v4_23 Nov 2018

Clinical Trial Protocol:

The Vaccination Infant Supplementation (VISS) Study

Randomised placebo-controlled trial investigating the effect of 8 weeks supplementation with probiotics and vitamin D around routine childhood immunisation on infant's ear temperature, growth, and sleeping pattern in 4-24 month-old infants.

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The Vaccination Infant Supplementation (VISS) Study

Randomised placebo-controlled trial investigating the effect of 8 weeks supplementation with probiotics and vitamin D around routine childhood immunisation on infant's ear temperature, growth, and sleeping pattern in 4-24 month-old infants.

Infant vaccination numbers in the city of Stonnington and Bundoora have declined over that past decade due to concern over the effect of vaccination. There has been increasing concern that vaccination may adversely affect a child's immune system. However, given the nature of vaccine preventable diseases it is understood that the advantages of disease prevention and vaccination in the community are a priority.

Recent evidence shows that Vitamin D and probiotic supplementation has a beneficial effect on a child's immune system.

This project aims to examine the effect of Vitamin D and probiotics on a child's immune function before and after vaccination in a placebo and treatment group. This project will assess temperature daily for 2 months, pre and post immunisation in a supplemented (Vitamin D and probiotic) and placebo group.

AUSTRALIAN IMMUNISATION SCHEDULE [1]
The current Australian routine immunisation schedule is:

Birth	• Hepatitis B (hepB)[a]
2 months	• **Infanrix hexa**: Hepatitis B, diphtheria, tetanus, acellular pertussis (whooping cough), *Haemophilus influenzae* type b, inactivated poliomyelitis (polio) (hepB-DTPa-Hib-IPV) • **Prevenar 13:** Pneumococcal conjugate (13vPCV) • Rotavirus
4 months	• **Infanrix hexa:** Hepatitis B, diphtheria, tetanus, acellular pertussis (whooping cough), *Haemophilus influenzae* type b, inactivated poliomyelitis (polio) (hepB-DTPa-Hib-IPV) • **Prevenar 13:** Pneumococcal conjugate (13vPCV) • Rotavirus
6 months	• **Infanrix hexa:** Hepatitis B, diphtheria, tetanus, acellular pertussis (whooping cough), *Haemophilus influenzae* type b, inactivated poliomyelitis (polio) (hepB-DTPa-Hib-IPV) • **Prevenar 13:** Pneumococcal conjugate (13vPCV)
12 months	• *Haemophilus influenzae* type b and meningococcal C (Hib-MenC) • Measles, mumps and rubella (MMR)
18 months	• Diphtheria, tetanus, pertussis (whooping cough) (DTPa) • Measles, mumps, rubella and varicella (chickenpox) (MMRV)
4 years	• Diphtheria, tetanus, acellular pertussis (whooping cough) and inactivated poliomyelitis (polio) (DTPa-IPV)

VITAMIN D
Vitamin D is an important immune regulator. Vitamin D receptors are present on immune cells such as B cells, T cells, and dendritic cells. Vitamin D can therefore modulate the innate and humoral immune responses. Patients deficient in Vitamin D are both more susceptible to infection as well as more susceptible to autoimmunity (Aranow 2011).[2]

Infants who are breastfed are recommended 400IU Vitamin D per day as per the American Academy of Paediatrics. This recommendation is based on expert opinion and recent clinical trials measuring biomarkers of vitamin D status (Greer 2004).[3] The Natural Medicines Database Safety information on Vitamin D states: When used long-term, doses should not exceed the tolerable upper intake level

(UL). Infants from 0-6 months should not exceed the UL of 1000 IU daily. Infants aged 6-12 months should not exceed the UL of 1500 IU daily. Children aged 1-3 years should not exceed the UL of 2500 IU daily.[4]

PROBIOTICS

Probiotics are also important in both the innate and humoral immune responses. Probiotics can modulate the function of dendritic cells, macrophages and B and T cells (Yan 2011).[5] Probiotics given to infants during vaccination may increase their seroconversion rate (del Giudice 2014).[6]

