Makani Science:

Pivotal Clinical Study for the Makani Science Respiration Monitoring System

Statistical Analysis Plan

Version 1.1 – 05 Aug 2022
Based on Protocol CP-0001 (18 Jul 2022)

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1. SYNOPSIS

The purpose of this study is to demonstrate that the Makani Science Respiration Monitoring System (RMS) can accurately measure respiratory rate in adult subjects compared to manual respiration rate counting by a trained individual (reference). The Makani Science RMS is a non-invasive system intended to monitor a patient's breathing. It graphically displays respiration versus time and reports an approximate value of respiratory rate. Its measurements are used as an adjunct to other clinical information sources. The Makani Science RMS is intended to be used by healthcare professionals in healthcare facilities and dental offices on adult patients.

This study is designed to evaluate the accuracy, bias, and precision of the Makani Science RMS as compared to manual counting for measuring respiratory rate in adult subjects in both supine and reclined positions. This is a prospective, non-blinded, open-label, single-arm study. Participants will follow the protocol-specified breathing procedures and respiratory data will be simultaneously recorded for each participant from: (1) Makani Science RMS, and (2) manual respiration counting performed by a trained individual. The planned enrollment is 30 subjects, and eligible participants will be healthy adults 22-99 years of age with body mass index (BMI) between 15 and 35 kg/m^2. Enrollment will be targeted to balance normal (BMI < 25) and overweight (BMI ≥ 25) subjects. An interim analysis is planned after 50% of subjects have completed the study.

2. ABBREVIATIONS

Abbreviation	Description
AE	Adverse Event
BMI	Body Mass Index
CV	Coefficient of variation
ITM	Intent to Monitor
PG	Performance Goal
PP	Per Protocol
RMS	Respiration Monitoring System
RMSPE	Root Mean Squared Percentage Error
RR	Respiratory Rate
SD	Standard Deviation

3. STUDY OBJECTIVES

The primary objective of the study is to evaluate the accuracy of the Makani Science RMS as compared to manual counting by a trained individual (reference) for measuring respiratory rate (RR) in adult subjects in both supine and reclined positions. The secondary objectives of the study are to evaluate the precision and bias of the Makani Science RMS as compared to manual counting.

4. ENDPOINT DEFINITIONS AND PERFORMANCE GOAL DERIVATIONS

4.1 Endpoint Definitions

Endpoints for this study include RR accuracy, percentage bias, and precision.

RR accuracy will be estimated using root mean squared percentage error (RMSPE). RMSPE will be calculated using the paired difference between log-transformed Makani Science and reference RR measurements analyzed in a mixed-effects linear model with random subject effects to account for repeated measurements. The RMSPE will be estimated as the sum of the squared bias, the within-subjects variance and the between-subjects variance, back-transformed with the usual equation for the coefficient of variation (CV), $\sqrt{e^{\sigma^2} - 1}$, and expressed as a percentage:

$$\text{RMSPE} = 100 * \sqrt{e^{(bias^2 + \sigma^2_{betw} + \sigma^2_{wi})} - 1}$$

RR percentage bias and precision (CV) will be calculated using a similar mixed-effects linear model as above and expressed as percentages. The bias is the estimated geometric mean paired difference from the model, back-transformed and expressed as a percentage:

$$\text{Bias} = 100 * (e^{bias} - 1)$$

and the precision is the CV or the standard deviation of the square root of the sum of the within-subjects variance and the between-subjects variance, back-transformed and expressed as a percentage:

$$\text{Precision} = 100 * \sqrt{e^{(\sigma^2_{betw} + \sigma^2_{wi})} - 1}$$

4.2 Performance Goal (PG) for Accuracy

The performance goal (PG) for both primary endpoints is set at 20%, based on Atkinson et al. (1) using the ExSpiron respiratory volume monitor by Respiratory Motion Incorporated.

5. SAMPLE SIZE

It is estimated that 28 subjects are needed to achieve an 80% probability of rejecting the null hypothesis at a significance level of 0.025, assuming the standard deviation of the RMSPE is 4.51% as observed in a pilot study for data collected in the supine position, and the observed RMSPE in this Pivotal study is 17.5% compared to a performance goal of 20%. This was calculated using the One Sample T-tests Procedure of NCSS Power Analysis Statistical Software, Version 20 (PASS 20, (2)). Assuming a potential 5% dropout rate, 30 subjects (28/0.95) will be enrolled. An interim analysis is planned once 50% of the subjects have completed the study to verify these assumptions and re-estimate the sample size if needed.

Enrollment will be targeted to balance normal (BMI < 25) and overweight (BMI ≥ 25) subjects.

6. INTERIM ANALYSIS

Once 50% of the planned subjects have completed the study, an interim analysis of RR accuracy will be performed and sample size re-estimated. The conditional probability of demonstrating accuracy in the supine position at the end of the trial, given the interim results, will be calculated. If this conditional probability is less than 50%, the study will continue until 30 subjects are enrolled per the current protocol; if the conditional probability is greater than or equal to 50%, the study will proceed to completion with the new sample size, capped at a maximum of 50 participants. The new sample

size will be calculated as the sample size necessary to yield an 80% conditional probability of trial success (i.e., meeting both primary endpoints), given the interim results. If the study continues following the interim analysis, the sponsor will remain blinded to the primary endpoint results and no overall alpha adjustment is required. This procedure has been shown by Gao et al. to control the overall significance level at 5% [3].

