Study protocol

Project Title: The Effect of Peri-Transplant Oral Use of Food for Special Medical Purposes on Nutrition and Intestinal Function in Patients Undergoing Hematopoietic Stem Cell Transplantation

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I. Rationale and Research Significance

Rationale

In addition to factors such as the primary disease, age, techniques for hematopoietic stem cell (HSC) collection, storage, and infusion, and pre-transplant chemotherapy conditioning, good nutritional status is an indispensable key factor for the success of Hematopoietic Stem Cell Transplantation (HSCT). Both medications used to treat the primary disease and antibiotics used for infections can damage the gastrointestinal mucosa and lead to gut microbiota dysbiosis. Patients often experience symptoms like mucosal ulcers, anorexia, and diarrhea, which not only limit oral intake but also exacerbate the loss of nutrients through feces. Post-transplant gastrointestinal Graft-versus-Host Disease (GvHD) further worsens these symptoms. Previous studies often measured nutritional status at a single time point, failing to reflect the dynamic changes in the nutritional status of HSCT patients.

Some retrospective studies abroad have shown that the implementation of oral/enteral nutrition helps maintain the nutritional status of HSCT patients, thereby improving clinical outcomes. Furthermore, a few studies indicate that supplementing with probiotics or prebiotics can restore the disordered gut microbiota in HSCT patients, alleviating gastrointestinal symptoms like diarrhea. However, due to the inherent limitations of retrospective studies, their conclusions are difficult to generalize widely. Further confirmation through prospective Randomized Controlled Trials (RCTs) is needed. Secondly, due to the immunocompromised state of HSCT patients, the safety of probiotic supplementation is questioned. Therefore, this study aims to observe the impact of Foods for Special Medical Purposes (FSMP) containing prebiotics on the nutritional status and clinical outcomes of HSCT patients.

Research Significance

1. Understanding the dynamic changes in nutritional indicators (weight, handgrip strength, serum protein levels) and their influencing factors in HSCT patients will facilitate early intervention and management of malnutrition.
2. Through this study, observe the impact of FSMP supplementation on nutritional status and clinical outcomes (hospital-acquired infections, length of stay in the transplant unit, and total hospital stay).
3. Through this study, investigate the effect of FSMP containing prebiotics on gastrointestinal function in HSCT patients. Gastrointestinal function will be

assessed both microscopically (using 16s rRNA technology to evaluate gut microbiota diversity; blood/urine/fecal microbiota metabolites) and macroscopically (frequency of diarrhea, stool consistency, and volume).

4. The tolerance of HSCT patients to the implemented FSMP can be assessed.

II. Domestic and International Research Background (with Key References)

Hematological malignancies are a general term for a major class of malignant tumors originating from the hematopoietic system. Among the top ten common malignancies in China, acute leukemia and lymphoma (both hematological malignancies) are ranked within the top ten. Furthermore, the overall incidence has shown an increasing trend in recent years, with a tendency towards affecting younger individuals. Due to the complex classification and subtypes of hematological malignancies, and significant individual differences in diagnosis and treatment, the diagnosis and treatment of hematological malignancies pose severe challenges. Survey data published in *The Lancet* in 2018 showed that the five-year survival rate for lymphoma patients in China is about 38.3%, while for myeloma it is only 24.8% [1].

Hematopoietic Stem Cell Transplantation (HSCT) is an active and effective intervention for treating hematopoietic dysfunction, immune deficiencies, and some hematological malignancies. Attal et al. [2] included 200 elderly (≥65 years) patients with newly diagnosed, previously untreated multiple myeloma to evaluate the therapeutic effects of HSCT versus conventional chemotherapy. They found that the response rate was 81% in patients receiving HSCT, compared to only 57% in those receiving conventional chemotherapy ($p<0.001$); significant differences were also observed in the 5-year progression-free survival (28% vs. 10%, $p=0.01$) and overall survival (52% vs. 12%, $p=0.03$) between the two treatment groups. Another large RCT screened 1966 patients with acute myeloid leukemia (≤55 years) from 13 medical institutions in the UK, New Zealand, and Iceland, randomly assigning them to receive conventional chemotherapy or HSCT with follow-up, ultimately including 381 patients for analysis. The relapse rate was significantly lower in the HSCT group compared to the conventional chemotherapy group (37% vs. 58%, $p=0.0007$), and the disease-free survival rate was significantly higher (53% vs. 40%, $p=0.04$); however, there was no significant difference in overall survival [3].

