Study Protocol - Effects of Simethicone and the Multi-strain Synbiotic Multilac Baby on Fecal Calprotectin in Colicky Babies

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Cover page

1. General Information

Infantile colic or extensive crying is a major burden for newborns, their parents and healthcare providers [Landgren and Hallström, 2011]. In most of the cases infantile colic will disappear after the first three to five months of life [Sung, 2018]. Nevertheless, due to its stressful nature for parents, infant colic is among the leading causes to consult a health care professional during early infancy [Lucassen, 2010]. The parental burden associated with infantile colic finally results in enormous pressure on pediatricians to prescribe at least some kind of remedy. Diagnosis of infantile colic can be based on the Wessel's criteria, defined by crying and restlessness for more than three hours a day for more than three days per week for more than three weeks [Wessel et al., 1954]. Depending on diagnostic details, occurrence rates between 3 to 40% of all infants have been found [Lucassen et al.2001, Helseth and Begnum, 2002].

2. Background Information

The etiology of infant colic remains unclear [Sarasu et al., 2018], with a variety of potential causes (gastrointestinal, hormonal, neurodevelopmental, and psychosocial) discussed. This has resulted in a broad range of therapeutic approaches aiming to address the problem [Lucassen, 2010, Mai et al, 2018].

Simethicone [Simethicone], a mixture of dimethicone and SiO_2, is a rather old product that, it is claimed, acts as a topical barrier for protecting the gut mucosa against irritants. It is not absorbed and is virtually non-toxic. While its use in diagnostic procedures is well-established, the therapeutic effects in a number of gastroenterological indications are contradictory [Meier and Streuwald, 2007]. In infantile colic, a number of smaller simethicone trials [Metcalf et al., 1994, Sethi and Sethi, 188, Danielsson and Hwang, 1985] have been published, but evidence did not reach the threshold of significance. Despite the absence of evidence for beneficial effects, simethicone is widely used for the treatment of infantile colic in some countries.

There is growing evidence that the gut microbiotas of colicky infants significantly differ from those of non-colicky babies [Savino et al., 2004, Savino et al., 2005, Savino et al., 2009]. In the gut microbiota of colicky babies a lower level of commensal bacteria like lactobacilli and bifidobacteria and higher numbers of proteobacteria have been found. Among these proteobacteria were Escherichia and Klebsiella bacteria [Savino et al., 2011], which are well known for their gas-producing properties, as well as the potential production of inflammatory lipopolysaccharides (LPS). In addition, it has been described that the gut microbiota of colicky infants exhibits a slower bacterial colonization, a reduced microbiota diversity and a lower microbiota stability [De Werth et al., 2013]. These findings have triggered a number of studies investigating the effect of supplementation of the gut microbiota of colicky

babies with products containing probiotic bacteria without a prebiotic component (probiotics), or with (synbiotics).

Although infantile colic is in most cases a self-limiting condition, pediatricians are writing a prescription for most of the affected infants. Results from surveys, performed among German and Polish pediatricians, revealed that in these two countries simethicone and pro-/synbiotics were the most frequently prescribed products for infantile colic [Sommermeyer et al., 2020]. In a recently published study it was found that the multi-strain synbiotic, Multilac Baby, antagonizes the in-vitro growth of the pathogenic bacteria Escherichia coli EPEC, Shigella sonnei, Salmonella typhimurium, Klebsiella pneumoniae and Clostridioides difficile [Piatek et al. 2020]. A clinical study evaluating effects of simethicone and Multilac Baby demonstrated that the synbiotic significantly improved the crying behavior of colicky babies when compared with the effects of simethicone [Piatek et al., 2020, accepted for publication in Beneficial Microbes].

