Statistical Analysis Plan

A randomized clinical trial of **s**implified dietary education versus **i**ntensive dietary education on **n**utritional status after **g**astrectomy (**SING** study)

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I. Introduction

The purpose of the statistical analysis plan (SAP) is to describe the procedures, statistical methods, and planned analyses that will be used to analyze and report results for the study. This SAP includes the details of study sample size calculation and statistical methods for analyses of primary outcome and secondary outcomes.

This study is a double-blind, parallel-assigned, randomized, prospective controlled trial (Samsung Medical Center IRB protocol No. 2020-11-016) "A randomized clinical trial of simplified dietary education versus intensive dietary education on nutritional status after gastrectomy (SING study)".

The SING study is divided into two separate study.

I. **Study including only patients who underwent subtotal gastrectomy**

II. Study including only patients who underwent total gastrectomy

Patient enrollment, data cleaning, and statistical analysis will be conducted separately in each study. This SAP is the plan for the study including only patients who underwent subtotal gastrectomy. This will be done regardless of the total gastrectomy study.

II. Study objectives

1. Primary objective

To evaluate a difference for the percent of weight loss (%) at 12 months after surgery between the simple nutrition counseling and the intensive nutrition counseling group for early stage (IA or IB).

2. Secondary objectives

1) Nutritional parameters, Patient-Generated Subjective Global Assessment (PG-SGA)
2) Intake (kcal, protein) and eating habits (meal frequency/meal speed) assessed by 24-hour dietary recall.
3) Albumin (Unit: g/dL)
4) Hemoglobin (Unit: g/dL)
5) Iron panel [Total Iron-Binding Capacity (TIBC) (Unit: μg/dL), Ferritin (Unit: ng/mL)]
6) Absolute lymphocyte count (ALC) (Unit: $10^3/\mu L$)
7) Vitamin B12 (Unit: pg/mL)
8) Homocysteine (Unit: μmol/L)
9) Quality of life using the European Quality of Life questionnaire (EQ-5D)

III. Study populations

1. Inclusion criteria

1) Histologically confirmed gastric cancer

2) Patients who were diagnosed as T1N0, T1N1, or T2N0 by preoperative CT scan and pathologically confirmed after gastric resection (AJCC 8th classification)

3) Tumors located in the antrum, angle, lower body, or mid-body of the stomach

4) No evidence of metastasis from other cancers

5) Age between 20 and 75 years

6) Patients who have not previously received chemotherapy or radiation therapy, and who have not undergone gastric resection

7) Patients with appropriate physical function based on blood tests:

   (a) WBC 3000/mm^3 – 12,000/mm^3,

   (b) serum hemoglobin > 8.0 g/dL,

   (c) serum platelets > 100,000/mm^3,

   (d) serum AST < 100 IU/L,

   (e) serum ALT < 100 IU/L,

   (f) total bilirubin < 2.0 mg/dL,

8) Patients who have signed informed consent

2. Exclusion criteria

1) Active synchronous or metachronous malignancy (within 5 years prior to enrollment), excluding carcinoma in situ lesions (lesions equal to intraepithelial or intramucosal cancer)
2) Presence of residual gastric cancer
3) T3, T4 stage according to preoperative staging
4) N2 or higher according to CT scan or pathological findings (presence of 3 or more metastatic lymph nodes)
5) Histologically rare variants of gastric cancer, such as adenosquamous, hepatoid, squamous cell, undifferentiated, and neuroendocrine carcinoma, according to the WHO classification
6) Pregnant or breast feeding women
7) Individuals with diagnosed mental illness according to medical records
8) Individuals with uncontrolled angina or a history of myocardial infarction within the past 6 months
9) Individuals with uncontrolled hypertension or with chronic kidney or liver disease
10) Individuals with diabetes requiring insulin therapy
11) Individuals with severe respiratory diseases requiring continuous oxygen therapy
12) Individuals who have undergone previous abdominal surgery
13) Individuals who have experienced complications after surgery

3. Definition of population for analysis

1) Full Analysis Set (FA set)

We will evaluate the primary outcome "percent of weight loss (%) at 12 months after surgery between the simple nutrition counseling and the intensive nutrition counseling group" in the FAS population. In the FAS population, all randomized subjects are included, except following subjects.

