# Statistical analysis plan

## Sample size

*A priori* sample size calculation based on the expected difference between groups for size of breast hardness area over 3 days (unpublished data from previous study by primary supervisor Leanda McKenna) revealed a sample of n= 156 (52 per group) has 80% power to show an effect size difference f= 0.20 (based on mean difference= 2.0cm2, standard deviation (SD)= 5.0cm2), in a linear mixed model with 3 groups and 4 timepoints (G\*Power V3.1.9.4)1. The expected difference in breast milk sodium levels produced similar mean and SD values, thus we are adequately powered for breast milk analysis.

A blinded interim analysis will be conducted by the trial’s statistician when this minimum sample size (n= 156) has been reached, specifically to assess the distribution of antibiotic use across the trial groups. If significant disproportion exists, further recruitment may be necessary to allow statistical comparison. At any time throughout the trial, women will be able to seek medical treatment, given ICLBs can rapidly progress in severity to a serious illness2, 3.

## Statistical methods

Data analysis will be blinded and completed according to intention-to-treat (Figure 1, Appendix 1). Stata (StataCrop, College Station, TX) will be used to perform a generalised linear mixed model (GLMM) analysis of the relationship between the 3 different TUS treatments and the outcomes listed in Table 1 (Appendix 2). Antibiotic use, for the current ICLB episode, will be entered as a fixed effect into the model. Treatment fidelity will be inspected and potentially entered into the model as confounders. Visual inspection of residual plots will be undertaken to evaluate normality and homogeneity of variance, and 95% confidence intervals and p-values will be obtained by likelihood ratio tests.4

## References

1. Faul F, Erdfelder E, Buchner A, Lang AG. Statistical power analyses using G\*Power 3.1: tests for correlation and regression analyses. *Behavior Research Methods.* 2009;41(4): 1149-1160.

2. Harris AD, McGregor JC, Perencevich EN, Furuno JP, Zhu J, Peterson DE, et al. The use and interpretation of quasi-experimental studies in medical informatics. *Journal of the American Medical Informatics Association : JAMIA.* 2006;13(1): 16-23.

3. Osterman KL, Rahm V-A. Lactation Mastitis: Bacterial Cultivation of Breast Milk, Symptoms, Treatment, and Outcome. *Journal of Human Lactation.* 2000;16(4): 297-302.

4. Tango T. *Repeated Measures Design with Generalized Linear Mixed Models for Randomized Controlled Trials*. New York: Chapman and Hall/CRC; 2017.

## Appendices

### Appendix 1

#### Figure 1: Modified CONSORT flow diagram

Graphical user interface, application, Word

Description automatically generated

Graphical user interface, application

Description automatically generatedGraphical user interface, application

Description automatically generated

### Appendix 2

#### Table 1: Outcomes

|  |  |  |
| --- | --- | --- |
|  | Outcome | Timepoint |
| Primary outcome [1] | **Size of breast hardness area (in cm2),** measured via tracing the area of hardness on to cling wrap with a surgical pen. Cling wrap tracings will be scanned into Adobe and the area calculated electronically. This measures area of local inflammatory symptoms. | *Timepoint 1*: baseline (day 1, pre intervention).  *Timepoint 2*: 1 day after intervention commencement (day 2, post intervention).  *Timepoint 3*: 2 days after intervention commencement (day 3, post intervention) - primary timepoint.  *Timepoint 4*: 10 days after intervention commencement. |
| Secondary outcome [1] | **Breast milk sodium and potassium levels** as measured by an ion selective electrode (ISE). This is to measure local and systemic inflammatory symptoms. | *Timepoint 1*: baseline (day 1, pre intervention).  *Timepoint 3*: 2 days after intervention commencement (day 3, post intervention).  *Timepoint 4*: 10 days after intervention commencement. |
| Secondary outcome [2] | **Breast Inflammatory Symptom Severity Index (BISSI) scores** - a patient reported outcome measure (copyright: Melinda Cooper, physiotherapist), with face and content validity established. This is to measure local and systemic inflammatory symptoms. | *Timepoint 1*: baseline (day 1, pre intervention).  *Timepoint 2*: 1 day after intervention commencement (day 2, post intervention).  *Timepoint 3*: 2 days after intervention commencement (day 3, post intervention).  *Timepoint 4*: 10 days after intervention commencement. |