Findings of a disturbed gut-microbiota in colicky babies have triggered interest in investigating gut-inflammation in these babies. An established marker for the level of gut inflammation marker is calprotectin [Stríz & Trebichavský, 2004]. Calprotectin is a 24 kDa dimer of calcium binding proteins S100A8 and S100A9 [Pthirana et al., 2018]. The complex accounts for up to 60% of the soluble protein content of the neutrophil cytosol. The complex is resistant to enzymatic degradation, and can be easily measured in feces. Inflammatory processes result in an influx of neutrophils into the bowel lumen [Walsham & Sherwood, 2016] Since calprotectin comprises as much as 60% of the soluble protein content of the cytosol of neutrophils, it can serve as a marker for the level of intestinal inflammation Measurement of fecal calprotectin has been shown to be strongly correlated with 111-indium-labelled leucocytes - considered the gold standard measurement of intestinal inflammation [Costa et al., 2003; Gisbert & McNicholl, 2009]. So far, there is only one study published which has characterized the effects of the probiotic L. reuteri on the level of fecal calprotectin in colicky babies [Savino et al., 2018]. Based on the findings of this study it was concluded that probiotics have the potential to improve gut-inflammation in babies with infantile colic.

3. Objectives/Purpose

The objective of the study is to compare the effects of simethicone and the multi-strain synbiotic Multilac Baby on calprotectin levels in colicky and non-colicky babies.

4. Study Design

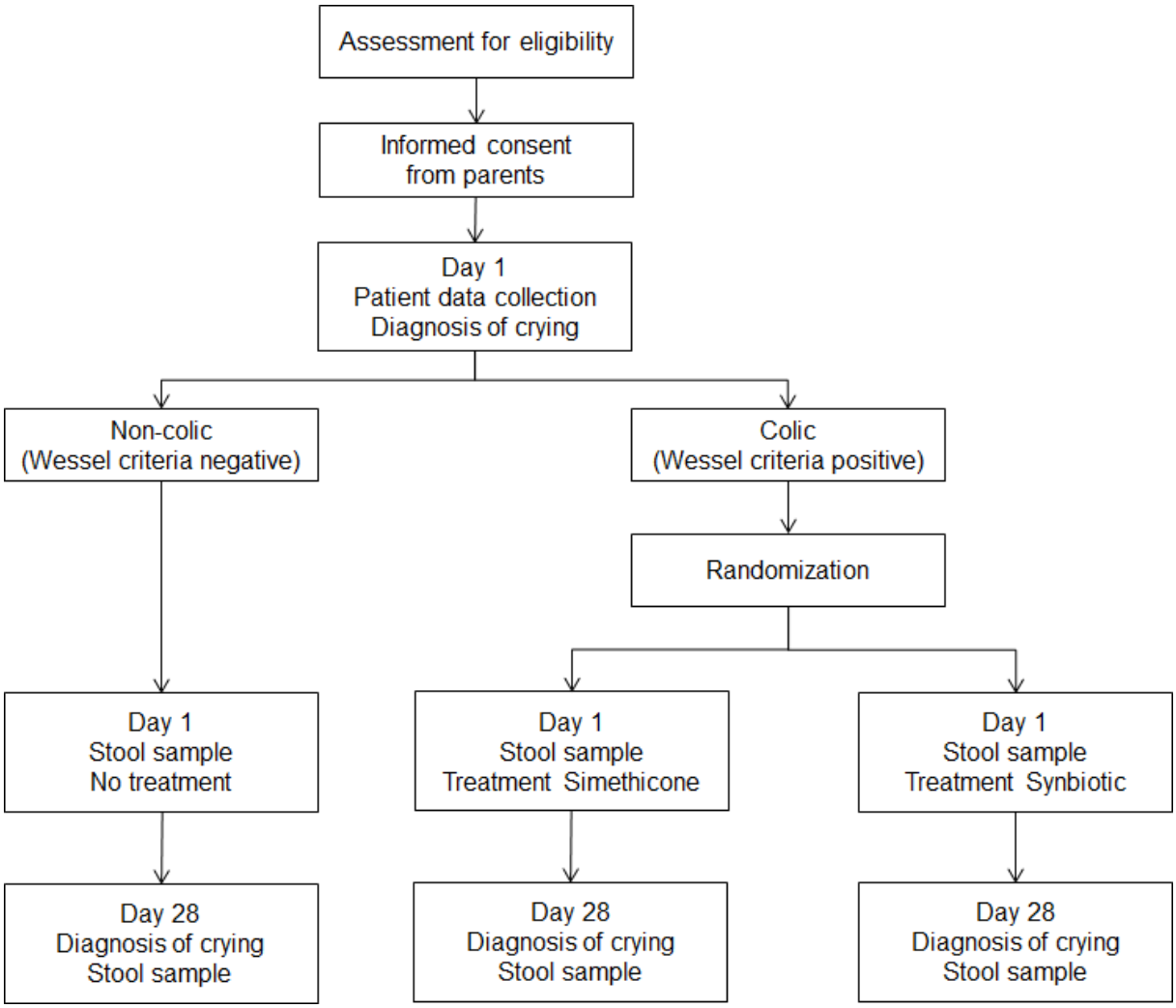
In a first step, the crying behavior of newborns shall be assessed by employing a parental daily diary for three weeks. Diagnosis of infantile colic will be based on the Wessel criteria (Rule of Three) [Wessel et al., 1954]: extensive evening crying for at least three hours per day, during at least three days per week, during the last three weeks.

