered by plaster, since the colour of the solvent changes after reconstitution.

2. The intervention/placebo is immediately delivered blinded to the second study staff person, who administers the injection and registers the administration.

Handling and reception of intervention and placebo

The intervention and placebo are received and stored at the hospital pharmacy at Rigshospitalet and Herlev Hospital. Proper control of receipt is performed at the investigator sites by specifically trained pharmacy personnel, who will also document the receipt and contact the sponsor/investigator in order for them to double check the receipt. Control of receipt is defined as: confirmation that the storage conditions of the intervention and placebo have been complied with during shipment, confirmation that the required documentation is included and that the delivered products comply with what has been ordered, confirmation that the delivered products are safely stored and orderly registered.

Recording of intervention

At each study site the study staffs have the responsibility of keeping electronic track of all injections administered, i.e. data (date, time, batch number, unique number label) on each injection administered will be computerised shortly after the administration. The file also contains data on packages destroyed due to exceeded expiry dates, end-of-study, or other reasons.

Allocation code

The allocation code is generated at randomisation in the e-crf within REDCap without involvement by sponsor, investigators, or study staff. Only in case of suspected unexpected serious adverse reactions the allocation code of the relevant study participant will be revealed.

Advantages for trial participants who get MMR VaxPro

Children who get randomised to the intervention have the chance of getting early protection against measles, mumps and rubella. Further, children randomised to the intervention may gain early positive non-specific effect of the measles vaccine (please see section Trial background and aims).

Screening population

All approximately 42,000 children born November 2018 - December 2020 in Region Hovedstaden who are alive and healthy at 3 months of age. Approximately half (48.6%) of the screening population is born at the study sites, since Herlev Hospital has 9000 births in two years and Rigshospitalet has 12,600. The screening population will be identified using the registries (names, addresses) of pregnant families in Rigshospitalet and Herlev in combination with monthly updated information (names, addresses) from CPR (via forskerservice, Sundhedsdatastyrelsen) on the families of all children born in region and alive at 3 months of age.

Trial population

All families in Region Hovedstaden with a 3 months old child will be invited by letter when the child is 3-4 months of age (Appendix 1, Appendix 2). According to the power calculation 6426 children need to be included. Among those approximately 6500 individuals, a maximum of 7% is expected to be born preterm with a gestational age of 32 weeks (inclusion criteria) – 36 weeks. Thus, we may include a maximum of 455 preterm children among the trial participants.

Samples

A subgroup of 600 children (300 MMR, 300 placebo) will be bleed at inclusion, 1 month after intervention and 16 months of age. The biological mothers of these 600 children will be asked for a blood sample too. All the 600 mothers and children will be asked for hair, saliva, nasal swaps, and urine samples. Families which do not allow for collection of samples can still participate in the trial.

3. Trial background and aims

Measles is a highly contagious, serious disease, and a recent study found that long-term measles-induced immunomodulation may increase overall childhood infectious disease mortality (19). The disease remains one of the leading causes of death among young children globally despite the availability of a safe and effective vaccine (20). Due to sub-optimal routine vaccine coverage and probably also declining level and duration of passively acquired maternal antibodies, outbreaks of measles in high-income countries have been observed with increasing frequency (21;22); also in Denmark (23). It has high priority to optimise the timing of the primary measles vaccine and the vaccine coverage.

The vaccine against measles, mumps, and rubella (MMR) age was introduced in Denmark in 1987 and is routinely administered to Danish children at 15 months of age. The timing of primary MMR vaccination is complex, driven by the need to provide protection prior to a time when the infant is likely to be exposed to

disease, and by the possibility of interference with vaccine-induced immunity by passively acquired maternal antibodies (24;25). A recent study in China found no change in seroconversion rates (specific IgG against measles, mumps and rubella) when MMR was administered at age 8 months compared to 12 months (26), but another study on early measles vaccination suggested that infants vaccinated at age 6 months might have an age-related delay in maturation of humoral immune response to measles vaccine, unrelated to passively transferred maternal antibody, compared with infants vaccinated at age 9 or 12 months (25;27). However, markers of cell-mediated immune response to measles vaccine were equivalent when infants were vaccinated at age 6, 9, and 12 months, regardless of presence of passive antibodies (28). Although the cell-mediated immune response to the first dose of measles vaccine alone might not provide full protection among all vaccine recipients, it might prime the humoral response to the second dose (29). Data indicate that revaccination of children first vaccinated as early as age 6 months will result in vaccine-induced immunity, although the response might be associated with a lower antibody titer than titers of children vaccinated at age 9 or 12 months (28). Moreover, mothers who received MMR vaccine tend to have a lower concentration of measles virus-specific antibodies than mothers who naturally acquired measles (30-33). Thus, infants born to measles-vaccinated mothers are likely to have lower levels maternal antibodies at birth and a shorter period of protection than infants of mothers who acquired measles naturally. A lower duration of protection by maternal antibodies against measles might provide a motivation to lower the age at which the first dose of measles vaccine is administered to infants, but the degree and duration of immune response is uncertain when the vaccine is administered to infants below 12 months of age.

Besides the disease-targeted effects, vaccines may affect morbidity and mortality to unrelated infections by changing the general level of resistance toward infections, the so-called non-specific effects of vaccines (34-36). In low-income countries, live vaccines like Bacille Calmette–Guérin (BCG) against tuberculosis and measles vaccine may have beneficial effects on all-cause child mortality (35;37-41). Non-specific effects of vaccines have been observed to possibly also decrease the incidence of infectious diseases and admission rates in both low-income (42-45) and high-income countries (46). Recently, MMR was found in observational studies to reduce the rate of admissions related to infections in Danish children (47), and we found MMR to reduce hospitalisation due to severe infection with respiratory syncytial virus (48), the single leading cause of hospitalisation in infancy and early childhood (49). Further, MMR may reduce the risk of asthma and allergy in childhood (50;51). The rapid development of the immune system during the first two years in life leaves the system vulnerable for external stimuluses including stress, which may give rise to long lasting immune dysregulation (9;52-56). The type, the timing, the amount of stimulus and the child's genetic and epigenetic make-up may influence the antibody response to the vaccine and may thereby be important co-players in evaluation of the effect of early vaccination (5;57-60). Most studies on pollution and immune system is based on umbilical cord measures or in adults with fully developed immune system, likewise the influence of psychological stress on vaccine response is done in medical students also with fully-developed immune system (53).

Circulating pro-inflammatory cytokines of particular importance is interleukin (IL-) 1B, IL-6 and tumor necrosis factor alpha (TNF-alpha), have robustly been found to increase following acute stress —results from adult populations with markedly inter-individual variation (7). Little is known about the association between IL-1B, IL-6, and TNF-alpha during a stressful event and later function of the immune system.

Stress-induced cytokine secretion might constitute an important pathophysiological process of how stress translates into influencing the immune system and future diseases (61).

