Statistical analysis plan (SAP)

Section 1: Administrative information

1a. Trial and Trial registration

Full study title: Bowel And Bladder function in Infant Toilet Training (BABITT) - a randomized, two-armed intervention study, to evaluate assisted infant toilet training as prevention of functional gastrointestinal and urinary tract disorders in children up to the age of 4 years

Acronym: BABITT

1b. Trial registration number: ClinicalTrials.gov identifier: NCT04082689

2. SAP Version: 2. 3, 2023-10-29

3. Protocol Version: October 29, 2023, protocol version 2.3

4a. SAP revisions: No major revisions.

5. Roles and responsibility:

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Riccardo Lo Martire, Statistician at the Center for Clinical Research (CKF) Dalarna.

Section 2: Introduction

7. Background and rationale

In the last decades, the average age for toilet training has increased in the western world and it is suggested that the postponed initiation of toilet training is a contributing factor to problems related to bowel and bladder control. This randomized intervention study aims to investigate whether assisted infant toilet training can prevent functional gastrointestinal and urinary tract disorders in children in a Swedish primary care setting.

8. Objectives

The overall objective is to obtain knowledge on whether assisted infant toilet training, initiated during the first year of life, affects the period prevalence of functional gastrointestinal or urinary tract disorders up to 4 years of age.

Primary research question

- Is the period prevalence of functional gastrointestinal disorders (constipation, infant dyschezia and infant colic) reduced in children introduced to assisted infant toilet training at 0-3 months of age (**Group A**), as compared to children who are ***not*** introduced to assisted infant toilet training (**Group B**), up to 9 months of age?

Secondary research questions

- Is the period prevalence of functional gastrointestinal disorders (constipation, gastrointestinal symptoms and/or stool toileting refusal) reduced in children introduced to assisted infant toilet training at 0-3 months of age (**Group A**), compared to children

introduced to toilet training at 9-11 months of age (**Group B**), when followed up until 4 years of age?

- Is the period prevalence of urinary tract disorders (bladder dysfunction and/or urinary tract infections) reduced in children introduced to assisted infant toilet training at 0-3 months of age (**Group A**), compared to children introduced to toilet training at 9-11 months of age (**Group B**), when followed up until 4 years of age?

- Does infant-to-mother attachment differ in families when initiating assisted infant toilet training at 0-3 months of age (**Group A**), compared to families ***not*** practicing assisted infant toilet training (**Group B**), up to 9 months of age?

- Does parental stress differ in families when initiating assisted infant toilet training at 0-3 months of age (**Group A**), compared to families ***not*** practicing assisted infant toilet training (**Group B**), up to 2.5 years of age?

- Are there differences regarding the toilet training process in children introduced to assisted infant toilet training at age 0-3 months (**Group A**), compared to children introduced to assisted infant toilet training at age 9-11 months (**Group B**), when followed up until 2.5 years of age?

- What are the overall parental experiences of assisted infant toilet training initiated during the first year of life (**Group A** and **Group B**), up until 2.5 years of age?

Section 3: Study Methods

9. Trial design:

The **BABITT**-study is designed as a randomized, controlled, investigator blinded, two-armed intervention study with follow-up until 4 years of age. It targets several well-defined clinical outcome measures with a superiority hypothesis in favor for assisted infant toilet training.
The intervention consists of parents performing assisted infant toilet training, Group A starting at inclusion to the study (but no later than the age of 3 months) and Group B starting at 9-11 months of age.

10. Randomization

Participants are randomly assigned to either **Group A** or **Group B** with a 1:1 allocation, using stratified block randomization. The randomization sequence is generated by the researchers responsible for inclusion by using a computer program designed by MediCaseAB. Allocation concealment will be ensured since randomization is only possible after the researchers have completed all the baseline data in the electronical clinical research form (eCRF) provided by MediCaseAB. All activity in the eCRF is available by electronic logs and audit trails. The randomization is stratified by the gender of the child with a nondisclosed block size within strata to ensure concealment. Because of the nature of the intervention, the parents are informed of the group allocation directly.