After the diagnosis has been performed (Day 1), calprotectin shall be determined in non-colicky babies (Arm 1, n=30-40) and colicky babies (Arms 2&3, each n=50-60). Based on the results from this steps it will be possible to determine if average calprotectin levels of colicky babies are different compared to those of non-colicky babies.

Colicky babies will then randomly assigned to a treatment with simethicone (Arm 2, n=50-60) and treatment with Multilac Baby (Arm 3, n=50-60). Treatment duration will be four weeks (until Day 28). Crying behavior will be assessed with a parental daily diary during the last three weeks before the end of treatment. At the end of the treatment (Day 28), calprotectin level in feces will be performed by using a point of care test (Buehlmann Quantum Blue Calprotectin).

5. Selection and Exclusion of Subjects

Work stream



Inclusion criteria

The study is aiming to recruit babies aged 3 to 6 weeks of aged.

Exclusion criteria

- Organic causes for crying
- Previous treatment with antibiotics
- Previous treatment with probiotic
- Previous treatment with synbiotic

6. Treatment of Subjects

Calculation

https://www.sealedenvelope.com/power/binary-superiority/

Settings

Significance level (alpha)	5%
Power (1-beta)	80%
Percentage 'success' in control group	50%
Percentage 'success' in experimental group	75%
Sample size required per group	55
Total sample size required	110

Randomization

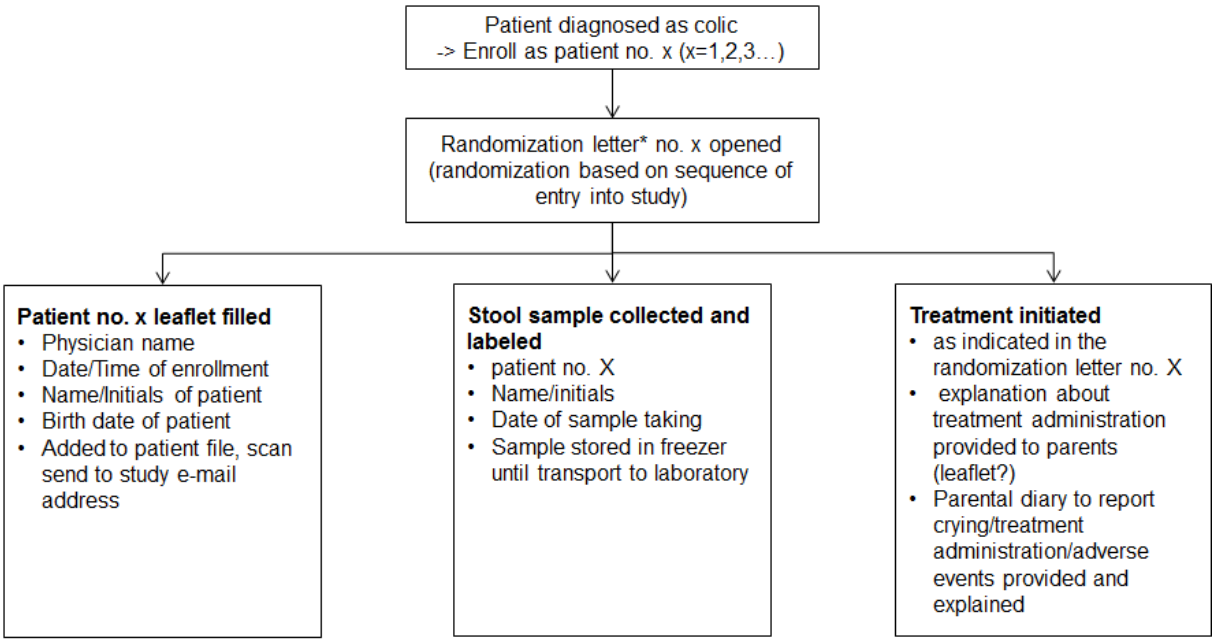
A two-treatment equal allocation randomization scheme will be used. Product will be allocated to patient based on the randomization scheme by the pediatrician on entry into the study.