Infants from 3 months recommended 2.3g (1 tsp) of probiotic blend containing: 6 billion Lactobacillus rhamnosus, 3.75 billion Lactobacillus acidophilus and3.75 billion Bifidobacterium lactis. There are multiple studies which have included these probiotics in infants 3months and over which have demonstrated safety of use (Gritz 2015, Saavedra 2007).[7,8]

References

1. AUSTRALIAN IMMUNISATION SCHEDULE 2018 http://www.immunise.health.gov.au/internet/immunise/publishing.nsf/Content/Handbook10-home
2. Aranow C. Vitamin D and the Immune System. Journal of investigative medicine 2011; 59 (6): 881–886.
3. Greer FR. Issues in establishing vitamin D recommendations for infants and children. Am J Clin Nutr 2004; 80 (6 suppl):1759S–1762S.
4. EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)2, Scientific Opinion on the Tolerable Upper Intake Level of vitamin D1 EFSA Journal 2012;10(7):2813.
5. Yan F & Polk DB. Probiotics and Immune Health. Current opinion in gastroenterology 2011; 27 (6): 496–501.
6. del Giudice M, et al. Probiotics and Vaccination in Children. J Vaccines and Vaccination 2014; 5:3.
7. Gritz E & Bhandari V. The Human Neonatal Gut Microbiome: A Brief Review. Frontiers in Pediatrics 2015; 5 (3): 17.
8. Saavedra JM. Use of Probiotics in Paediatrics: rationale, mechanisms of action, and practical aspects. Nutrition in Clinical Practice 2007; 22 (3): 351-65.

STUDY DESIGN

Randomised parallel double-blind placebo-controlled trial of 2 months duration investigating the effect of supplementation with vitamin D and probiotics on behavioural and physiological response in infants during routine childhood immunisation.

Participants

Inclusion criteria

- Group 1 (preferred): Infants who have not yet been immunised (age range: 4-24 months).
- Group 2: Infants who have been immunised (age range: 4-24 months).
- Parents have agreed to have their child immunised.
- Normal infant growth as per WHO growth charts: http://www.education.vic.gov.au/Documents/childhood/parents/mch/mchgrowthboy24mths.pdf
- http://www.education.vic.gov.au/Documents/childhood/parents/mch/mchgrowthgirl24mths.pdf
- No tympanic temperature > 38 degrees Celsius in the preceding 4 weeks prior to immunisation
- Infants currently breastfed (e.g. exclusively or breast and formula fed)
- Infants currently not taking supplements (Vitamin D and/or probiotics)
- Infants who will remain in the same routine with primary carers for the 2 month study period
- Willing to contribute 50% ($30) of the cost price for ear thermometer

Exclusion criteria

- Infant has any severe allergies (particularly corn or maize products, as probiotics and placebo powder contains maltodextrin)
- Infant has a disease that lowers their immunity (ie. leukaemia, cancer, HIV/AIDs)
- Infant is having treatment that lowers immunity (ie. steroids, immunotherapy, chemotherapy)
- Infant's mother is receiving highly immunosuppressive therapy (ie. biological disease modifying anti-rheumatic drugs (bDMARDs)
- Infant has had an immunoglobulin injection, received any blood products or blood transfusion)
- Infant has had a past history of Guillain-Barre syndrome
- Infant has a chronic illness
- Infant has a bleeding disorder
- Infant is ATSI (Aboriginal Torres Strait Islander)
- Infant does not have functional spleen
- Infant lives with someone who is immunocompromised (ie. leukaemia, cancer, HIV/AIDS, chemotherapy)

If any of the factors listed in the exclusion criteria (e.g. bleeding disorders, allergies to maize) are unknown at the beginning of the study and are discovered during the study, the infant has to withdraw from the study, and standard medical care and follow-up will be provided.