11. Sample size

Sample size was determined from a power calculation of the period prevalence of functional gastrointestinal disorders (functional constipation, infant dyschezia or infant colic) at age 9 months.

- *Functional constipation*: the reported prevalence ranges between 0.7-30% and unpublished data from an ongoing Swedish cohort indicate 7% prevalence at 1 year of age (*GC*, personal communication).
- *Infant dyschezia:* the reported prevalence ranges between 0.9-3.2% and unpublished data from an ongoing Swedish cohort indicate 18% at 2 months of age and 4% at 6 months of age (*GC*, personal communication).
- *Infant colic:* the reported prevalence ranges between 17-25% at 6 weeks of age.

Based on this information, we argue that the prevalence of at least one of these disorders could be expected to 15% in the general population (congruent with **Group B)** combined with an assumed prevalence of 4% in children who have been introduced to infant toilet training (congruent with **Group A**). With 80% power and a significance level of 0.05 in a two-sided test for comparing two independent proportions, 121 children are needed in each intervention arm. Allowing for 10% missingness, 134 (=121×1.1) children are needed in each group, which amounts to totally 268 children in the **BABITT**-study.

12. Framework

The study is a randomized two armed intervention study with several well-defined clinical outcome measures with a superiority hypothesis testing in favor for assisted infant toilet training.

13a. Statistical interim analysis and stopping guidance

None planned or executed.

14. Time frame

Web surveys are conducted at 2, 3, 6, 9, 12, 18 months and at 2, 2.5 and 4 years. A 28-day window (defined as 14 days before and 14 days after the due date), is allowed for the 2, 3 and 6-month survey. At 9 months, a 44-day window is allowed (14 days before and 30 days after the due date) and at 12, 18 months and at 2 and 2.5 years a 60-day window is allowed (30 days before and 30 days after the due date). At 4 years of age a 120-day window will be allowed (30 days before and 90 days after the due date).
For the rectal ultrasound measurement at 9 months of age, a 60-day window will be desired (14 days before and 46 days after the due date). At 4 years of age, a 120-day window will be desired (30 days before and 90 days after the due date).

Analyses for the primary outcome measure will be performed once all participants has completed the 9 months web survey. Other secondary outcome measures will be performed when the participants have passed the time window stated in the study protocol. Last web survey and ultrasound measurement is at 4 years of age.

Table 1. Time frame overview.

		t_{-1}	t_0	t_1	t_2	t_3	t_4	t_5	t_6	t_7	t_8	t_9
				Web surveys (month from birth)								
Activity/assessment	**Staff**	**Eligibility screening**	**Intervention allocation**	**2**	**3**	**6**	**9**	**12**	**18**	**24**	**30**	**48**
Enrolment												
Screening interview	Physician	X										
Informed consent	Physician	X										
Demographics questionnaire	Physician	X										
Intervention allocation	Physician		X									
Assessments												
Infant colic				X	X							
Infant dyschezia				X	X	X	X					
Functional constipation				X	X	X	X	X	X	X	X	X
Use of laxatives				X	X	X	X	X	X	X	X	X
Rectal diameter							X					X
Gastrointestinal symptoms (PedsQLGastro)												X
Stool toileting refusal												X
Bladder dysfunction												X
Urinary tract infections							X			X	X	X
Infant-to-mother attachment					X		X					
Parental stress					X		X		X		X	
Intervention adherence				X	X	X	X	X	X	X	X	
Statistical analysis												
Primary analysis: Gastrointestinal disorders combined							X					
Secondary analyses							X	X	X	X	X	X

Section 4: Analysis

16. Analytical principles

Data will primarily be analyzed according to the intention-to-treat principle, whereby participants are coded in the analyses according to their assigned intervention. Secondarily, data will be analyzed according to the per-protocol principle, whereby only participants that adhered to their assigned intervention are included in the analysis. Analyses will be based on a population model, with alpha set at 0.05 and two-sided hypothesis tests and confidence intervals. Secondary outcome analyses will not be multiplicity adjusted.