https://www.sealedenvelope.com/simple-randomiser/v1/lists

Settings

Treatment groups	Group A, Group B
Block sizes	2,4,6,8,10
List length	100

Randomization process



***Content of randomization letter (labeled with study title and sequential number)**

- Patient leaflet with information about randomly allocated treatment
- Information leaflet for parents about the treatment administration (either synbiotic or simethicone)
- Parental diary for monitoring crying, administration of treatment, adverse events

Control Group – Non-colicky Babies (Arm 1)

It is planned to include 30-40 babies to this arm to determine the calprotectin levels in non-colicky babies.

Simethicone (Arm 2)

Treatment for four weeks with simethicone (Espumisan®, 100 mg/ml, Berlin-Chemie / Menarini Polska Sp z o.o., Warsaw, Poland). Simethicone administered 3-6 times per day with each treatment comprising 6 drops of the 100 mg/ml emulsion. It is planned to include 50-60 patients to this arm.

Multilac Baby (Arm 3)

Treated for four weeks with one stick pack of a multi-strain synbiotic (Multilac® Baby, Vivatrex GmbH, Aachen, Germany) per day. Each stick pack of Multilac® Baby contains a total of 10^9 colony forming units (CFU) with equal CFU amounts of the following probiotic bacteria: L. acidophilus LA-14, L. casei R0215; L. paracasei Lpc-3; L. plantarum Lp-115; L. rhamnosus GG, L. salivarius Ls-33, B. lactis Bl-04, B. bifidum R0071, B. longum R0175 and 1.43 g of the prebiotic fructooligosaccharides. It is planned to include 50-60 patients to this arm.

7. Assessment of Efficacy

Crying behavior

Crying behavior will be assessed by using a parental diary (24-hour Parental Daily Report). Primary outcome measures are (i) “crying days last 3 weeks” and (ii) “average of daily evening crying duration last 3 weeks” and (iii) change of fecal calprotectin level between (Day 1 and Day 28).

Gut calprotectin level

Calprotectin will be measured in feces samples taken before start of treatment (Day 1) and at the end of treatment (Day 28) with Simethicone and Multilac Baby.

8. Assessment of Safety

Safety will be assessed by using a parental diary (24-hour Parental Daily Report).

9. Adverse Events

Adverse Events will be reported by the standard procedure established to report adverse events of pharmaceutical products by physicians.

10. Discontinuation of the Study

The study will be discontinued in case of:

- occurrence of adverse events,
- failure to recruit patients.