① Subjects who do not meet the inclusion/exclusion criteria

② Subjects who have never received the nutrition counseling during the study period

③ Subjects who did not provide any data after baseline

④ Subjects diagnosed with other severe diseases affecting weight or receiving additional treatment (ex. Surgery, chemotherapy, etc.) after enrollment

2) Per-Protocol Set (PP set)

PPS consists of data from subjects included in FAS who received all nutrition counseling and completed the study per protocol without major violation.

IV. Sample size considerations

The primary objective is to clarify the difference for the percent of weight loss (%) at 12 months after surgery between the simple nutrition counseling and the intensive nutrition counseling group. The percent of weight loss (%) in the simple nutrition counseling group would be about 10% with standard deviation of 5.407% [*reference*]. We expected that the intensive nutrition counseling group would reduce by 20% in simple nutrition counseling group, which would result in a percent of weight loss(%) of 8% in the intensive nutrition counseling group.

We calculated that 116 patients in each group would provide a power of 80% to determine the difference for percent of weight loss (%) in intensive nutrition counseling group compared to the simple nutrition counseling group (8% vs. 10%) with a standard deviation for both groups of 5.407%. The comparison will be made using a two-sided, two-sample equal-variance t-test, with a significance level of 0.05. After considering 10% loss to follow-up, we determined the total enrollment of 258 patients (129 in each group). An interim analysis was not planned.

[*reference*]*: Ryu K, Bae J, Kim E, An JY, Choi M, Lee JH, et al. Long-term effect of simplified dietary education on the nutritional status of patients after a gastrectomy. PloS One (2021) 16(5):E252168. doi: 10.1371/journal.pone.0252168*

V. Data Analysis Plan

The Full Analysis Set will be used for the primary efficacy analyses, and the Per-Protocol set will be used for supportive efficacy analyses.

The statistical methods of characteristics will be determined according to the type of variables. Continuous variables will be given as mean ± standard deviation or median (interquartile range, IQR) and compared with a Student's t-test or Wilcoxon rank-sum test, depending on the normality of the distribution. For categorical variables, comparisons between groups will be summarized as frequency and percentile, and performed using a Chi-squared test or Fisher's exact test, as appropriate. To assess the association between two continuous variables, the correlation analysis will be performed and presented with scatter plots. In addition to, the standardized mean difference (SMD) will be calculated to estimate the difference for the mean values between two groups.

The primary objective of this study is to clarify the difference for the percent of weight loss (%) at 12 months after surgery between the simple nutrition counseling and the intensive nutrition counseling group. The difference for the percent of weight loss (%) at 12 months from baseline between two groups will be calculated, and the difference is tested using Student t-test. If the distribution for the percent of weight loss (%) violates the normality assumption, the non-parametric Wilcoxon rank-sum test will be used for sensitivity analysis. In addition, the percent of weight loss (%) at 1, 3, 6, 12 and 18 months will be analyzed using the generalized estimating equations (GEE) or mixed effects model to examine whether there is a difference between the two groups over time.

The repeated measures will be collected at baseline and each follow-up time points (1, 3, 6, 12, 18 months). All patients who had surgery and completed at least one baseline and

one follow-up (at any time point) measurement will be included. To estimate the difference between groups in change at each time points from baseline, the generalized estimating equations (GEE) or mixed effects model will be applied including time-by-group interaction term with the main effects. GEE method allows the inclusion of all participants, regardless of whether they have missing data at any follow-up time points, and will be adopted to take into account the within-patient correlation. The following effects will be estimated using GEE or mixed effects model:

1) differences in the percent of weight loss (%) between the two groups
2) whether the percent of weight loss (%) change over time within each group
3) whether there are differences in the percent of weight loss (%) changes over time between the two groups.

Missing data are expected due to drop outs and missed visits. Although substantial effort will be employed to minimize the missing data, it is inevitable that some missing data will occur. Various statistical analyses will be employed to perform statistical inferences that properly account for statistical uncertainty due to missingness. The percent of weight loss (%) is a subject-level variable and missing assessments would influence tests relative to treatment-by-time interactions. The primary analysis described above will be performed using all data. No patients will be deleted from the analysis because of partial data. To assess the sensitivity of results, weight loss will be analyzed using:

1) All patients regardless of missing data
2) Only patients who participated in all assessment visits and have no missing data
3) Rubin's multiple imputation