Intervention/ Comparison

Sample size: n=50
Active group: n=25
Placebo group: n=25

Active group: 1000 IU Vitamin D & 2.3g Probiotics daily for 2 months
a) Vit D3 Metagenics 1000 IU with 0.25ml dropper
Metagenics Vitamin D3 90 mL oral liquid (360 days)
Storage: Store below 30°C. After opening store at 2°C to 8°C. (Refrigerate. Do not freeze.)

b) Probiotics High Strength Inner Health for Kids (Metagenics)
Each 2.3 g dose (1 level metric teaspoon) contains a blend of probiotic strains:
6 billion *Lactobacillus rhamnosus* (GG) organisms (LGG®)
3.75 billion *Lactobacillus acidophilus* (NCFM) organisms
3.75 billion *Bifidobacterium lactis* (Bi-07) organisms

Placebo group:
a) Placebo liquid contains medium-chain-length triglycerides (Metagenics R0121)
b) Placebo powder contains maize maltodextrin (Metagenics R0232)

TIME LINE

Appointments with doctor:

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0 weeks 4 weeks 8 weeks
Enrolment Vaccination Final visit
Start of daily trial supplementation End of trial supplementation
Daily temperature/ sleep pattern recorded

Outcome measures

Primary (1), Secondary (2)	**Outcome measures**	**Tests**	**Equipment**	**Lab/ location**	**Time points**
1	Tympanic ear temperature	Temp	Thermometer	NIIM, home	Daily incl 0, 4, 8 weeks
1	Weight, Growth	scales	Growth chart, weight chart	NIIM	0,4,8 weeks
2	Sleep / cry	Q/ diary	Daily diary for infant collection	NIIM, home	Daily
2	Other symptoms	Q	Colds, mouth ulcers, rashes	NIIM, home	0,4,8 weeks
2	Mood/ Stress	Bond Lader Q	Main carer	NIIM	0,4,8 weeks
2	Sleep	Q	Main carer	home	Daily

Assessments:

Baseline data collection:

- Infant's mother's Vit D supplementation & plasma levels (if known) – during pregnancy and current
- Birth week of gestation, prematurity
- Vaginal, or caesarean birth
- Vit K given to infant at birth?
- Any genetic screening or other test results
- Infant's medication, e.g. PPI for reflux

Growth: Weight and size
For size and weight measurements, standard scales will be use available at the clinic at all appointments.
Other symptoms Questionnaire
Incl. Common Cold Symptoms, mouth ulcers, rashes, other

Tympanic ear temperature
Daily 7pm measurements using the Infrared Thermometer ET100B, available from Medshop Australia.

Sleep/Cry questionnaire for infants
Daily diary booklet assessing sleep and cry pattern of the infant will be provided to participants.

Sleep/ Mood of carer
The **Bond Lader Mood Q** will be administered to the main carer at NIIM at each visit at 0, 4 and 8 weeks.
In addition, a **sleep diary** will be provided to the main carer, to record a summary of the daily sleep pattern including length and quality of sleep.
These Sleep & Mood Data of the main carer are collated as an indirect measure of the child's health, as the infant can't express in words about their feelings.

STUDY PLAN

PARTICIPANT RECRUITMENT

Patients will be recruited through the NIIM clinic newsletter, NIIM website, facebook, flyers, and advertisements in Melbourne (maternal outreach Booroondara and Stonnington).

Enrolment & randomisation

The doctor and chief investigator TN will meet patients at the NIIM clinic to confirm eligibility and to collect the infant's care givers informed consent. Two groups will be recruited into this pilot study, group 1: vaccination-naïve (not yet immunised) infants 4-24 months into the study, and group 2: vaccinated infants 4-24 months.

Eligible infants will be enrolled and randomised. A computer-generated permuted block randomisation schedule prepared by a researcher not involved in patient recruitment and data collection will be used to allocate patients to the active or placebo groups.

Trial medication

The active treatment group (n=25) will be allocated to 1000 IU Vitamin D (Metagenics) + 2.3 g probiotics (Inner Health Kids, Metagenics) daily for 2 months. The control group (n=25) will be allocated to matching placebo drops and powder. Placebo drops and powder, supplied by Metagenics, will be matched in quantity and appearance (odour and colour). Care givers of participating infants will be advised to provide the trial supplements with food in the morning.