Factors influencing the success of HSCT, besides HSC collection, cryopreservation, reinfusion, and conditioning regimens, also include nutritional status. Numerous factors affect the nutritional status of patients undergoing HSCT, including: ① The primary disease and its treatment. Studies focusing on children showed that approximately 25% of children had energy intake below recommended levels, and almost all children had

significantly lower intake after treatment compared to intake at diagnosis [4]. Studies on adult leukemia patients also found that their daily energy intake decreased from 1396 kcal before chemotherapy to 1046 kcal after chemotherapy, and daily protein intake decreased by about 20g [5]. ② The impact of induction chemotherapy prior to HSCT or Total Body Irradiation (TBI) on nutritional status. High-dose melphalan or melphalan combined with immunomodulatory drugs for induction has become a standard regimen before HSCT [6-7]. These drugs undoubtedly damage rapidly proliferating intestinal mucosal cells, leading patients to prone to gastrointestinal symptoms like anorexia, nausea, vomiting, and diarrhea. High-dose TBI also easily causes damage to the gastrointestinal mucosa. Chemotherapy and radiotherapy not only affect appetite, leading to decreased intake, but associated diarrhea also increases nutrient loss [8]. ③ Graft-versus-Host Disease (GvHD) causing digestive and absorptive dysfunction. Secondary infections post-HSCT may further increase the patient's energy expenditure.

The incidence of malnutrition or nutritional risk in HSCT patients varies greatly depending on factors such as study sample size, disease type, assessment timing, and HSCT type [9]. Ding Xiaoping et al. reported a combined malnutrition incidence of 61.1% determined by SGA in 55 patients undergoing HSCT, but this study did not report the type of HSCT received [10]. Also using the PG-SGA method, Hao Sujuan et al. reported a malnutrition incidence of 29.3% before conditioning in 75 patients receiving allogeneic HSCT, which significantly increased to 85% at two and four weeks post-conditioning [11]. However, some studies suggest that overnutrition (overweight and obesity) is more common before HSCT rather than undernutrition. Brauer et al.'s recent study analyzed the weight status at diagnosis and pre-HSCT in 662 acute myeloid leukemia patients (median age 59.4 years, range 16.3-74.9 years), finding that the rates of underweight (BMI<18.5 kg/m²) were only 1% and 3% respectively, while the cumulative rate of overweight and obesity exceeded 50% [12]. Unlike studies on preoperative nutritional status and disease, dynamic weight changes around HSCT are more closely related to survival and relapse rates. A Japanese study involving 145 leukemia patients who received their first allogeneic bone marrow transplant found that, using weight loss of <5%, 5-10%, and ⩾10% as criteria for well-maintained, moderate, and severe malnutrition respectively, the rates of moderate and severe malnutrition reached 32.4% and 31.0%. The severity of weight loss was not significantly correlated with the incidence of GvHD, but the risk of patient death increased more than twofold (HR=2.04, 95% CI: 1.12-3.71) [13]. Another follow-up study included 182 patients with acute leukemia (15-69 years) with a mean follow-up of 26.3 months, showing that 23.0% of patients had severe weight loss (a 13.2% decrease in weight at HSCT compared to initial weight); multivariate regression analysis showed that weight loss was significantly associated with 2-year survival (39.9% vs. 65.8%). Those with weight loss had a 2.06-fold increased

risk of death (95% CI: 1.00-3.07) [14]. Brauer et al.'s study also confirmed that weight loss (defined as a BMI decrease of ⩾2.0 kg/m²) was an independent risk factor for increased mortality (HR=1.23, 95% CI: 1.05-1.43), while BMI at diagnosis was not significantly associated with clinical prognosis [12]. A systematic review focusing on children, adolescents, and young adults (<24 years) also confirmed that lean body mass significantly decreased in patients receiving HSCT + TBI compared to those receiving HSCT alone, and body composition analysis before and after HSCT showed a significant decrease in lean tissue mass [15]. Although in autologous HSCT the stem cells come from the patient themselves, thus avoiding graft rejection and GvHD, nutritional issues can still exist. Horsley et al. used PG-SGA to analyze the nutritional status of 66 patients undergoing peripheral blood stem cell transplantation (7 allogeneic, 59 autologous; mean age 58.7±12.0 years) two weeks before transplantation, finding rates of 73%, 23%, and 4% for well-nourished, moderately malnourished, and severely malnourished status, respectively. Well-nourished patients had an average hospital stay 7 days shorter than malnourished patients [16].

