Statistical Analysis Plan

Protocol Title

Measurement of Oxygen Saturation in healthy human volunteers before, during and after hyperemic events using Multi-Modal Techniques: Spatial Frequency Domain Imaging (SFDI), transcutaneous oxygen measurement (TCOM), Pulse Oximeter, and Apple Watch.

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Jan. 18, 2022

Analysis will be performed based on search of answers for the questions designed.

Q1: What are the correlations between the measurements we've taken? Are those correlations significant?

The repeated measures correlations for the measurements. Use repeated measures correlation because we have repeated measures for our data (we get values for each measurement four separate times).

Q2: What are the differences between males' and females' measurements, over time?

We have 20 subjects (13 male, 7 female). We have 5 parameters for each subject: StO2, HbO2, HbR, HbT1, and HBT2. Each measurement will be recorded at 4 time points: baseline, occlusion, reactive hyperemia, and recovery. Plots showing how subjects' measurements changed over time will be obtained. The mean measurement value for males and females, over time will be obtained. Hypothesized that males' and females' measurements follow similar patterns over time. StO2 for males will have higher average HbO2, HbR, HbT1 and HbT2 measurements than females, over all time points compared to females.

Q3. Is this male/female gap in measurements statistically significant, though?

To answer this, I fit multivariate linear regression models at each time point. Results of multivariate linear regression models, for each time point will be obtained. Multivariate models are basically just regression models with multiple outcome variables; in this case, the outcomes are the 5 measurements. The regression coefficients for the intercepts are basically the estimated average measurement for males, for that measurement, at that time point; the p values for the intercepts don't really matter. The regression coefficients for female is just the estimated average difference between males and females, for that measurement, at that time point.

Example:

For StO2 at time 1, it's estimated that males have an StO2 value of 62, with a 95% confidence interval of 54 to 70. It's estimated that females have StO2 values at time 1 that are 4 higher than males, on average [95% confidence interval ranges from 11 lower than males, to 19 higher than males]. That confidence interval [-11 to 19] contains 0, so there's a strong enough possibility that the difference between male and female StO2 values at time 1 is 0 (i.e. there's no difference). Because of that, the p value for Time 1/StO2/Female is 0.581, which is greater than .05, which

means we can't reject the idea the females and males have the same StO2 values at time 1.

Q2: Instead of gender, what about differences between skin type (II and III vs. IV)?

Plot of measurements over time for subjects with skin type II and III vs. skin type IV will be analyzed. The data represent the mean measurement value for skin types 2 and 3 vs skin type 4, over time. Based on the above plots, it appears that (on average) those with skin type 4 have lower StO2 and HbO2 over time, compared to those with skin type 2 and 3. Skin type 4s appear to have higher HbR and HbT1 over time, compared to skin type II and III. There's a lot of variation in HBT2 over time, and both skin type groups have similar mean values over time. Like for gender, I fit separate multivariate linear regression models for each time point. The regression coefficients for the intercepts are basically the estimated average measurement for the skin type II + III group, for that measurement, at that time point; the p values for the intercepts don't really matter. The regression coefficients for skin type IV is just the estimated average difference between 4s and 2s + 3s, for that measurement, at that time point.

Example:

For StO2 at time 1, it's estimated that type 2s and 3s have an StO2 value of 71, with a 95% confidence interval of 63 to 78. It's estimated that type 4s have StO2 values at time 1 that are 20 lower than type 2s and 3s, on average [95% confidence interval ranges from 33 lower than 2s and 3s, to 7 lower than 2s and 3s]. That confidence interval [-33 to -7] does not contain 0, meaning we're confident that the difference between 4s and 2s/3s is not 0. Because of that, the p value for Time 1/StO2/Female is 0.004, which is less than .05, which means we can reject the idea that 4s and 2s/3s have the same StO2 values at time 1. Most of the 5 measurements are correlated with one another. Females and males had similar measurements at baseline, but started to differ in HbO2, HbR, HbT1, and HbT2 from inflation onwards. Those with skin type 4 had lower StO2, higher HbR, and higher HbT1 compared to those with skin type 2 and 3, at all-time points; the two groups didn't significantly differ in regard to HbO2 and HbT2 at any time.

Abbreviation

StO2= tissue oxygen saturation

HbO2=oxy hemoglobin

HbR= deoxy hemoglobin

HbT1= superficial perfusion hemoglobin

HbT2= deep perfusion hemoglobin

TCOM=trans-cutaneous oxygen measurement

SFDI=spatial frequency domain imaging