HLA alleles explain 30% of the interindividual variability in vaccine response, other genes of interest are genetic variation in cytokines, cytokine receptors or cell-surface receptors. Especially, cellular measles receptors (CD46, SLAM and PVRL4) have shown interesting results. The genetic influence on measles vaccine response is especially true in the very young (13). New methods for genetic testing (genome-wide association studies (GWAS)), open for the opportunity to detect new common genes involved in the stress and immune response (62) and compare high and low stress responders. GWAS detects only variance with minor allele frequency > 4-5%, which means, that rare variants are not detected. Genetic background, gene expression profiles and epigenetic changes influences the immune system as well as the human stress response (57;63). Glucocorticoid resistance may exist because of epigenetic changes in the glucocorticoid receptor and mineralocorticoid receptor (64) encoded by the genes NR3C1 and NR3C2 respectively. Methylation of the NR3C1 gene silences the expression of GRs in the hippocampus when cortisol levels remain high for too long, thereby damaging the negative feedback loop that turns down the HPA axis. This way downregulation of the genes leads to resistance to anti-inflammatory signals (65) and thereby contribute to loss of inflammatory regulation (66-71). Furthermore, oxytocin receptor genotype and epigenetic changes in the expression of the oxytocin receptor may be involved (72-74).

Therefore, this study opens a unique opportunity for studying the effect of common variants in genetic profile, toxic pollution exposures, and possible modulating factors as breastfeeding as well as acute and chronic stress on the vaccine response and thereby the immune system (75).

Maternal measles immunity was thought to suppress the overall immune response and the efficiency of measles vaccination in infants; however that assumption has recently been questioned (76): on the contrary, early vaccination in the presence of maternal measles antibodies was found to be associated with large reductions in non-measles child mortality among West African children, indicating that vaccination in the presence of specific maternal immunity may be important for the development of beneficial non-specific effects of measles vaccine (77). Since the majority of Danish mothers are either exposed to or vaccinated against measles and have measles antibodies, it is relevant to test the hypothesis of positive non-specific heterologous effect of the MMR vaccine in Danish children and at the same time study the influence of passively acquired maternal immunity on the specific immune response in the child in order to examine if primary MMR-vaccination could be brought forward to 6 months of age.

Spring 2020, Europe became the epicentre of the new COVID-19 airway infection. Information about low- or a-symptomatic spread of COVID-19 among healthy adults and children is warranted. This information can easily be generated by analysing plasma from samples already collected for the present project, and with the collection of additional nasal swap tests.

The trial has two co-primary aims:

Aim in relation to the specific effect of the MMR vaccine. Sub-group study among 600 children.

1. To measure the level of specific immunity, measured as level of measles neutralising antibodies by plaque-reduction neutralisation test 1 month after experimental MMR vaccination at 6 months of age.

Aim in relation to the potential non-specific, heterologous effect of the MMR vaccine. All 6426 children.

2. To test if MMR administered to healthy Danish children at 6 months of age decreases non-measles childhood morbidity defined as hospitalisation for infection between 6 and 12 months of age before the third DTaKPHib is scheduled according to the Danish child vaccination programme.

4. Trial design

Endpoints, primary and secondary

Primary endpoint 1

Level of specific immunity, measured as level of measles neutralising antibodies by plaque-reduction neutralisation test 1 month after experimental MMR vaccination at 6 months of age. Level of specific immunity, measured as level of measles neutralising antibodies by plaque-reduction neutralisation test at inclusion will be taken into consideration when reporting this primary outcome.

The plaque reduction neutralisation test, (PRNT) which measures the serum dilution capable of preventing 50% of plaque formation induced by measles virus in cell cultures, has been considered the most reliable criterion for the serologic evaluation of measles immunity. For PRNT the protective cutoff titer is defined to be >120. A frequency of 95% seroconversion rate, i.e. children mounting a protective level of humoral immunity according to the abovementioned cutoff value after MMR-vaccination at 6 M of age will be considered sufficient to implement MMR at 6 M in the Danish vaccination programme.

We will use standard World Health Organization cutoff criteria to define the levels of protective humoral immunoglobulin G, measured by commercial ELISA tests. Diagnostic cutoffs for discriminating between susceptible and immune individuals are levels of 0.2 IU/mL anti-measles antibody for measles, 10 IU /mL anti-mumps antibody for mumps, and 10 IU/mL anti-rubella antibody for rubella.

Secondary endpoints in relation to primary endpoint 1

- Level of specific immunity, measured as level of measles neutralising antibodies by plaque-reduction neutralisation test 1 month after routine MMR vaccination at 15 months of age.
- Level of specific immunity, measured as level of IgG and IgM against measles 1 month after experimental MMR vaccination at 6 months of age. Level of specific immunity, measured as level of IgG and IgM against measles at inclusion will be taken into consideration when reporting this secondary outcome.
- IgM and IgG against mumps and rubella 1 month after experimental MMR vaccination at 6 months of age, and again at 1 month after routine MMR vaccination at 15 months of age. Level of specific immunity, measured as level of IgG and IgM against mumps and rubella at inclusion will be taken into consideration when reporting these secondary outcomes.

- The associations between level of maternal specific immunity against measles, mumps and rubella and the children's specific immunity against measles, mumps and rubella will be studied.
- Measles, mumps and rubella-specific cellular immunity (T-cells) inclusive T-cell epitope specificity at base-line (randomisation), 1 month after experimental MMR vaccination at 6 months of age, and again at 1 month after routine MMR vaccination at 15 months of age.
- Hyman leucocyte antigen (HLA)-typing of the children using buccal swaps since HLA-typing is prerequisite to define the MMR-specific cellular immunity.
- The influence of acute maternal and child stress levels (salivary cortisol and salivary alpha-amylase) on specific immunity measured as level of IgG and IgM against measles 1 month after experimental MMR vaccination at 6 and 15 months of age.
- The influence of chronic maternal and or child stress levels (hair cortisol) on specific immunity measured as level of IgG and IgM against measles 1 month after experimental MMR vaccination at 6 and 15 months of age.
- The influence of maternal levels of perfluoroalkyl substances (PFASs) on specific immunity measured as level of IgG and IgM against measles 1 month after experimental MMR vaccination at 6 and 15 months of age.
- The influence of child's immune markers (IL-1B, IL-6, and TNF-alpha) on specific immunity measured as level of IgG and IgM against measles 1 month after experimental MMR vaccination at 6 and 15 months of age.
- The association between common single-nucleotide polymorphism (SNP's) and the MMR-vaccine response
- The association between common SNP's and chronic stress markers
- The association between common SNP's and pollution markers
- Serological immune memory of common infant infections before and after randomization to MMR at 6 months of age

COVID-19 exposure detected in nasopharyngeal swap and plasma **Primary endpoint 2**

Hospitalisation for infection between 6 and 12 months of age (after the second and before the third routine DTaKPHib vaccine) defined by data obtained from The Danish National Patient Register (78;79).

Secondary endpoints in relation to primary endpoint 2

- Use of antibiotics defined as in our prior study (80) using data obtained from The Danish Register of Medicinal Product Statistics (81;82).
- Atopic disease defined as in our prior study (83) using data from The Danish National Patient Register and The Danish Register of Medicinal Product Statistics.
- The influence of maternal acute and chronic stress levels on hospitalisation for infection between 6 and 12 months of age.
- The influence of maternal acute and chronic stress levels on the use of antibiotics defined as in our prior study using data obtained from The Danish Register of Medicinal Product Statistics.

Further, it is planned to continuously follow-up all the children included for morbidity in future registry-based studies.

Design

Randomised double-blind placebocontrolled trial with 2-4-6 block randomisation stratified by site, sex and prematurity defined as gestational age < 37 weeks.

Bias

The risk of bias is reduced by the use of randomisation and double blinding; however, the blinding may be partly compromised by adverse reactions (Table 1) being more frequent among children randomised to MMR. We will be able to follow-up 100% of study participants through the public registers. Follow-up data from the public registers are collected independently of the trial staff.