Although HSCT patients face numerous nutritional problems, there is currently a lack of unified standards on how to manage these issues. A survey of 83 Italian stem cell transplant centers showed significant differences among centers in nutritional risk screening, monitoring, and choice of nutritional support methods [17]. Although both US and European guidelines recommend oral or enteral nutrition (EN) when gut function permits, over 90% of stem cell transplant centers tend to use parenteral nutrition (PN) [17]. Supplementing natural food with a whey and soy protein mixture (0.5g/kg/day) or tube feeding EN can improve nutritional status, reduce mortality, and decrease GvHD [18-19].

Besides insufficient energy and protein intake, HSCT patients also experience gut microbiota dysbiosis. Analysis of 8767 fecal samples from 1362 patients undergoing allogeneic HSCT showed that gut microbiota diversity was significantly reduced from 30 days before transplant to 6 days before transplant compared to healthy volunteers (n=246) [20]. Patients receiving autologous HSCT also showed reduced gut microbiota diversity [21]. More importantly, reduced gut microbiota diversity is associated with transplant-related mortality [20-21]. Exogenous administration of probiotics and prebiotics helps restore disordered gut microbiota, but evidence for their use in HSCT patients remains limited [22-23]. Observational studies showed that yogurt intake (150g/d) promoted faster neutrophil recovery [24]. Another RCT found that daily probiotic supplementation (*Lactobacillus rhamnosus GG*, 1×10^{10}/d) did not alter gut microbiota diversity, and the incidence of GvHD was not significantly different [25]. A prospective study showed that compared to patients not using prebiotics, administering

oral prebiotics to allogeneic HSCT patients during the peri-transplant period significantly shortened the duration of moderate to severe mucosal damage (11d vs. 14d), significantly increased the rate of patients without diarrhea (17% vs. 7%); prebiotic supplementation significantly reduced 100-day GvHD [26].

In summary, this study plans to enroll all adult multiple myeloma patients undergoing HSCT at our hospital from January 1, 2022, to December 31, 2023. Patients will be stratified by disease course (newly diagnosed vs. relapsed) and HSCT type (autologous vs. allogeneic) and then randomly assigned to two groups: conventional treatment + homogenized meal replacement powder (control group) and conventional treatment + FSMP supplementation (treatment group). We intend to use Abbott's Ensure as the FSMP supplement. Ensure is a complete nutrient-type FSMP containing fermentable prebiotics (inulin + fructooligosaccharides, 4.3g per 100g), which can promote the proliferation of the patient's own colonizing probiotics. Supplementation will continue throughout the pre-transplant induction chemotherapy phase. Through this study, we aim to achieve the following objectives: ① Dynamic changes in nutritional status [at admission, pre-induction chemotherapy, post-induction chemotherapy (i.e., pre-transplant), post-HSCT, and at discharge] and their influencing factors; ② The impact of pre-transplant oral FSMP on nutritional indicators (anthropometric measurements, handgrip strength, serum protein levels); ③ The impact of pre-transplant oral FSMP on hospital-acquired infections, length of stay in the transplant unit, and total hospital stay; ④ The impact of FSMP containing prebiotics on intestinal function (diarrhea, gut microbiota diversity, and microbiota metabolites). Based on the scale of HSCT in our hospital's hematology department, approximately 100 patients undergo HSCT annually, about 70% of whom are multiple myeloma patients. During the study period, we can enroll 140 patients. Excluding those unwilling to participate or ineligible, we can ensure a sample size of 50 patients per group.

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III. Research Plan (Including Research Objectives, Research Content, and Key Issues to be Addressed)

Research Objectives

1. To evaluate the impact of peri-transplant oral FSMP on nutritional indicators in HSCT patients.
2. To evaluate the impact of peri-transplant oral FSMP on intestinal function in HSCT patients.
3. To evaluate the impact of peri-transplant oral FSMP on clinical outcomes such as infections.
4. To assess the tolerance of FSMP use in HSCT patients.

