STATISTICAL ANALYSIS PLAN

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Statistical considerations and power calculation

BA-FMD is the primary outcome of this randomized controlled trial. The change in BA-FMD from baseline to 12 months after randomization will be compared between the curcumin and placebo groups. Other secondary outcomes include aPWV, systemic and endothelial markers of inflammation and oxidative stress, and cognitive function measured by the NIH Cognition Toolbox Battery. Power and sample size calculation [1-3] is based on two-sample t-test of change in FMD by using PASS [4]. Preliminary data provides estimate of the mean and standard deviation (SD) of BA-FMD change as 1.25 and 0.88 under curcumin (n=17) and 0.08 and 0.40 under placebo (n=13), respectively. This provides a large between-conditions difference (i.e. 1.25 - 0.08 = 1.13) and effect of 1.7 taking account heterogeneity. Given the sample mean of 0.08 under control condition of usual care in Van Craenenbroeck, *et al*. [5], we have assumed 0.10 as mean change in BA-FMD under placebo conditions. While there is no published literature to estimate the mean change in BA-FMD under curcumin in CKD, we believe it should be smaller than 1.25; in other words, the between conditions difference should be smaller than 1.13. Based on Van Craenenbroeck, *et al*. [5] and Thambyrajah, *et al*. [6] where the between-conditions difference was 0.33 and 0.80, respectively, we assumed a difference of 0.6 between curcumin and placebo, considering difference between studies in intervention, study population, duration of study, and the observed difference in preliminary data. In summary we have the following assumptions: mean and SD of FMD change from baseline to 12 months after randomization are 0.70 and 0.90 under curcumin and 0.10 and 0.40 under placebo, respectively. With target power of 95% and 2-sided alpha 0.05, calculation by PASS indicates a sample size of 37 patients per group. We are going to enroll a total of 88 patients assuming approximately 18% potential loss to follow-up and equal randomization to the curcumin and placebo groups with 44 participants in each group. Power for cognitive function and other secondary outcomes: Based on the preliminary data from our pilot study of 12 weeks treatment (curcumin *vs.* placebo) with small sample size (17 patients per group), improved cognitive function was observed in the curcumin group but not in placebo. The effect size for episodic memory can be estimated as 0.8 for both picture sequence memory (data showed 0.77) and fluid composite (data showed 0.83). This implies that 37 patients per group will provide more than 90% power, based on calculation by PASS, with 2-sided significant level of 0.05 by using the two-sample t-test. Similarly, for other secondary outcomes, including change in aPWV and systemic and endothelial markers of inflammation and oxidative stress, 37 patients per group will provide more than 90% power if the effect size is 0.8 or larger. The power will be at least 95% if 44 patients per group can be included and effect size is 0.8.

Statistical analysis

Descriptive statistics will be provided for demographic characteristics, baseline measure, and outcome variables as appropriate. For example, mean and SD will be provided for continuous variables and proportion for categorical variables. 95% confidence intervals will also be provided if appropriate. We don't anticipate necessary data transformation but will confirm it before analysis.

All outcome analyses will be based on the "intent-to-treat (ITT)" principle. For Aim 1a, change in BA-FMD from baseline to 12 months after randomization will be compared between curcumin and placebo groups by using two-sample t-test. The same analysis will be performed for aPWV. As an additiaonl analysis, repeated measures analysis will be employed to compare the two groups in the change in both BA-FMD and aPWV from baseline to month 6 and 12, and the compound symmetry covariance structure will be specified. Treatment group indicator, time, and their interaction will be included as fixed effects in the model. We will explore the impact of sex, race, baseline CKD stage, and physical activity by including them as covariates in a regression model. Of note, we are stratifying by age and DM status (as noted above). For Aim 1b, repeated measures analysis will be performed for change in both BA-FMD and aPWV before and after vitamin C infusion at all three time points, the same analysis as the previous one in Aim 1a except that we now have three time points and that other covariance structures will also be explored. In addition, correlation analysis will be performed to examine the association between change in vascular function (BA-FMD and aPWV) and change in levels of inflammatory and oxidant markers including systemic measures (high-sensitivity CRP, IL-6, MCP-1, and oxLDL) and endothelial measures (eNOS and eNOS phosphorylated at Ser 1177, NF-κB, NAD(P)H oxidase,

and nitrotyrosine). Finally, repeated measures analysis will be employed to compare between groups the change in inflammatory and oxidant markers from baseline to month 6 and 12. For Aim 2, the same analysis as in Aim 1a will be performed to evaluate the treatment efficacy on cognitive function, including correlation analysis to examine the association between change in vascular function (BA-FMD and aPWV) and cognitive function. Based on our pilot study, we don't anticipate severe adverse events but we will tabulate all observed events. Data of pill count will be summarized and analyzed as appropriate. All analyses will be performed by using SAS 9.4, SAS Institute Inc., Cary, NC, USA [7].

Loss to follow-up and missing data: We anticipate loss to follow-up and missing values will occur, but based on our experience and track record we do not anticipate large number of loss to follow-up. We thus plan to derive conclusions based on complete case analysis by using two-sample t-test of the outcome variables. We will tabulate baseline characteristics to examine any difference between those lost to follow up and those complete the study. In the unlikely event of >10% missing data, we will perform multiple imputation under assumption of missing at random (MAR) mechanism [8] and examine any change in conclusions.

References

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