11. Statistics

The statistical analyses will be conducted with GraphPad Prism software version 8.2 (GraphPad Software, Dan Diego, California, USA) or MedCalc Statistical Software version 19.2.1 (MedCalc Software Ltd., Ostend, Belgium). The Student t-test will be used to compare mean values of continuous variables approximating a normal distribution. For non-normally distributed variables, the Mann-Whitney U test will be used. The X2 test or Fisher exact test will be used, as appropriate, to compare percentages. The MedCalc Statistical Software version 19.2.1 will be used to calculate the relative risk (RR) and number needed to treat (NNT) [Altman, 1998], all with a 95% confidence interval (CI). The difference between the treatment groups will be considered significant when the p-value will be less than 0.05, when the 95% CI for RR will not include 1.0, or when the 95% CI for mean difference will not include 1.0, or when the 95% CI for mean difference will not include 0. One-way ANOVA followed by Dunnett's multiple comparisons test as well as Cochran-Armitage Chi-squared test for trend will be performed using GraphPad Prism software. All statistical tests will be two-tailed and will be performed at the 5% level of significance. All analyses will be conducted on an intention-to-treat basis, including all patients in the groups to which they will be allocated.

12. Quality Control and Assurance

According to local GCP SOPs.

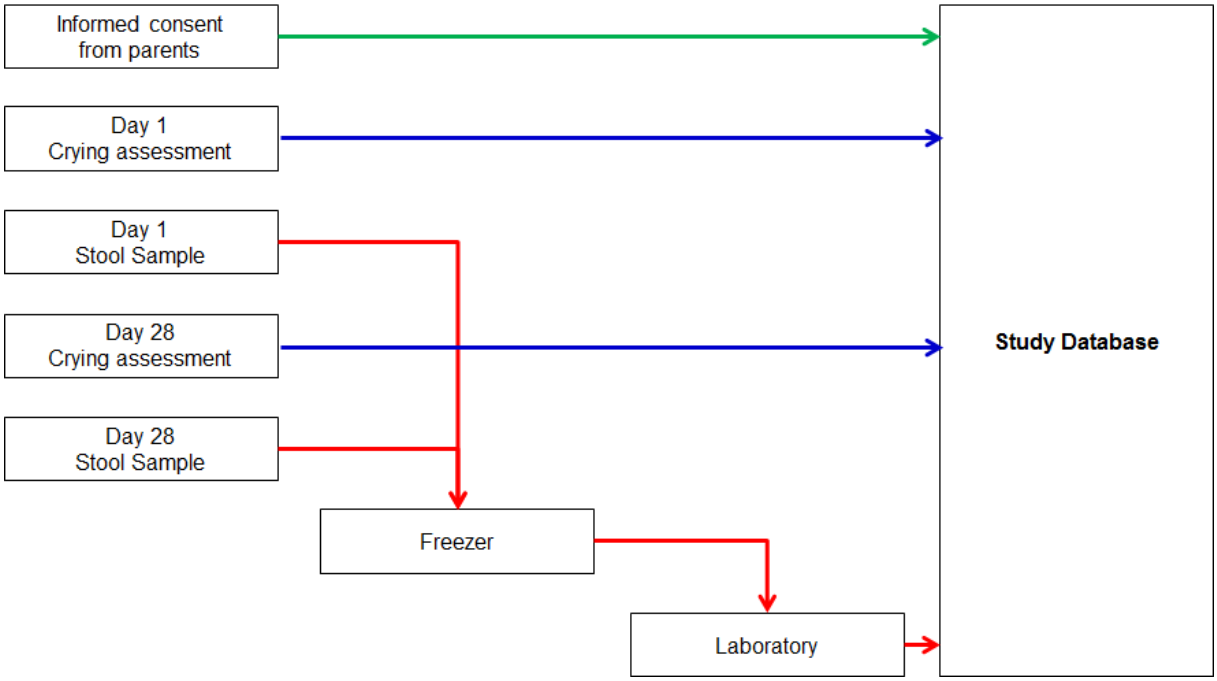
13. Ethics

Study will have to be approved by the Ethics Committee of The President Stanisław Wojciechowski State University of Applied Sciences in Kalisz.

Informed consent of parents of colicky babies will be collected from parents in writing before enrolling of the babies into the study.

14. Data handling and Recordkeeping

Data will be handled by study investigators monitored and supervised by the principle investigator of the study (Prof. Dr. Hanna Kraus). Records will be kept at the office of the principle investigator applying all local regulations of data protection provisions.