Research Content

1. This study is a single-center, randomized, controlled, subject-blind RCT.
2. The FSMP used is Ensure, supplemented at a dose of 500 kcal/day (complete nutritional formula, providing 430 kcal energy and 15.9g protein per 100g, containing 4.3g fermentable dietary fiber), administered orally in two divided doses. The control group will supplement with ordinary homogenized meal replacement powder (complete nutritional formula, providing 440 kcal energy

and 19.8g protein 3.0g dietary fiber per 100g) also at 500 kcal/day, administered orally in two divided doses. We will accurately weigh 57g of Ensure powder and ordinary homogenized powder respectively each morning and afternoon, mix with warm water to 250ml to prepare a standard energy density (1.0 kcal/ml) liquid, seal it in cups, and distribute it to subjects for consumption.

3. **Primary outcome measures:** Changes in body weight, lean body mass (by bioelectrical impedance analysis), and handgrip strength.

4. **Secondary outcome measures:**
   (1) Changes in PG-SGA score;
   (2) Changes in serum protein levels;
   (3) Incidence of diarrhea;
   (4) Incidence of infection;
   (6) Incidence of adverse events and serious adverse events;

5. **Gut microbiota:** Gut microbiota diversity assessed by 16s rRNA sequencing and blood levels of gut microbiota metabolites.

Key Issues to be Addressed

1. **Improving nutritional status in bone marrow transplant patients through peri-transplant oral FSMP.**
   Previous research has mostly focused on the effects of pharmaceutical-type enteral nutrition on bone marrow transplant patients, lacking studies on the role of FSMP. Under the background of DRG payment, nutritional support must effectively improve patients' nutritional status without increasing medical insurance expenditures; FSMP is a more suitable choice.

2. **Establishing a standardized FSMP usage protocol.**
   Although US and European guidelines recommend Oral Nutritional Supplements (ONS), foreign surveys indicate that over 90% of stem cell transplant centers still prefer PN. Compared to PN, ONS aligns better with physiological characteristics, is more acceptable, has lower costs, and fewer complications. Furthermore, ONS is more conducive to maintaining gut barrier function, reducing bacterial translocation, and lowering infection rates. We plan to learn from care pathways for other diseases to establish a standardized protocol for "screening-assessment-protocol development" for oral FSMP use in HSCT patients.

3. **FSMP and Intestinal Function.**
   Due to the immunocompromised status of HSCT patients, the safety of

exogenous probiotic supplementation is questioned. Promoting the proliferation of the patient's own colonizing bacteria by supplementing soluble dietary fiber, whose metabolites like short-chain fatty acids benefit the repair of damaged intestinal mucosa, can improve gastrointestinal symptoms and reduce bacterial translocation. Abbott's Ensure contains fermentable fructo-oligosaccharides and inulin, which can promote the proliferation of the patient's own beneficial colonizing bacteria, thereby resolving safety concerns.

4. **Safety of FSMP use in bone marrow transplant patients.**
   Determined based on the incidence of adverse events and serious adverse events.

IV. Research Methods and Technical Pathway

4.1 Study Subjects

This is a single-center randomized controlled study. All multiple myeloma patients undergoing HSCT at our hospital from January 1, 2022, to December 31, 2023, will be initially screened.

- **Inclusion Criteria:**
  1. Age ⩾18 years and <75 years.
  2. Clearly clinically diagnosed with multiple myeloma and scheduled to undergo HSCT.
  3. No contraindications to oral intake.
  4. Willing to participate in the study and provide informed consent.
- **Exclusion Criteria:**
  1. Patients with known contraindications to enteral nutrition.
  2. Patients with known infectious diarrhea.
  3. Patients with other concurrent malignancies or having undergone intestinal surgery within the past year.
  4. Patients who have taken other probiotics, prebiotics, or synbiotics within the recent month (1 month).
  5. Patients with immune deficiency or dysfunction, such as HIV or long-term use of corticosteroids (⩾3 months).