Drops and powder can be mixed into the infant formula. If infant is exclusively breastfed, the drops can be given directly into the mouth, the powder can be mixed into water and supplied by spoon.

Compliance & Tolerability

Compliance and tolerability will be assessed at each visit by Questionnaire and count of remaining trial supplements (drops/powder).

Allocation & blinding

Active and placebo drops and powder will be packaged and labelled offsite in identical containers by the manufacturing company. Patients, as well as doctors and investigators will be blinded to the group allocation. Coding will remain with the manufacturer until completion of the trial. Blinding success of patients will be evaluated at the end of the trial by questionnaire.

Visits & Assessments

Visit 1 – week 0 - baseline

Enrolment, informed consent by GP and chief investigator TN.

Baseline data collection:

- Infant's mother's Vit D supplementation & plasma levels (if known) – during pregnancy and current
- Birth week of gestation, prematurity
- Vaginal, or caesarean birth
- Vit K given to infant at birth?
- Any genetic screening or other test results
- Infant's medication, e.g. PPI for reflux

Assessments: weight, growth, temperature, other symptoms, Carer: Mood Q

Supply of supplements for 4 weeks – and discussion of administration

Supply of diaries: Daily 7pm temperature, infant sleep/cry = infant home diary; Carer sleep diary = Length and Quality of sleep recorded daily)

Visit 2 – week 4 – vaccination
The infant will only be eligible to be vaccinated if its temperature had been less than 38 degrees Celsius in the 4 weeks prior vaccination and since enrolment (at week 0).
Eligibility will be assessed by phone call 1-2 days before the scheduled appointment by NIIM receptionists.
If the infant had run a temperature > 38C in the last 4 weeks, the appointment will be postponed until 4 weeks after the fever.
If eligibility criteria are met, the original appointment will be confirmed.

1) **Assessments:** Weight, growth, temperature, other symptoms, collection of infant home diary and carer sleep diary; Carer: Mood Q; Tolerability & compliance Q

2) **Vaccination:** first immunisation of the Australian standard paediatric immunisation schedule:
a) **Infanrix hexa: c**ombined Diphtheria-Tetanus-acellular Pertussis (DTPa), hepatitis B, poliovirus and haemophilus influenzae type B vaccine
b) Prevenar 13: Pneumococcal polysaccharide conjugate vaccine, 13-valent adsorbed
Infanrix hexa and Prevenar 13 Information sheets will be provided by TN to the participating carers.

- Immunisation to be given by same medical doctor and investigator TN for all participants.