Time line for participants

Overall

All families in Region Hovedstaden with a 3 months old child will be invited by letter when the child is 3-4 months of age. When the child is 4-5 months old the families will be contacted by telephone once (however with no less than five trials of reaching the family by phone), informed about the study, and preliminary informed consent will be collected. The families will be invited to the nearest study site for signed informed consent, interview regarding baseline characteristics of the family and child, clinical examination of the child, and randomisation and intervention/placebo procedures. All children receive 1 injection once so procedures for preliminary end-of-treatment are not relevant.

To keep track of the participants, they are allocated a unique randomisation number in the e-crf when they are randomised into the trial. The randomisation number is linked to the allocation code (intervention/placebo). In case of emergency, the allocation can be un-blinded by the sponsor using the randomisation number.

Participants, who will not participate with blood samples, are attending this one visit only, and will be followed-up using data from the public Danish health registers.

Blood samples

From a subgroup of 600 children blood samples (14ml) will be collected at inclusion, 1 month after intervention, and 1 month after having had the MMR-VaxPro-vaccine at 15 months of age as part of the Danish child vaccination programme. I.e. mothers and children who participate with blood samples are attending a total of three visits. At inclusion, the biological mothers of the 600 children will be asked for a blood sample (25ml) to be able to study the associations between the immunity against measles, mumps and rubella in the mothers and their children; included in the 25 ml is blood for three dried blood spot samples to be collected to the biobank for additional immune markers (IL-1B, IL-6 and TNF-alpha) and cortisol. A personal schedule for these 600 children will be made at the inclusion visit and the families will receive an e-mail reminder during the week before the dates of sampling.

From March 2020, mothers will be asked for a blood sample (25 ml) at the second and third visit (1 month after intervention, and 1 month after having the MMR-VaxPro-vaccine at 15 months of age) to be able to study the serological progression of Covid-19.

Nasal swap

From March 2020 a subgroup of 350 mother-child pairs will be offered to have a nasal/nasopharyngeal swap collected for COVID-19 detection.

Urine samples

All mothers and children will be offered the opportunity to give a urine sample, buccal swap and hair samples. The urine sample will be collected on the day of vaccination preferable before vaccination is performed (min. of 50 ml from the mother and 10 ml from the child).

Salivary samples

Two buccal swaps from mother and one from the child will be performed to carry out HLA-typing and measure salivary cortisol and salivary alpha-amylase as indicators of physiological stress response.

DNA is genotyped using the Illumina or Affymetrix array. These array genotype 500-800,000 common genetic variants and will allow optimal genetic differentiation between high and low stress responders. GWAS detects only variance with minor allele frequency > 4-5%, which means, that rare variants are not detected.

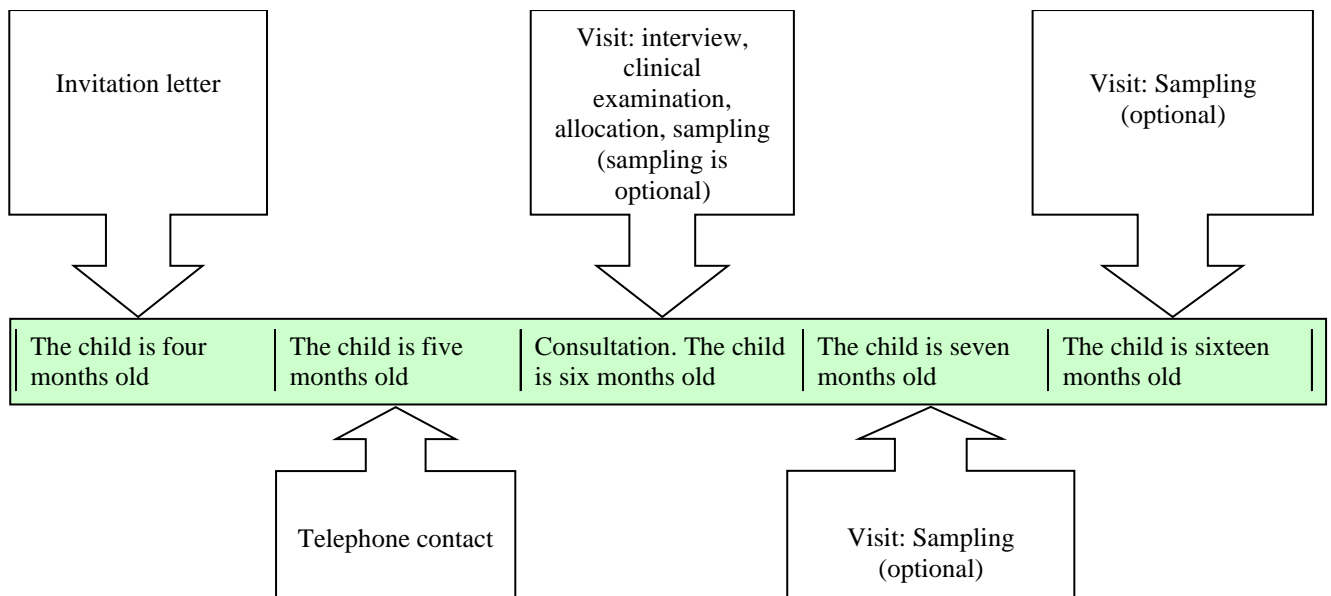
In addition, detailed HLA genotypes for the following HLA loci (HLA-A, -B, -C, -DPA1, -DPB1, -DQA1, -DQB1, -DRB1, -DRB3, -DRB4, and -DRB5) can be imputed from these arrays. The genetic analysis is done in collaboration with Professor Flemming Pociot and Simranjet Kaur at Steno Diabetes Center

Hair samples

A minimum of 10 mg of hair from the proximal to the scalp with a length no greater than 3 cm will be collected from both mother and child for measures of cortisol as a marker of chronic physiologic stress (9).

In case any quantity of the samples remains after the planned analyses, they will be stored in a biobank. The primary purpose of the biobank is to be able to carry out genotyping if analyses for antibody, leukocyte and cytokine responses indicate that information of genotype differences would be valuable.

The Committees on Biomedical Research Ethics will be asked for permission to use the remaining biologic material (samples) from the biobank. If not used 5 years after End-of-Trial, the biobank samples will be destroyed.



In figure: Sampling, i.e. bleeding, urine, hair and buccal swap is optional

Data

The data is directly computerised via the password protected safe data portal, the e-crf, which also contain a system of constantly logging every procedure (audit trail). The e-crf will be considered data source for the trial, which further includes data regarding the results of

- Data to identify the screening population (for the flow chart)
- Data to identify study participants and their parents
- Data on base-line characteristics of study participants (Appendix 3)
- Data on exclusions
- Data on adverse events

Pseudonymised data on the results of immune tests on blood samples delivered safely from Eric Simoes to sponsor in a password protected electronic file is considered data source for the trial.

Pseudonymised follow-up data obtained from CPR and the Danish health registries is considered data source for the trial. The data is accessed by digital personal keyfile ("digital medarbejdersignatur") via the password protected safe data portal "forskermaskinen" at Sundhedsdatastyrelsen (84).

5. The selection and information of participants

Normal, healthy, immunocompetent Danish children born in Region Hovedstaden will be included at approximately 6 months of age (range 5 months to 7 months).

Inclusion criteria

Gestational age of 32+ weeks, birth weight of 1000+ grams, signed informed consent from the parents. Children with gestational age < 32 weeks and birthweight < 1000 grams are excluded due to potential low immunogenicity of vaccination (85).