6. Pregnant or lactating female patients.
7. Patients with known allergy to any ingredient in the trial product, or those judged by the investigator to require restricted dietary fiber intake.
8. Other patients deemed unsuitable for participation by the investigator, e.g., ① unwilling to participate; ② severe organ dysfunction, shock, active gastrointestinal bleeding, or obstruction.

4.2 Sample Size Calculation

The primary objective of this trial is to compare the change in body weight before and after treatment between the control group and the FSMP supplementation group. The sample ratio is 1:1, with a two-sided significance level (α) of 0.05 and power (1-β) of 85%. Based on our previous work, the conventional treatment group is expected to have a weight loss of -1.2kg, while the conventional treatment + FSMP group is expected to have a weight loss of -0.5kg. Approximately 37 subjects are needed per group, requiring 74 subjects in total for both groups. Considering compliance and loss to follow-up, the sample size for each group will be increased by 20%, requiring a total of 100 subjects. All enrolled patients will be stratified by disease course (newly diagnosed vs. relapsed) and HSCT type (autologous vs. allogeneic) and then randomly divided into two groups (n=50 each):

① **Control Group:** According to the hematology department's routine, provide a low-residue semi-liquid diet, supplemented with ordinary homogenized meal replacement powder (500 kcal/day, orally in two divided doses).

② **Oral FSMP Supplementation Group:** According to the hematology department's routine, provide a low-residue semi-liquid diet, additionally supplemented with Ensure (500 kcal/day, orally in two divided doses).

Oral FSMP supplementation will start from the beginning of the pre-transplant preparation phase and continue until discharge from the transplant unit.

4.3 Data Collection Indicators

4.3.1 Demographic indicators: Gender, age, ethnicity.

4.3.2 Disease-related indicators: Primary disease and its treatment, disease course, previous HSCT history, chronic disease history (diabetes, hypertension, dyslipidemia, cardiovascular/cerebrovascular diseases, fatty liver, etc.), medication use.

4.3.3 Nutrition-related indicators

4.3.3.1 Body weight and body composition analysis (by bioelectrical impedance, TANITA TBF-410).

4.3.3.2 Handgrip strength.

4.3.3.3 PG-SGA score.

4.3.3.4 Dietary intake: Weighed food record method; each patient will be provided with a food scale to weigh and photograph food.
4.3.3.5 Laboratory tests: Complete blood count, liver/kidney function, blood glucose, lipids, electrolytes, serum calcium/phosphorus/magnesium.

4.3.4 Intestinal function assessment

4.3.4.1 Gastrointestinal symptoms: Frequency of nausea, vomiting, diarrhea.
4.3.4.2 Stool consistency and volume. Collect 2ml of stool, vacuum dry and freeze; measure gut microbiota diversity using 16s rRNA sequencing.
4.3.4.3 Gut microbiota metabolites in blood and urine.

4.3.5 Clinical outcome-related indicators

4.3.5.1 Length of stay in the transplant unit and total hospital stay (days).
4.3.5.2 Incidence of infections during hospitalization.

Statistical plan

Paired t-tests will be used for within-group changes in continuous data at baseline, and independent samples t-tests for between-group comparisons. Chi-square tests will be used for categorical data. Multiple linear regression will be used to analyze factors related to weight, handgrip strength, transplant unit stay, and hospital stay. Multiple logistic regression analysis will be used to analyze factors related to hospital-acquired infections. Confounding factors included in the models will be based on univariate regression analysis results and clinical experience.

1. Patient daily energy requirements will be calculated using the Schofield equation for Resting Energy Expenditure (REE). For age 18-30: Male: 63*Weight(kg) + 2896; Female: 62*Weight(kg) + 2036. For age 30-60: Male: 48*Weight(kg) + 3653; Female: 34*Weight(kg) + 3538. Stress factors will be considered based on the formula result.

2. According to the Chinese Medical Association Parenteral and Enteral Nutrition Branch guidelines for adult Oral Nutritional Supplementation, the recommended single-day ONS dose for adults is 300-900 kcal, hence the dose is set at 500 kcal/day. Supplementation starts from the pre-transplant preparation period and continues until discharge from the transplant unit.

3. Dietary intake will be assessed using 24-hour dietary recall combined with the weighed food record method.

4. Microbiota metabolites will be analyzed using metabolomics.