The primary outcome is period prevalence of functional constipation, infant dyschezia and infant colic up to 9 months of age defined according to the ROME IV criteria. The sample size calculation was made on estimated period prevalences of functional gastrointestinal disorders (functional constipation, infant dyschezia and infant colic) in Group A and Group B at 9 months of age. Thus no adjustments for multiple analyses will be performed.

19. Adherence and Protocol deviations

19a. Definition of adherence to the intervention and how this is assessed including extent of exposure

In each web survey the parents are asked to what extent they perform assisted infant toilet training. Parents of children allocated to group B are asked in the web survey at 2, 3, 6 and 9 months of age, if they are obeying the group allocation and not undertaking assisted infant toilet training. To be active in assisted infant toilet training is defined by the making of at least one attempt a day (without the requirement of a successful outcome) on at least 5 out of 7 days per week for Group A at the 3 month survey and for Group B at the 12 month survey.

19b. Description of how adherence to the intervention will be presented

For all the web surveys adherence to assisted infant toilet training for the specific age will be presented until the child is reported using diapers less than 25% of the time daytime per day. Information of number of attempts to assisted infant toilet training will be presented (e.g. 8 or more attempts per day, 4-7 attempts per day or 1-3 attempts per day).

19c. Definition of protocol deviations for the trial

It will be presented the amount of children in Group B that perform assisted infant toilet training regularly before 9 months of age (e.g. not adhering to the allocated group of starting between 9-11 months of age) at the 2, 3, 6 and 9 months web surveys. All participants that state in the web surveys that they are not performing assisted infant toilet training (less than one attempt a day less than 5 out of 7 days per week) will be presented; for Group A starting at the 3 month survey and for Group B starting at the 12 month survey until the child is considered diaper free (using diapers less than 25% daytime). Parents are encouraged to leave comments in a free text section in the web surveys, in case of non-adherence to the intervention.

19d. Description of which protocol deviations will be summarized

Deviation from the definition of being active in assisted infant toilet training, deviation of timing in starting assisted infant toilet training depending on group allocation and withdrawal from participation (and if known reason) of the study will be presented at each time point.

20. Analysis populations

The primary analysis will be performed in accordance with intention-to-treat principles. For hypothesis generating reasons, per protocol analyzes will also be presented for primary and secondary analyses.

Section 5: Trial Population

22. Eligibility

Inclusion Criteria

- Full-term infant (born at gestational week 37+0 to 41+6)

Exclusion Criteria

- Infants with malformations or disorders that may affect the gastrointestinal or urinary tract in any relevant way
- Infants born small for gestational age (SGA), <-2 SD
- Parents with insufficient understanding of the Swedish language
- Infants older than 2 months, 1 week and 6 days at inclusion

Inclusion and exclusion criteria were chosen so the study population would represent normal healthy children in a Swedish child care setting. Premature born and born small for gestational age are often affected of initial gastrointestinal trouble, therefor they were excluded. Understanding of the Swedish language is necessary since the questionnaires are in Swedish and correct scientific translation to various languages was not feasible.

23. Recruitment

Information of all new born children during the study period at the participating CHC centers will be presented, and when know reason for declining participation, numbers of enrolled participants, lost to follow up or discontinued (with reasons when known) and analyzed or excluded from analysis (with reasons) will be presented in a CONSORT flow diagram.

24a. Withdrawal/ Follow-up

Participants withdrawn or lost to follow-up will be presented for each group an each time point in the CONSORT flow diagram.

24b. Timing of withdrawal/lost to follow-up data

Timing of withdrawal or loss to follow-up will be presented for each group and each time point in the CONSORT flow diagram.

24c. Reasons and details of how withdrawal/lost to follow-up data will be presented

Reasons and details will be presented in a CONSORT flow diagram and in text in the results.

25a. Baseline characteristics

Data on participating children (gender, weight at birth, gestational age at birth) as parent reported information at the inclusion in the study. **Socio-demographic data on parents** (age of the parents, country of birth, educational level, presence of siblings and family history of functional constipation) will be collected at baseline, as parent reported information at the inclusion in the study.