Exclusion criteria

Immune-deficiency (primary or acquired) or –suppression, and/or intake of immune modulating medicine (including high doses of corticosteroids) (M-M-RVAXPRO is not contraindicated in individuals who are receiving topical or low-dose parenteral corticosteroids, *e.g.* for asthma prophylaxis or replacement therapy), signs of severe illness or major malformation, no Danish-speaking parent. Children with a history of anaphylactic, anaphylactoid, or other immediate reactions (*e.g.*, hives, swelling of the mouth and throat, difficulty breathing, hypotension, or shock) subsequent to egg ingestion are excluded. Children with known fructose intolerance, thrombocytopenia or any coagulation disorder will be excluded. Children who received blood or plasma transfusions, or administration of human immune serum globulin within the last 3 months will be excluded.

Further, children are excluded from the trial if any contraindication is suspected (10): history of hypersensitivity to any measles, mumps, or rubella vaccine, or to any of the excipients, including neomycin. Children with active untreated tuberculosis, blood dyscrasias, leukaemia, lymphomas of any type, or other malignant neoplasms affecting the haematopoietic and lymphatic systems will be excluded.

Information of participants

The parents will be informed about the trial in writing (invitation letter with “information for participants”) and orally (telephone call and consultation) by specially trained study staff (nurse, physician, medical student). The following topics are mentioned both in the written and oral information:

- Aims
- Risks and potential side effects
- Benefits
- That the informed consent includes consent to authorities to monitor and audit the data collection in accordance with the Danish laws

Withdrawal of consent

The parents can withdraw their consent for the participation of their child at any stage of the study without any consequences. The blood samples of children and mothers, for whom the consent is withdrawn, will be destroyed. All study participants will participate in the registry-based follow-up.

6. Measures of effect

The study has two co-primary outcomes, primary endpoint 1 and 2:

1. Level of *specific* immunity, measured as level of measles neutralising antibodies by plaque-reduction neutralisation test 1 month after experimental MMR vaccination at 6 months of age as a measure of the sufficiency of the *specific* immunity to the MMR vaccine
2. Hospitalisation for infection from 6 to 12 months of age as a measure of the potential positive *non-specific* effect of the vaccine.

Each co-primary outcome has secondary endpoints. Below arguments for all endpoints are listed.

Primary endpoint 1

Level of specific immunity, measured as level of measles neutralising antibodies by plaque-reduction neutralisation test 1 month after experimental MMR vaccination at 6 months of age.

According to the Danish child vaccination programme, Danish children are MMR-vaccinated at 15 months of age because of theoretical considerations about suppression of the specific immune-response by maternal antibodies. If we find that children mount a sufficient protective level of specific immune response to MMR already at 6 months of age, the MMR vaccine can be brought forward to 6 months of age to decrease the risk of disease transmission from infants and to increase vaccine coverage.

Secondary endpoints in relation to primary endpoint 1

- Level of specific immunity, measured as level of measles neutralising antibodies by plaque-reduction neutralisation test 1 month after routine MMR vaccination at 15 months of age.
- Level of specific immunity, measured as level of IgG and IgM against measles 1 month after experimental MMR vaccination at 6 months of age. Level of specific immunity, measured as level of IgG and IgM against measles at inclusion will be taken into consideration when reporting this secondary outcome.
- IgM and IgG against mumps and rubella 1 month after experimental MMR vaccination at 6 months of age, and again at 1 month after routine MMR vaccination at 15 months of age. Level of specific immunity, measured as level of IgG and IgM against mumps and rubella at inclusion will be taken into consideration when reporting these secondary outcomes.
- Measles, mumps and rubella-specific cellular immunity (T-cells) inclusive T-cell epitope specificity at base-line (randomisation), 1 month after experimental MMR vaccination at 6 months of age, and again at 1 month after routine MMR vaccination at 15 months of age.
- Hyman leucocyte antigen (HLA)-typing of the children using buccal swaps since HLA-typing is prerequisite to define the MMR-specific cellular immunity.
- The influence of acute maternal stress levels (salivary cortisol and salivary alpha-amylase) on specific immunity measured as level of IgG and IgM against measles 1 month after experimental MMR vaccination at 6 and 15 months of age.
- The influence of acute maternal and child stress levels (salivary cortisol and salivary alpha-amylase) on specific immunity measured as level of IgG and IgM against measles 1 month after experimental MMR vaccination at 6 and 15 months of age.
- The influence of chronic maternal and or child stress levels (hair cortisol) on specific immunity measured as level of IgG and IgM against measles 1 month after experimental MMR vaccination at 6 and 15 months of age.

- The influence of maternal levels of perfluoroalkyl substances (PFASs) on specific immunity measured as level of IgG and IgM against measles 1 month after experimental MMR vaccination at 6 and 15 months of age.
- The influence of child's immune markers (IL-1B, IL-6, and TNF-alpha) on specific immunity measured as level of IgG and IgM against measles 1 month after experimental MMR vaccination at 6 and 15 months of age.
- The association between common single-nucleotide polymorphism (SNP's) and the MMR-vaccine response
- The association between common SNP's and chronic stress markers
- The association between common SNP's and pollution markers
- Serological immune memory of common infant infections before and after randomization to MMR at 6 months of age

COVID-19 exposure detected in nasopharyngeal swap and plasmalt is essential to know if the level of measles neutralising antibodies after routine MMR vaccination at 15 months of age is influenced by early measles vaccination at 6 months of age. IgG and IgM are supplemental measures of the specific immunity. Immunogenicity against mumps and rubella must be documented as well. The importance of the T-cellular arm of the immune response is increasingly acknowledged, and the planned analyses for cellular immune response add essential and new information about the early immune response after MMR-vaccination. HLA-typing is prerequisite for the definition of the T-cellular immune response (4).

Since the immune system is under rapid development in children smaller amounts of stimuli is needed to prime the developing immune system. This means that even a very low level of a chemical substance or minor stressors may have long-lasting effects on the immune system and thereby give rise to later childhood or adult diseases. Studies using umbilical cord blood is hampered by the influence of stress associated with the birth of a child. Many studies regarding the influence of stress and chemicals on the immune system and vaccine response is executed in older children or adults. Therefore, this study is a unique opportunity to get valuable insights into the environmental impact on a developing immune system. Currently there exist no international consensus regarding biochemical, psychological, or physiological methods for measuring a transient and/or persistent stress condition. We have chosen a multidimensional approach to identify the essential stress-measures that impacts the immune system. This means both use immune markers (IL-1b, IL-6 and TNF-alpha), and testing the HPA (Hypothalamic Pituitary Adrenal) axis as well as identifying single-nucleotide polymorphism (SNP) associated with the vaccine and stress response in 6 months old children and their mothers.

Primary endpoint 2

Hospitalisation for infection from 6 to 12 months of age (after the second and before the third routine DTaKPHib vaccine) defined by data obtained from The Danish National Patient Register (78;79).

If early MMR has non-specific effects and reduces the risk of hospitalisation for infection, this is of clear public health interest and argues for earlier administration of MMR in the Danish child vaccination programme.

Secondary endpoints in relation to primary endpoint 2

- Use of antibiotics defined as in our prior study (80) using data obtained from The Danish Register of Medicinal Product Statistics (81;82).