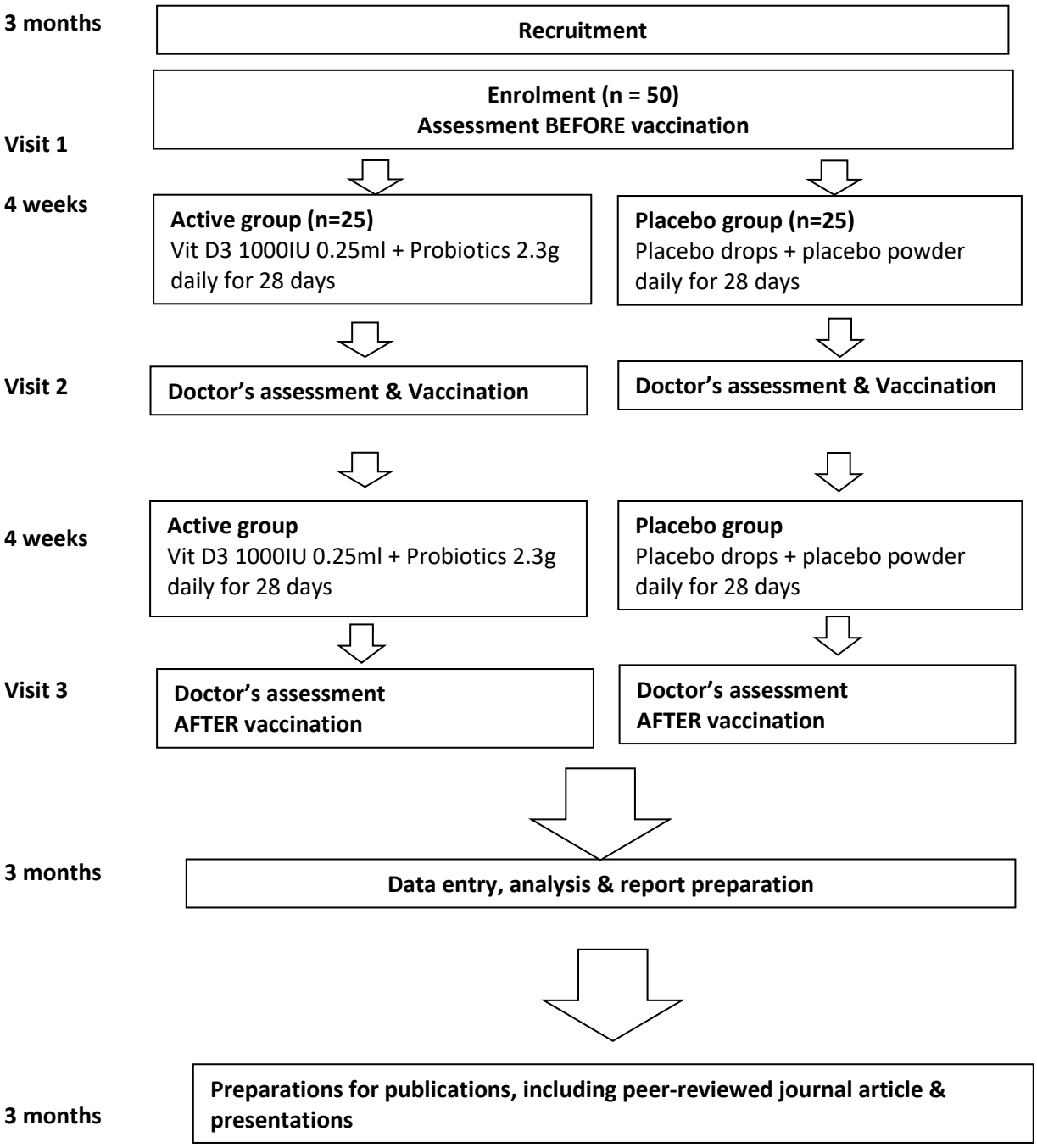
3) Supply of supplements for 4 weeks – and discussion of administration
Supply of diary: Daily 7pm temperature, infant sleep/cry

Visit 3 – week 8 – final visit
1) **Assessments:** Weight, growth, temperature, other symptoms, collection of infant home diary and carer sleep diary; Carer: Mood Q; Tolerability & compliance Q
& Blinding

Sample size & Justification
A sample size of **50 participants**: Active group: Vit D + probiotic (n=25); Placebo group (n=25) suggested for this pilot study to obtain baseline values and variance for a larger study.

Analysis
Analyses will be performed using SPSS (PASW version 18). Statistical significance will be set at $p<0.05$. Between-group differences will be analysed by ANOVA comparing data after vaccination at 4 weeks (mean of daily data 4-8 weeks incl temperature, sleep/cry, carer sleep; or infant weight/growth data or carer mood data at 8 weeks) to data before vaccination (mean of daily data 0-4 weeks; or at 0 and 4 weeks).

FLOW CHART


3 months
Recruitment
Visit 1
Enrolment (n = 50)
Assessment BEFORE vaccination
4 weeks
Active group (n=25)
Vit D3 1000IU 0.25ml + Probiotics 2.3g daily for 28 days
Placebo group (n=25)
Placebo drops + placebo powder daily for 28 days
Visit 2
Doctor's assessment & Vaccination
Doctor's assessment & Vaccination
4 weeks
Active group
Vit D3 1000IU 0.25ml + Probiotics 2.3g daily for 28 days
Placebo group
Placebo drops + placebo powder daily for 28 days
Visit 3
Doctor's assessment AFTER vaccination
Doctor's assessment AFTER vaccination
3 months
Data entry, analysis & report preparation
3 months
Preparations for publications, including peer-reviewed journal article & presentations