25b. Details of how baseline characteristics will be descriptively summarized

Data will be presented as absolute and relative frequencies for period prevalence. Categorical variables will be presented as mean ± SD and non-normally distributed variables as median ± IQR .

Section 6: Analysis

26. Outcome definitions

26a. Specification of outcomes and timings

Primary outcome

The primary outcome measure is the period prevalence of functional gastrointestinal disorders (i.e., either infant colic, infant dyschezia or functional constipation, defined according to ROME IV criteria) from birth and to the age of 9 months, as parent-reported symptoms in the web surveys (web survey at 2, 3, 6, 9 months of age).

Secondary outcomes

The secondary outcomes are the period prevalence's of functional gastrointestinal disorders (defined as constipation, gastrointestinal symptoms and/or stool toileting refusal) and urinary tract disorders (defined as bladder dysfunction and/or urinary tract infections) up to the age of 4 years, with the specific secondary outcome measures as described below:

1a. The period prevalence of functional constipation (defined according to ROME IV criteria) will be evaluated up to 4 years of age, as parent-reported symptoms in the web surveys (web surveys at 2, 3, 6, 9, 12, 18 months and at 2, 2.5 and 4 years)

1b. The use of laxatives as treatment for functional constipation will be evaluated as parent reported information in the web surveys (and if necessary medical journal will be revised). Children diagnosed and/or prescribed laxatives will be counted as functional constipation, since symptoms may appear in between the web surveys (web surveys at 2, 3, 6, 9, 12, 18 months and at 2, 2.5 and 4 years).

1c. Rectal diameter, as a complementary measure of functional constipation, will be measured by abdominal ultrasound at 9 months and 4 years of age. A rectal diameter > 30mm is considered a sign of functional constipation at 4 years of age. The method is adapted from a manual by the University of Gothenburg (personal communication). To date, there is no knowledge of what is considered normal rectal diameter at the age of 9 months, but studies are ongoing (personal communication). An inter-intra-rater agreement analysis will be performed on the measurement results of the specialized staff of the BABITT-study.

2. Gastrointestinal symptoms will be measured with a Pediatric Quality of Life Inventory Gastrointestinal Symptoms Module (PedsQLGastro) at 4 years of age. The Swedish validated version of PedsQLGastro is used. PedsQLGastro contains 58 items. The items are reverse-scored and linearly transformed to a 0-100 scale (0=100, 1=75, 2=50, 3=25, 4=0), so that lower scores demonstrate more gastrointestinal symptoms, and hence, lower disease specific health-related quality of life. The scale scores are computed as the sum of the items divided by the number of items answered.

3. The period prevalence of stool toileting refusal will be evaluated at the age of 4 years, as parent-reported symptoms in the web survey. Stool toileting refusal is defined as children who can urinate in the potty/toilet without difficulties, but refuse to have a bowel evacuation in the potty/toilet, during a period of more than one month.

5. The period prevalence of bladder dysfunction will be evaluated at the age of 4 years, as parent-reported symptoms in the web surveys. For bladder dysfunction the ICCS terminology is used and for diagnosis a bladder symptom questionnaire is used, validated in a Swedish population with a scoring system and cut-off for dysfunction. When parents report that their child is diaper free (using diapers less than 25% of the time daytime per day) they will be able to answer the bladder symptom questionnaire at the questionnaires before the age of 4 years.

6. The period prevalence of urinary tract infections will be evaluated up to 4 years of age as parent-reported information in the web surveys (web survey at 9 months, 2,5 and 4 years). If stated, the

medical record will be reviewed. Diagnosis of urinary tract infection is defined by the diagnostic numbers N.30.0 (acute cystitis), N30.9 (cystitis UNS) and N10.9 (acute pyelonephritis).

7. Infant-to-mother attachment, will be measured with the Maternal Postnatal Attachment Scale (MPAS) at 3 and 9 months. The MPAS scale contains 19 items regarding mothers' emotional response to their infants and their attachment. Each item is scored 1-5. Total score is calculated by adding scores on all items. Scale range for total score is 19-95. A higher score indicates higher infant-to-mother attachment.