- Atopic disease defined as in our prior study (86) using data from The Danish National Patient Register and The Danish Register of Medicinal Product Statistics.
- The influence of maternal acute and chronic stress levels on hospitalisation for infection between 6 and 12 months of age.
- The influence of maternal acute and chronic stress levels on the use of antibiotics defined as in our prior study using data obtained from The Danish Register of Medicinal Product Statistics.

The use of antibiotics is high and steadily increasing in Denmark, and atopic disease affects nearly 30% of our population. If MMR reduces the risks of need of antibiotics and/or atopic disease, this is of clear public health interest and argues for earlier administration of MMR in the Danish child vaccination programme.

7. Safety (10)

MMR VaxPro is a safe vaccine used routinely in Danish children at 15 months and 4 years of age as part of the Danish child vaccination programme. All participating children will be urged to follow the Danish child vaccination programme, inclusive routine MMR vaccination at 15 months of age. Besides the risk of side effects following each MMR vaccine, the application of a third MMR vaccine is not considered a safety issue. A third dose of MMR have been recommended by Center for Disease Control and Prevention during outbreaks, this leading to no serious adverse events (87), however, to our knowledge no literature describing the safety of a third MMR vaccine in children is yet available. The vaccine may not be injected intravascularly; this will be checked in the trial using SOP for administration of vaccine. Children are not considered at risk of any severe consequence caused by participation in the trial.

The collection of blood samples is safe, however, participants will be offered local anaesthetics against the potential pain caused by the sampling, and informed about potential bruising and the low risk of local infection following a blood sample. The collection of swaps, urine and hair is safe.

Special warnings and precautions (10)

Appropriate medical treatment will always be readily available at each trial site in case of rare anaphylactic reactions following the administration of the vaccine. Since live measles vaccine and live mumps vaccine are produced in chick embryo cell culture, children with a history of anaphylactic, anaphylactoid, or other immediate reactions (*e.g.*, hives, swelling of the mouth and throat, difficulty breathing, hypotension, or shock) subsequent to egg ingestion may be at an enhanced risk of immediate-type hypersensitivity reactions. Due caution should be employed in administration of MMR VaxPro to persons with individual or family history of convulsions, or a history of cerebral injury. Families with a history of convulsions will be recommended to treat fever >38.5 with a standard dose of paracetamole (12.5mg/kg/6hours). The families should be alert to and will be instructed about how to handle the temperature elevation that may occur following vaccination. The vaccine contains 14.5 mg of sorbitol as an excipient. Patients with rare hereditary problems of fructose intolerance should not take the vaccine. Individuals with current thrombocytopenia may develop more severe thrombocytopenia following vaccination. In addition, individuals who experienced thrombocytopenia with the first dose of M-M-RVAXPRO (or its component vaccines) may develop thrombocytopenia with repeat doses.

Interaction with other medicinal products (10)

Administration of immune globulins concomitantly with MMR VaxPro may interfere with the expected immune response. Vaccination should be deferred for at least 3 months following blood or plasma transfusions, or administration of human immune serum globulin. Administration of measles, mumps, or rubella antibody-containing blood products, including immune globulin preparations, should be avoided within 1 month after a dose of M-M-RVAXPRO unless considered to be essential.

Registration of adverse events

The parents will be informed about adverse events following vaccination by specially trained study staff (nurse, physician, medical student). The reference document of adverse events is the MMR VaxPro product information (10), pages 6-8. At inclusion, the families will receive a diary for notes of adverse events, inclusive the list of adverse effects expected to happen in more than 1/1000 of participants (Table 1). The children will be followed for adverse events for 6 weeks post allocation, and at this time point all families will be contacted by telephone by specially trained study staff (nurse, physician, medical student) to collect and register data on adverse events in the e-crf. Likewise, if parents contact the trial staff within 6 weeks post allocation with information on adverse events, this information will be recorded in the e-crf. The e-crf will contain a specific scheme including the elements of Table 1 and Appendix 4 for the registration of adverse events. The e-crf automatically generates an email alert to the sponsor when the registration of an AE fulfils the criteria of a SAE.

Definitions

- Adverse events (AE) are defined as any untoward medical occurrence, which does not necessarily have a causal relationship with the intervention, in trial participants.
- Related adverse events (AR) are defined as any untoward and unintended response to the intervention.
- Serious adverse events (SAE) are defined as any untoward medical occurrences that results in death, is life-threatening, results in persistent or significant disability/incapacity, or requires intervention to prevent permanent impairment or damage. Information on number and duration of hospital contacts is collected separately as an outcome. Since hospital contacts for infection is a primary study outcomes, information on hospital contacts will not be collected as part of the registration of adverse reactions. However, detailed information for all study participants on hospital contacts by intervention will be made available and published.
- Serious adverse reactions (SAR) are defined as serious adverse events judged to be related to the intervention.
- SUSARs (suspected unexpected serious adverse reactions), i.e. suspected SAR which is considered unexpected according to the Summary of Product Characteristics.

Responsibilities regarding adverse events

Investigator

The investigator's responsibilities entail immediate (within 24 hours) reporting of serious adverse events to the sponsor. The reporting takes place via the registration of SAE in the e-crf, which automatically generates an email alert to the sponsor when the registration of AE fulfils the criteria of a SAE.

Sponsor

The sponsor's responsibilities entail:

- To ensure the recording of adverse events in the e-crf.
- To ensure that all relevant information about SUSARs, which are fatal or life-threatening, is recorded and reported to the DKMA as soon as possible and no later than 7 days after the sponsor is informed of such a suspected adverse reaction. No later than 8 days after the reporting, the sponsor must inform the DKMA of relevant follow-up information on the sponsor's and the investigator's follow-up action to the reporting.
- To ensure that other SUSARs are reported to the DKMA no later than 15 days from the time when the sponsor is informed about them.
- When an event may be a SUSAR the blind will be broken by the sponsor only for that specific subject.
- To ensure the competence of investigator regarding the registration and reporting of adverse events.
- To work out annual safety reporting and final safety report after End-of-Trial to the national competent authority and the Ethics Committee.
- To continuously weigh anticipated benefits and risks of the clinical trial.

8. Study power and statistics

Detailed statistical analysis plans will be worked out before analysis start. The original analysis plan will be stored at an independent third party before analysis start. Potential *post hoc* changes to the analysis plan must be discussed by Lone Graff Stensballe and Jannet Svensson, and the reason why such *post hoc* change was decided upon will be written down in meeting minutes. Potential *post hoc* analyses will be described as "explorative" when the results are presented and published.

Power: blood samples. Specific effect of MMR on the level of measles neutralising antibodies

To detect a clinically significant difference of ≥ 0.3 SDS in the level of measles neutralising antibodies between case children who received MMR at 6 months of age and control children who received placebo, we need to collect a total of 500 samples per each of the three sample time points (at inclusion (500 children and 500 mothers), 1 month after experimental MMR vaccination at 6 months of age, and again 1 month after the MMR booster scheduled at 15 months of age). To account for 20% drop-out in follow-up blood samples 1 month after intervention and at 16 months of age, 600 mother-child pairs will be included in the collection of blood-samples.

Statistics: blood samples. Specific effect of MMR on the level of measles neutralising antibodies

A 5%-significance level will be used. In each randomisation group, we will report the proportion of children with a non-protective level of antibodies according to standard cut-off values for each vaccine. In addition, we will report the antibody geometric mean titre (GMT) in each randomisation group. The primary estimate of the MMR-effect is the geometric mean titre ratio (GMR) with 95% confidence interval obtained as the anti-logged coefficient from a linear regression with log-titre as outcome and randomisation group, sex, study site and gestational age as covariates. In case the antibody measurements assay is subject to upper-

or lower limits of detection, the GMR will be obtained by Tobit regression, which is a censored normal method. Non-detectable levels are not given a specific value but are instead regarded as censored with the true value being below/above the lower/upper limit. A prior study applied these methods to similar data on measles antibodies (88). Level of specific immunity (measles neutralising antibodies by plaque-reduction neutralisation test) at inclusion will be adjusted for.