7. ANALYSIS POPULATIONS

All subjects who meet the inclusion and exclusion criteria and are enrolled in the study will be considered Enrolled Subjects.

Since it is required that both Makani Science RMS and reference RR measurements are available to calculate the study endpoints, only time frames in which both were properly measured will be utilized. All Enrolled Subjects with simultaneous measurements for any amount of time will be included in the Intent to Monitor (ITM) population.

8. STATISTICAL METHODS

The number of observations, mean, standard deviation (SD), median, minimum and maximum will be calculated for continuous variables, unless otherwise stated. The number of significant digits reported will be as follows: minimum and maximum will be reported with the same number of significant digits as the raw data; the mean, median, and SD will be reported with one more significant digit than the raw data. Frequencies and percentages will be calculated for categorical data using one significant digit for percentages.

As a routine function in applying statistical procedures, the assumptions underlying those procedures will be evaluated. Tests for normality will not be done for continuous variables, but data will be inspected for symmetry through histogram plots. Parametric test procedures will be supported by non-parametric procedures as appropriate.

Statistical procedures specified in this analysis plan include mixed effects linear modelling to estimate RMSPE, percentage bias, and precision, as well as bootstrapping methods to calculate confidence intervals.

A per-protocol analysis will be performed for each monitoring position (supine and reclined). Each analysis will include subjects who completed the full breathing protocol for the position.

8.1 Data Pooling

This study will be conducted at one study site, thus data pooling is not a consideration.

8.2 Missing Data

The primary analysis uses a mixed-effects repeated measures model, which assumes data are missing at random and incorporates all available information from all subjects.

8.3 Multiplicity

Types of multiplicity to address in the analyses of this study include:

- multiple hypothesis testing: two primary endpoints
  Refer to the detailed description of the planned primary analysis in Section 11.2.
- multiple hypothesis testing: interim and final analyses
  Refer to the detailed description of the planned interim analysis in Section 6.
- subgroup analyses
  Subgroup analyses of primary and secondary endpoints will be presented by subject BMI (normal: BMI < 25 and overweight: BMI ≥ 25). These analyses are considered exploratory, and results will be analyzed as detailed in Section 13.

9. SUBJECT ENROLLMENT AND ACCOUNTABILITY

The number of subjects Enrolled and in the ITM population will be presented, along with the number and percentage of ITM subjects in the PP population.

The number and percentage of study subjects who completed/did not complete the study will be presented, and any protocol deviations will be listed.

10. DEMOGRAPHIC AND BASELINE CHARACTERISTICS

Demographic and baseline characteristics will be summarized descriptively for the ITM population. Demographic characteristics include age, sex, weight, height, BMI, abdomen circumference, and torso circumference.

11. PRIMARY ENDPOINT

11.1 Primary Endpoint

The primary endpoint is RR accuracy of the Makani Science RMS as compared to manual counting, which will be estimated using root mean squared percentage error (RMSPE).

11.2 Primary Endpoint Analysis

The null and alternative hypotheses for the primary endpoint are:

$$H_{01}: \sigma_1 \geq PG \quad \text{v.} \quad H_{a1}: \sigma_1 < PG,$$

$$H_{02}: \sigma_2 \geq PG \quad \text{v.} \quad H_{a2}: \sigma_2 < PG$$

where σ_1 is the RR accuracy estimated by the RMSPE from data collected in the supine position, σ_2 is the RMSPE from data collected in the reclined position, and PG is the performance goal, as defined in Section 4. The primary hypotheses will be sequentially tested using a gate-keeping approach, each with a one-sided significance level of 0.025. If H_{01} is rejected, H_{02} may be tested. If H_{01} is not rejected, H_{02} will not be tested.

Bootstrapped confidence intervals for RMPSE will be calculated by randomly sampling subjects with replacement and including all of the selected subjects' replicates in the analysis. Using the sequential gate-keeping approach, statistical testing will proceed as follows:

1. If the upper one-sided 97.5% confidence limit for the supine position RMSPE is less than the performance goal, then *H_{01}* is rejected and accuracy can be claimed for this position.
2. If *H_{01}* is rejected, statistical testing of *H_{02}* will proceed. If the upper one-sided 97.5%

confidence limit for the reclined position RMSPE is less than the performance goal, then H_{02} is rejected and accuracy can be claimed for this position.

12. SECONDARY ENDPOINTS

Secondary endpoints for this study include RR percentage bias and precision of the Makani Science RMS as compared to manual counting. For both supine and reclined positions, percentage bias and precision will be estimated using a mixed-effects linear model as described in Section 4 and bootstrapped 95% confidence intervals will be calculated.

