Study Protocol with

Statistical Analysis Plan

With Research Title:

Intravenous hydrogen nanobubbles effect on cardiac physiology and quality of life: a single blind, dose-response study

Malang, Indonesia

19th September, 2022

Study Title

Intravenous hydrogen nanobubbles effect on cardiac physiology and quality of life: a single blind, dose-response study

Study Design

This study is a randomized, single-blind, dose-response clinical trial conducted to assess the effects of intravenous hydrogen nanobubbles (HNBs) on multiple health outcomes, including cardiac physiology, quality of life, and blood biomarkers. Participants were randomly assigned to one of six groups and received either HNB infusion or normal saline (control) over a 5-week period (10 sessions total, twice per week). The intervention doses were 0 mL (control), 5 mL, 10 mL, 15 mL, 20 mL, and 25 mL of HNB diluted in 500 mL of normal saline.

Ethical Considerations

This study followed the ethical principles outlined in the Declaration of Helsinki. Ethical approval was granted by the Research Ethics Committee of State Polytechnic of Health Malang (Ref: 410/KPEK-POLKESMA/2022). All participants provided written informed consent, and participation was entirely voluntary. Blinding was maintained by using indistinguishable infusions for HNB and saline solutions.

Eligibility Criteria

Inclusion criteria: adults aged 18–65 years, stable health condition permitting participation, and ability to provide informed consent.
Exclusion criteria: known allergies to hydrogen, concurrent participation in other trials, congestive heart failure, stage 4 or higher kidney disease, or liver failure.

Intervention Details

Group A (Control): 500 mL normal saline
Group B: 5 mL HNB in 500 mL NS
Group C: 10 mL HNB in 500 mL NS
Group D: 15 mL HNB in 500 mL NS
Group E: 20 mL HNB in 500 mL NS
Group F: 25 mL HNB in 500 mL NS
Each group received 10 intravenous sessions over 5 weeks (2 sessions/week).

Outcome Measures

Primary outcomes were assessed at baseline (week 0) and post-intervention (week 5):
1. Cardiovascular physiology: systolic and diastolic blood pressure (mmHg), heart rate (bpm), LVMI (g/m^2), RWT (unitless), TAPSE (cm), and FMD (% change).
2. Quality of Life: measured using the SF-36 questionnaire.
3. Blood profile: inflammatory markers, lipid profile, liver function tests, and hematologic indices.

Statistical Analysis Plan

Statistical analyses were conducted to evaluate time-related changes and dose-dependent effects.

- Repeated Measures ANOVA was used to assess within-subject changes (pre- vs post-treatment) and interaction effects between dose and time.
- The Bayesian paired t-test was applied to complement the ANOVA results, providing Bayes Factors (BF_{10}) that quantify the strength of evidence supporting the treatment effect.
- A p-value < 0.05 was considered statistically significant. BF_{10} values > 3 indicated moderate evidence, > 10 strong evidence, and > 100 very strong evidence for the alternative hypothesis.
- Data analysis was performed using appropriate statistical software, and each outcome was treated independently without aggregation into composite indices.