Secondary analyses: potential effect-modifiers of MMR

Age at vaccination/randomisation, age at bleeding, atopic disposition, maternal MMR vaccination, maternal measles/mumps/rubella infection, paternal MMR vaccination, paternal measles/mumps/rubella infection, and vaccine batch. Effect-modifiers such as physiologic stress levels and pollution compounds will be analysed according to distribution and combined with questionnaire data using structural equation models. All secondary analyses will be adjusted for multiplicity.

Power: cohort study of non-specific effects of MMR

Sample size estimates are based on 95%-confidence intervals and 80% power. 10% of the child population in Denmark is expected to be hospitalised from 6 months to 1 year of age (89). To detect a 20% reduction in hospitalisations, 6426 children need to be included (3213 measles vaccinated children and 3213 control children).

Statistics: cohort study of non-specific effects of MMR

Cox proportional hazard models will be used to estimate hazard ratios of hospitalisations for infection after randomisation/vaccination by allocation. The results will be presented as hazard ratios (HR) with 95% confidence intervals (CIs) and p-values of differences between groups. P-values of < 0.05 is considered statistical significant. We will be able to follow-up 100% of the trial population in the public health registries. The children will be censored at migration, 12 months of age, or death, whichever comes first. The analyses will be stratified by sex, study site and prematurity in accordance with the randomisation procedure. In the main “intention-to-treat analysis” age at first hospitalisation within the period from randomisation to 12 months of age will be analysed according to randomisation group. We will also conduct a “per-protocol analysis” in which children who did not follow the allocation will be excluded and time to first hospitalisation will be defined as time since vaccination for the MFR-group and time since randomisation for controls. No interim analysis will be carried out, since data from the study will not be linked to data from the health registries, and no samples analysed until after End-of-Study.

Secondary analyses

We will estimate potential effect modification by breastfeeding, atopic disposition, maternal MMR vaccination, maternal measles/mumps/rubella infection, paternal MMR vaccination, paternal measles/mumps/rubella infection, maternal and child stress levels, maternal and child levels of chemical compounds and vaccine batch. All secondary analyses will be adjusted for multiplicity.

9. Data access

Sponsor and investigators can access all trial data via the e-crf. Study staff can access relevant part of data via the e-crf. For inspection/audit/monitoring, authorities can access all trial data via e-crf.

10. Quality assurance and guarantee

The trial is approved by the Danish Data Protection Board (J.no. 2015-41-4508) and the Committees on Biomedical Research Ethics (Protocol no. H-16041195). Permission is obtained from Lægemiddelstyrelsen/ European Medicines Agency. The trial is registered at www.ClinicalTrials.gov. The trial will be monitored by the Good Clinical Practice Unit of Region Hovedstaden in accordance with §§ 3 and 4 in “GCP– bekendtgørelsen”.

11. Ethics

MMR VaxPro is a widely used, generally well tolerated, safe, and effective vaccine indicated for simultaneous vaccination against measles, mumps, and rubella in individuals from 12 months of age (10;11). MMR VaxPro is used routinely at 15 months in the Danish child vaccination programme (12). All participating children will be urged to follow the Danish child vaccination programme, inclusive routine MMR-vaccination at 15 months of age. Statens Serum Institute recommends of-label vaccination as early as 6 months of age in outbreak situations (15). The rate of adverse events after MMR is lower if the children are vaccinated at 6-8 months of age (18). The World Health Organization recommends 6 months of age as earliest age for measles vaccination (90), Center for Disease Control and Prevention, USA, recommends infant vaccination from 6 months of age before international travelling (91), and the rate of having a protective level of measles antibodies after vaccination at 4-8 months of age was found to be high, 88-89% (92;93). Children with potential vaccine failure because of early vaccination will be routine MMR-revaccinated at 15 months of age. Any child with confirmed or suspected immune suppression will be excluded from the trial.

If the MMR vaccine could be brought forward to 6 months of age in Denmark, this would decrease the risk of disease transmission from infants and increase vaccine coverage. Further, if early measles vaccine has positive non-specific effects and decrease early childhood morbidity, routine early MMR vaccine among Danish infants will reduce early childhood morbidity in Denmark. Moreover, if positive non-specific effects of early measles vaccine are observed among Danish children, the finding may be generalisable to children in other high-income countries. In low-income countries, early measles vaccination would probably move measles infections to older children with milder disease and lower case fatality rates (94).

All parents will be informed written and orally about the MMR-vaccine, inclusive about potential side effects and about how to react if they suspect potential side effects. Written consent from all parents will be obtained before randomisation (Appendix 5, Appendix 6), and the parents can withdraw their consent at any stage without any consequences. Animal models or a trial including adults could not provide the results of the present trial.

Ethical considerations according genetic testing in children as well as in their mothers have been carefully evaluated and discussed. GWAS analysis offers a precise approach and is more likely to reveal novel information. GWAS analysis of SNP-arrays detecting common genetic variances of SNP's associated with immune response, stress and pollution is not an ethical concern, as GWAS only detect variance with minor allele frequency > 4-5%, which means, that rare variants will not be detected. Furthermore, SNP-arrays for GWAS analyses use common SNPs that on their own have no predictive value for the individual, and therefore, these analyses are not covered by the term "extensive mapping of the individual's genome" (in Danish: "omfattet kortlægning af individets arvemasse").

12. Handling and archiving of data

All data will be entered electronically in the e-crf and stored in a pass-word protected safe data portal. After End-of-study, sponsor and investigator will receive an original data copy.

13. Funding

The study is initiated and planned by the researchers and funded by the two involved hospitals, the Innovation fund Denmark (23 million DKK). Trial participants and The Ethical Committee are informed about the funders and amount. The study participants are insured by the Patient Insurance Act. There will be no remuneration for participation in the trial.

14. Publication

The results will be published in international medical journals with the highest impact possible no matter if positive or negative. Lone Graff Stensballe will be the first/last author of the publications of the primary outcomes and the register-based outcomes. Jannet Svensson will be last author on the publications of data analysed at Herlev Hospital regarding the influence of stress and pollution on the immune response after MMR. Eric Simoes co-authors publications reporting the results of analyses from assays supervised by Eric Simoes.

Publications planned for the PhD-student at Rigshospitalet

1. Study protocol publication.
2. MMR at 6 months of age and the risk of hospitalisation for infection from 6-12 months of age.
3. MMR at 6 months of age and the use antibiotics from 6-12 months of age.
4. MMR at 6 months of age and the risk at atopic disease from 6-12 months of age.

Publications planned for the PhD-student at Herlev Hospital

1. Measles vaccine review.
2. MMR at six months of age and IgM and IgG antibodies against measles, mumps and rubella.
3. Adverse events after MMR at six months of age.

4. MMR at six months of age and the level measles neutralising antibodies.

Other publications

1. The associations between the mother's and her child's immunity against measles, mumps and rubella.
2. The influence of maternal stress levels on child's specific immunity response to measles, mumps and rubella vaccination.
3. The influence of maternal perfluoroalkyl substances levels in serum on child's specific immunity response to measles, mumps and rubella vaccination.
4. The influence of child's stress levels on child's specific immunity response to measles, mumps and rubella vaccination.