13. SUBGROUP ANALYSES

For each monitoring position (supine and reclined), subgroup analyses of the primary endpoint will be presented separately by subject BMI (normal: BMI < 25 and overweight: BMI ≥ 25). RMSPE will be estimated for each BMI category using the methods described in Section 4 and a randomization test will be employed to test whether subjects in the two BMI categories differ with respect to RMSPE. The difference in RMSPE between the two groups will be calculated and compared to the distribution of possible differences assuming a null distribution of homogeneity of groups. Specifically, subjects' BMI category will be randomly permuted to generate the null distribution of the differences. The p-value for BMI effect is defined as the proportion of replicates in the null distribution where the difference between BMI groups is greater than or equal to the observed difference.

Secondary endpoints will also be summarized for each monitoring position by BMI category using descriptive statistics with no formal statistical comparisons.

14. ADDITIONAL ANALYSES

Bland-Altman limits of agreement will be calculated to further evaluate differences in RR between Makani Science RMS and manual counting. Results will be tabulated presenting the lower and upper limits of agreement and associated 95% confidence interval. Limits of agreement will be calculated as described in Bland and Altman (2007) [4] using the mixed-effects model to estimate the percentage bias and the percentage total variance. Results displaying all measurements will be presented graphically.

A scatter plot of Makani Science RMS and manually counted RR and estimated regression coefficients will be presented on the log-transformed scale. The regression coefficients will be calculated using a repeated-measures linear model.

15. SAFETY ANALYSES

All adverse events (AE) reported during the study will be listed, including the event description, time of occurrence, severity, outcome, action taken, expectedness and relationship to the study procedure and/or device. Serious and study- or device-related AEs will be presented separately. AEs will be tabulated by event category if needed.

16. DATA HANDLING

The following participant data will be collected:

- Respiratory rate over time from the Makani Science RMS
- Respiratory rate over time manually counted by a trained individual (reference)
- Baseline subject information
- Adverse events
- Protocol deviations

Respiratory rate data will be acquired from the Makani Science RMS and manual counting and stored as described in the study protocol. Results will be provided in Microsoft Excel files for analysis.

Baseline subject data, AEs, and protocol deviations will be recorded by the clinical researcher and stored in Microsoft Excel files.

All study data will be imported into SAS datasets (or other format as needed) to perform the summaries and analyses detailed in this document.

17. STATISTICAL SOFTWARE AND QUALITY CONTROL

All statistical analyses will be generated using SAS® software, version 9.4 or later, or other appropriate statistical package. Statistical analyses will be independently quality checked by a second statistician to ensure that the Statistical Analysis Plan has been followed and programming errors are minimized.

18. REFERENCES

(1) Atkinson DB, Sens BA, Bernier RS, Gomez-Morad AD, Imsirovic J, Nasr VG. The Evaluation of a Noninvasive Respiratory Volume Monitor in Mechanically Ventilated Neonates and Infants. Anesth Analg. 2021 Apr 30. doi: 10.1213/ANE.0000000000005562. Epub ahead of print. PMID: 33929346.

(2) PASS 2021 Power Analysis and Sample Size Software (2021). NCSS, LLC. Kaysville, Utah, USA, ncss.com/software/pass.

(3) Gao P, Ware JH, Mehta C. Sample size re-estimation for adaptive sequential design in clinical trials. J Biopharm Stat. 2008;18(6):1184-1196. doi: 10.1080/10543400802369053. PMID: 18991116.

(4) Bland, J. Martin and Altman, Douglas G. Agreement Between Methods of Measurement with Multiple Observations Per Individual, Journal of Biopharmaceutical Statistics 2007, 17:4, 571 – 582. DOI: 10.1080/10543400701329422

19. TABLES AND FIGURES

The following tables, figures, and listings are planned:

Table	Title
1	Subject Enrollment and Study Populations
2	Subject Accountability
3	Demographic and Baseline Characteristics
4.1	Primary and Secondary Analyses of Respiratory Rate (RR) ITM Population
4.2	Primary and Secondary Analyses of Respiratory Rate (RR)

Table	Title
	Per Protocol Analysis
5.1	Subgroup Analyses of Respiratory Rate (RR) by BMI Category ITM Population
5.2	Subgroup Analyses of Respiratory Rate (RR) by BMI Category Per Protocol Analysis

Figure	Title
1.1	Bland-Altman Limits of Agreement comparing Makani Science RMS and manually counted RR: Supine Position
1.2	Bland-Altman Limits of Agreement comparing Makani Science RMS and manually counted RR: Reclined Position
2.1	Scatter plot and regression coefficients comparing Makani Science RMS and manually counted RR: Supine Position
2.2	Scatter plot and regression coefficients comparing Makani Science RMS and manually counted RR: Reclined Position

Data Listing	Title
1	Protocol Deviations
2	Adverse Events

20. DOCUMENT VERSION HISTORY

Version	Issue Date	Author	Significant changes from previous version
1.0	06Jun2022	Leslee Willes	NA
1.1	01Aug2022	Meredith Decker	Updated to reflect changes in study protocol related to respiratory rate reference data and subject positioning.
1.2	13Oct2022	Meredith Decker	Updated to clarify per-protocol analyses and source data format.
	26Oct2022	Leslee Willes	Updated interim analysis.