8. Parental stress will be measured with the Swedish Parenthood Stress Questionnaire (SPSQ) as parent-reported information in the web surveys (web survey at 3, 9 and 18 months and 2.5 years). SPSQ is an adapted Swedish version of the Parental Stress Index. It measures perceived stress in parenting in five dimensions (incompetence, role restriction, social isolation, spouse relationship and health problems) and contains 34 items, each scoring 1-5. Total score is calculated as an average of answers (1-5) on all items. Scale range for total score is 34-170. A higher score indicates higher parental stress.

Additional data collection

1. Data on intervention adherence and fidelity to allocation will be collected until the toilet training process is completed, as parent-reported information in the web surveys (web surveys at 2, 3, 6, 9, 12, 18 months and at 2, 2.5 and 4 years).

2. Data on day care services (the child's attendance at day care service and parental experience of the day care service's provided support concerning potty training and toilet habits) will be collected up to 4 years of age, as parent-reported information in the web surveys (web surveys 18 months and at 2, 2.5 and 4 years).

3. Parental experiences of the toilet training process up to 4 years of age will be explored, as parent-reported experiences in the web surveys (web surveys at 2, 3, 6, 9, 12, 18 months and at 2, 2.5 and 4 years). The web surveys contain one item with a 6-point rating scale and a free text area.

4. Breastfeeding and nutrition will be presented up to 12 months of age for each group in table format.

5. Data on evaluation of the intervention will be collected as parent-reported information in the web survey at 18 months.

26b. Specific measurement and units

Not applicable.

26c. Any calculation or transformation used to derive the outcome

The primary outcomes is defined according to the ROME IV criteria and the period prevalence in the two different groups is sought.

27. Analysis methods

27a. What analysis method will be used and how the treatment effects will be presented

Primary analysis:

Logistic regression will be used to compare the period prevalence of functional gastrointestinal disorders (i.e., the collapsed composite of infant colic, infant dyschezia, and functional constipation) from birth to 9 months of age between group A and group B, adjusted for the child's gender (the randomization stratification factor). Treatment effects will be presented in the form of the relative

risk ratio and the absolute risk difference. Point estimates with confidence intervals will be presented both for functional gastrointestinal disorders overall and for infant colic, infant dyschezia, and functional constipation individually.

Secondary analyses:
The period prevalence of functional constipation will be compared according to the primary analysis procedure per time point from 9 months and forward. In this analysis, both the parent-report period prevalence according to the ROME IV criteria, the parent-report use of laxatives, and physician-measured rectal diameter will be included as functional constipation indicators, tested both as a collapsed composite and individually.
For functional constipation and answers will also be analyzed with Mann-Whitney test according to the frequency of the symptoms stated by the parents in the web surveys- always (approximately 100 % of the times), often (approximately 75 % of the times), sometimes (approximately 50 % of the times), seldom (approximately 25% of the times).
The period prevalence of stool toilet refusal, bladder dysfunction, and urinary tract infection will be compared according to the primary analysis procedure at the time points specified in Table 1.

Gastrointestinal symptoms, infant-to-mother attachment, and parental stress will be compared with the Mann-Whitney test at the time points specified in Table 1.

Secondary analyses will not be multiplicity adjusted. Finally, supplementary analyses may be conducted for hypothesis-generative purposes.

27b. Any adjustment for covariates
Adjustments will be made for the stratification factor the child's gender.

27c. Methods used for assumptions to be checked for statistical methods
Model diagnostics will be conducted using a simulation-based approach of the scaled (quantile) residuals.

28. Missing data
Missing values will be replaced using multiple imputation. Sensitivity analysis will be used to evaluate the results robustness.

29. Additional analyses
For secondary outcomes analyses will be performed with similar methods as other continuous/ categorical variables.

30. Harms
If adverse effects are described by the parents in the free commentary areas in the web surveys or if reported by CHC nurse they will be described in text or table format. No serious adverse effects are expected.