15. Danish summary

Dansk protokolresumé

Mæslinge-fåresyge-røde hunde-vaccine ved 6 måneders alderen, immunologi, og morbiditet blandt danske småbørn

Sponsor og forsøgsansvarlig

Forskningsleder, børnelæge, ph.d. Lone Graff Stensballe, BørneUngeKlinikken 4072, Juliane Marie Centret, Rigshospitalet, Blegdamsvej 9, DK-2100 København Ø. Tlf.: 35459727. Email: lone.graff.stensballe@regionh.dk

Lone Graff Stensballe har initieret forsøget og er overordnet forsøgs- og data-ansvarlig.

Formålet med undersøgelsen

Mæslinge-fåresyge-røde hunde-vaccinen, (MFR-vaccinen, på apoteket benævnt MMR-VaxPro) er udviklet til at beskytte mod mæslinger, fåresyge og røde hunde. Undersøgelser tyder på, at MFR-vaccinen også beskytter børn mod astma og allergi samt børneinfektioner som forkølelse og lungebetændelse. I dag gives vaccinen ved 15-måneders alderen. Vores undersøgelse blandt 6500 børn skal vise om vaccinen beskytter barnet, hvis man giver den første dosis allerede ved 6-måneders alderen. Desuden vil vi undersøge, om MFR-vaccinen nedsætter barnets sygelighed, herunder hyppigheden af indlæggelser og medicinforbrug. Halvdelen, det vil sige cirka 3250 af børnene, vil ved tilfældig tildeling blive MFR-vaccinerede, når de er 6 måneder gamle og den anden halvdel vil få en vaccine uden aktivt stof. For 600 børn vil vi spørge forældrene, om vi må tage blodprøver på mor og barn, så vi kan bestemme niveauet af antistoffer mod mæslinger, fåresyge og røde hunde. Denne gruppe vil også få tilbudt indsamling af urin, sekret fra luftvejene, spyt og hår til måling af almindelige infektioner, forurening og stress, som potentielt påvirker vaccineresponset. Alle studiedeltagere vil blive opfordret til at følge det almindelige børnevaccinationsprogram.

Forløbet af undersøgelsen

Vi planlægger at sende invitationsbreve til alle cirka 42000 familier i Region Hovedstaden, der i perioden februar 2019 til februar 2021 har et barn, som er 3 måneder gammelt. 2-3 uger efter invitationsbrevet ringer vores sundhedsfaglige personale til familierne for at give mere information om forsøget og svare på spørgsmål. Hvis familien er interesseret i at deltage, inviterer vi familien til en konsultation hos specialuddannet sundhedsfagligt forskningspersonale (læge, sygeplejerske eller medicinstuderende) på Rigshospitalet eller på Herlev Hospital, når barnet er 6 måneder. Vi vil opfordre til at alle forældre er med ved konsultationen og til at forældrene kan tage en bisidder med til konsultationen. Ved konsultationen snakker vi igen om undersøgelsen, svarer på spørgsmål, og, såfremt familien fastholder at ønske at deltage, laver vi et kort interview om forældrenes uddannelse og økonomi, stress og om mæslinger, fåresyge, røde hunde, astma og allergi i familien, søskende, rygning og kæledyr. Vi vil også lave en børneundersøgelse.

Ved konsultationen bestemmer et computerprogram via tilfældig lodtrækning, om barnet skal have MFR-vaccine eller en lignende injektion uden aktivt stof, såkaldt placebo. MFR-vaccinen, der bruges, er den samme som ellers gives i det danske børnevaccinationsprogram, når børnene er 15 måneder. Placebo-injektionen indeholder præcis de samme komponenter som vaccinen, bare uden aktivt stof. Hverken vores personale eller forældrene får oplysning om der er givet aktiv vaccine eller placebo. Injektionen gives af vores personale i låret og tager kun sekunder at indgive. Når der er kommet 6500 børn med i undersøgelsen, stopper inklusionen.

Hvis ikke barnet skal have taget blodprøver, skal der herefter ikke ske mere. Alle børnene anbefales fremover at følge det danske børnevaccinationsprogram.

Beskrivelse af procedurer for afgivelse af mundtlig deltagerinformation

Konsultationen foregår uforstyrret i et dertil indrettet konsultationslokale. Ved konsultationen gennemgås formål med undersøgelsen, inklusions- og eksklusions-kriterier, plan for deltagere i undersøgelsen herunder for 600 familier som giver tilladelse til blodprøver på barnet og biologisk mor, tidsplan for undersøgelsen og bivirkninger igen med familien. Familien kan vælge at underskrive informeret samtykke med det samme eller betænke sig i mindst 24 timer. For familier, der har brug for betænkningstid, planlægges ny konsultationstid.

Skriftlig og mundtlig deltagerinformation omfatter information om:

- Formål med undersøgelsen
- Ulemper, bivirkninger og uventede bivirkninger
- Fordele
- At samtykke omfatter samtykke til at myndighederne kan tilgå de indsamlede oplysninger med henblik på audit og monitorering i henhold til dansk lovgivning

Tidsperioder for forskellige dele af undersøgelsen

- Invitationsbreve udsendes februar 2019 – marts 2021
- Inklusionskonsultation marts 2019 – april 2021
- Klinisk opfølgning-periode indtil børnene er 16 måneder februar 2020 – februar 2022
- Resultater forventes offentliggjort 2023-2025

Eventuelle prøver

Blandt 600 børn vil vi, såfremt forældrene accepterer det, gerne tage 3 blodprøver. Blodprøverne tages af rutineret sundhedspersonale og udgør ingen risiko for barnet. Før prøverne tages, får børnene lokalbedøvende plaster på. Den første blodprøve på 14ml tages ved konsultationen. Ved konsultationen aftales en dato for, hvornår barnet kan komme ind og få taget den anden blodprøve på 14ml en måned efter konsultationen, når barnet er 7 måneder. Den tredje blodprøve på 14ml tages, når barnet er 16 måneder gammelt, cirka en måned efter den MFR-vaccine, alle børn får, når de er 15 måneder. I blodprøverne undersøger vi barnets immunforsvar efter vaccinen og ser, hvor godt MFR-vaccinen beskytter barnet mod mæslinger, fåresyge og røde hunde. Herudover vil vi også undersøge blodet for almindelige infektioner samt for immunforsvar (antistoffer i blodet) mod COVID-19. Vi vil spørge, om vores specialuddannede sundhedsfaglige forskningspersonale (læge, sygeplejerske eller medicinstuderende) må indsamle spyt-, hår og urinprøver på barn og mor til bestemmelse af stressniveau og udsættelse for forurening. Ved konsultationerne vil vi også spørge biologiske mødre til børn, der skal have taget blodprøver, om vi også må tage en blodprøve på 25 ml fra mor. Prøverne fra mor vil blive brugt til at undersøge hvordan mors immunforsvar mod mæslinger, fåresyge og røde hunde påvirker barnets immunforsvar samt til undersøgelse for immunforsvar (antistoffer i blodet) mod COVID-19. Analyserne på blodprøverne vil blive udført i Danmark og USA.