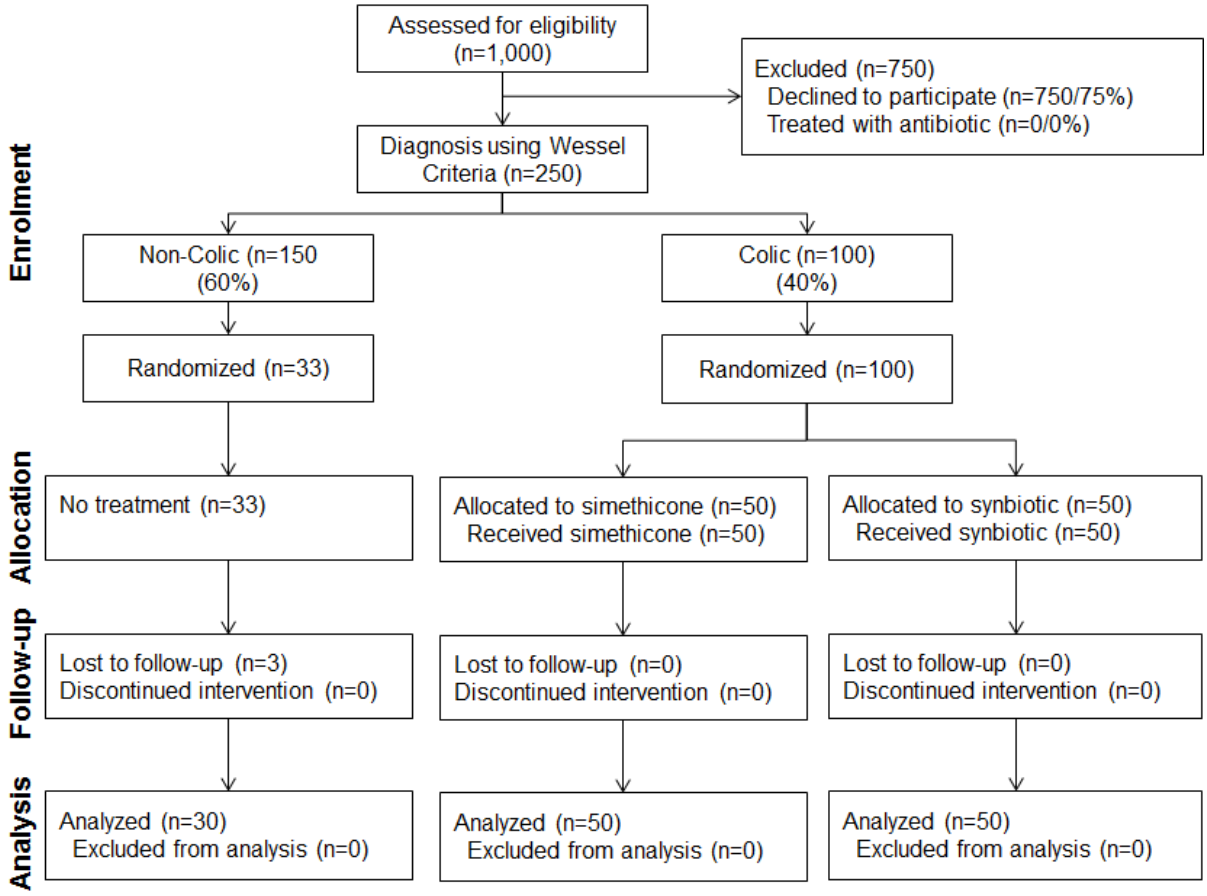
15. Publication Policy

It is planned to publish the study results in a peer-reviewed scientific/medical journal.

16. Project Timetable/Flowchart

Start of study after approval by the ethics committee and establishment of the calprotectin measurements. Start of the study is expected in 12/2020.

Patient enrollment and study progress:



17. References

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18. Supplements/Appendices

Randomization Letter

Parental Letter Multilac Baby

Parental Letter Espumisan

Patient Assessment Sheet Day 1 EN/PL

24 Hours Parental Diary Form EN/PL