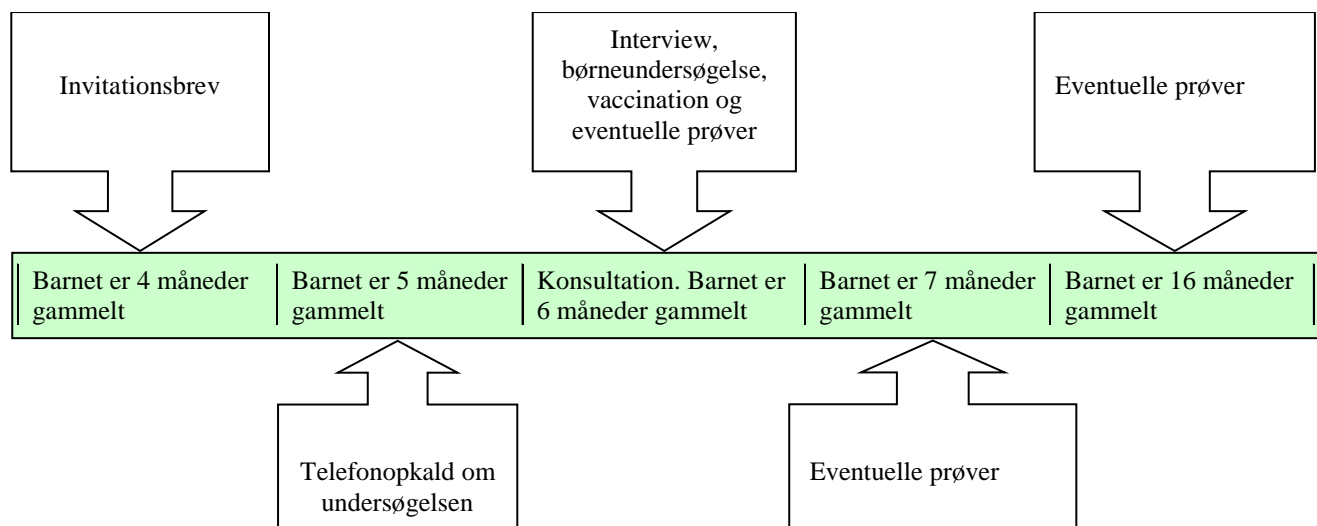
Spyt og hårprøverne vil blive brugt til at analysere cortisol og alfa-amylase, som er stress markører. Derudover vil spyt blive brugt til at genetiske analyser af betydning for immunsystemet, stress og forurening. Urin og serum vil blive brugt til at analysere forurenings markører (polychlorinated biphenyls og perfluoroalkyl substanser), og serum vil blive brugt til at analysere for inflammatoriske signalstoffer (cytokiner) samt almindelige infektioner. Vi vil tillige udføre næse-svælgpodninger på alle mødre og børn, som deltager i prøvetagning, med henblik på PCR-undersøgelse for COVID-19.

Prøverne vil herefter blive sikkert opbevaret i fryseskab i biobank i Danmark med henblik på mulige fremtidige analyser. Videnskabsetisk Komite vil blive ansøgt ved fremtidige analyser af blodet i biobanken. I kan når som helst bede om at blodprøven destrueres, så den ikke kan indgå i flere analyser.

Resultater

Vi følger børn, der også bidrager med blodprøver, indtil de er 16 måneder, for at undersøge deres immunforsvar mod mæslinger, fåresyge og røde hunde. Vi følger alle børnenes til de er 2 år for lægebesøg, indlæggelser og medicinforbrug i de danske sundhedsregistre. Resultaterne af undersøgelsen offentliggøres i internationale medicinske tidsskrifter. Når forsøget er afsluttet, vil man kunne følge studiet forløb og et resumé af resultaterne på studiets hjemmeside. Her vil også findes oplysninger om uventede bivirkninger eller andre risici. Studiet afsluttes december 2023 og resultaterne forventes offentliggjort fra 2023.

Plan for undersøgelsen vist som figur



Nytte ved undersøgelsen

Undersøgelsen vil give viden om vi kan give MFR-vaccinen allerede ved 6-måneders alderen og dermed beskytte børnene mod tidlig smitte. Desuden vil vi finde ud af, om MFR-vaccine gavner børnenes helbred udover at beskytte mod smitte. Deltagere, der vaccineres med tidlig MFR-vaccine forventes at opnå nogen, muligvis fuld, tidlig beskyttelse mod smitte med mæslinger, fåresyge og røde hunde.

Bivirkninger, risici, komplikationer og ulemper

MFR-vaccinen har været en del af det danske børnevaccinationsprogram siden 1987 og de mulige bivirkninger er velkendte. Der er ikke aluminium i MFR-vaccinen. Se Tabel 1 nedenfor og produktresumé på http://www.ema.europa.eu/docs/da_DK/document_library/EPAR_-_Product_Information/human/000604/WC500030170.pdf.

Alvorlige bivirkninger er yderst sjældne og deltagelse i forsøget vurderes derfor kun at medføre en meget lille men dog reel risiko, som opvejes af de mulige betydningsfulde fordele for deltagerne i form af tidlig beskyttelse mod mæslinger, fåresyge og røde hunde og nedsat risiko for astma og infektioner blandt deltagere, der får MFR-vaccine.

Studiedeltagerne er fuldt forsikrede i henhold til Patientforsikringsloven.

Bivirkning	Hypighed
Forkølelse	Ikke almindelig 1/1000 til 1/100
Løbende næse	Ikke almindelig 1/1000 til 1/100
Diaré og opkastning	Ikke almindelig 1/1000 til 1/100
Udslet	Almindelig 1/100 til 1/10

Feber (38.5°C eller højere). Rødme, ømhed og hævelse på injektionsstedet	Meget almindelig Mere end 1/10
Blåt mærke på injektionsstedet	Almindelig 1/100 til 1/10
Kløe på injektionsstedet	Ikke almindelig 1/1000 til 1/100
Feberkramper	3-16/10000 (16;17)
Fald i antal af blodplader i blodet (thrombocytopeni) som kan medføre blødningstendens	Ukendt

Der kan være risici ved forsøget, som vi endnu ikke kender. Vores specialuddannede sundhedsfaglige forskningspersonale (læge, sygeplejerske eller medicinstuderende) indsamler derfor også oplysninger om evt. problemer med helbredet den første 6 uger efter vaccination.

Hvem kan ikke være med i undersøgelsen

Børn født før 32. uge af svangerskabet og/eller med fødselsvægt < 1000gram, børn med tegn til defekt i immunforsvaret, syge børn med behov for intensiv behandling på sygehus, børn hvor ingen af forældrene taler dansk, børn som har kendt allergi overfor æg, børn med fruktose-intolerans, børn med trombocytopeni (lavt antal blodplader) eller anden blodsygdom, børn med koagulationsdefekt og børn som får eller har fået immunsupprimerende medicin eller blod/plasma/immunglobulin-transfusion indenfor 3 måneder udelukkes fra forsøget.

Gruppen bag undersøgelsen

Undersøgelsen ledes af børnelæge Lone Graff Stensballe fra BørneUngeKlinikken på Rigshospitalet og udføres i samarbejde med børnelæge, Jannet Svensson, fra Børne- og Unge-afdelingen på Herlev Hospital.

Oplysninger om økonomiske forhold

Undersøgelsen er planlagt af børnelæge Lone Graff Stensballe og er finansieret af fondsmidler fra Innovationsfonden (23 millioner kr.). Der ydes ikke vederlag for deltagelse i studiet. Der er ingen økonomisk tilknytning mellem de forsøgsansvarlige og støttegiverne.

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