The role of nutrition support for quality of life and clinical outcomes in adult recipients of allogeneic hematopoietic stem cell transplantation

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PhD thesis

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List of Papers

In this thesis the Papers will be referred to by their Roman numerals.

Paper I

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Effects of individualized nutrition after allogeneic hematopoietic stem cell transplantation following myeloablative conditioning; a randomized controlled trial.

Clinical Nutrition ESPEN 2018; 28: 59-66. <https://doi.org/10.1016/j.clnesp.2018.08.002>

Paper II

Kristin J. Skaarud, Marit B. Veierød, Simon Lergenmuller, Asta Bye, Per Ole Iversen, Geir E. Tjønnfjord.

Body weight, body composition and survival after one year: follow-up of a nutritional intervention trial in allo-HSCT recipients. *Bone Marrow Transplantation* 2019; 54: 2102-2109. <https://doi.org/10.1038/s41409-019-0638-6>

Paper III

Kristin J. Skaarud, Johannes R. Hov, Marius Trøseid, Simen H. Hansen, Martin Kummen, Jørgen Valeur, Asta Bye, Vemund Paulsen, Knut E. A. Lundin, Geir E. Tjønnfjord, Per Ole Iversen.

Microbial diversity and mortality after allogeneic hematopoietic stem cell transplantation: secondary analysis of a randomized nutritional intervention trial. Submitted; revised manuscript accepted for publication in Scientific Reports after submission of the thesis.

Abbreviations

aGVHD	Acute graft-versus-host-disease
allo-HSCT	Allogeneic hematopoietic stem cell transplantation
AML	Acute myeloid leukemia
cGVHD	Chronic graft-versus-host-disease
EN	Enteral nutrition
GVHD	Graft-versus-host disease
HSCT	Hematopoietic stem cell transplantation
I-FABP	Intestinal fatty acid binding protein
LBP	Lipopolysaccharide binding protein
MAC	Myeloablative conditioning
OTUs	Observed operational taxonomic units
PN	Parenteral nutrition
QoL	Quality of life
RCT	Randomized controlled trial
RIC	Reduced intensive conditioning
sCD	Soluble cluster of differentiation
SCFAs	Short chain fatty acids
TPN	Total parenteral nutrition

Summary

Patients with malignant blood disease treated with allogeneic stem cell transplantation (allo-HSCT) suffer from insufficient energy and protein intake leading to weight loss and malnutrition. Poor nutritional status has been associated with impaired quality of life and increased morbidity and mortality. International guidelines recommend nutritional screening and to provide early nutritional support to prevent weight loss and malnutrition. However, clinical and biological parameters to assess nutritional status are non-specific, and there is no agreement on food recommendations, the optimal time point to start nutritional support, the dose of nutritional support or the optimal administration route (parenteral or enteral). Finally, it is not known if a nutritional intervention can improve quality of life and clinical outcomes in allo-HSCT recipients.

In a two-armed randomized controlled trial (intervention: n=57 and control: n=60), we administrated an open intervention with optimized energy and protein intake combined with tube feeding aimed to improve quality of life and clinical outcomes. The intervention was delivered to adult patients with a malignant blood disease undergoing allo-HSCT at Oslo University Hospital.

We found no effect of the nutritional intervention on global quality of life, oral mucositis, acute graft-versus-host disease and other clinical outcomes at 3 months. Body composition during 1-year follow-up was the same in both groups. When we pooled the two groups, 1-year mortality was associated with weight gain (mostly fluid retention). Loss of microbial diversity at 3 weeks post-HSCT was associated with 1-year mortality in both groups. Short-chain fatty acids were of minor importance for clinical outcomes. Circulating markers of gut barrier function was not associated with clinical outcomes. Optimized energy and protein intake preferable by the enteral route had no effect on QoL and clinical outcomes, suggesting that other types of intervention are needed.

The different hematological malignancies may be classified according to the site and severity of the disease (2). Leukemia is the most frequent hematological malignancy in Norway and the number of new cases diagnosed with leukemia (all diagnosed according to ICD-10 numbers 91-95) in 2018 was 1,205. For the period 2014–18, the 5-year relative survival estimate (the observed survival proportion in a patient group divided by the expected survival of a comparable group in the general population) was 65% and 71% for male and female, respectively (3). The etiology of malignant blood disease is more or less unknown, but previous exposure to chemotherapy and irradiation increases the risk. Furthermore, genetic abnormalities may cause some rare variants of malignant blood disease (2). Treatments for malignant blood diseases include chemotherapy (major treatment modality for leukemia), radiation therapy, immunotherapy and/or hematopoietic stem cell transplantation, and is based on the specific features of the disease (4).

1.2 Hematopoietic stem cell transplantation

Hematopoietic stem cell transplantation (HSCT) became feasible in the USA in the early 1960s (5) and in Norway in the 1980s (6) and is a well-established treatment for certain hematologic diseases that cannot be cured with conventional treatment. The rationale behind HSCT is to first destroy the hematopoietic system (as this harbors the malignant cells), of the patients before reconstituting the patients with a new and healthy hematopoietic system (donor engraftment). The graft contains donor T cells that can be beneficial for the recipient by eliminating residual malignant cells (graft-versus-leukemia effect). In allogeneic HSCT (allo-HSCT) two principally different approaches exist to prepare the recipient for donor engraftment. Myeloablative conditioning (MAC) is the originally method, namely to treat the underlying disease with high-dose chemotherapy, sometimes in combination with total body irradiation. The aim is to

eradicate the patients' bone marrow cells, and without the support of HSCT the patient would succumb due to bone marrow failure. The aim of reduced intensity conditioning (RIC), also denoted non-myeloablative conditioning, is to induce sufficient immunosuppression by less toxic chemotherapy to allow donor engraftment, but retain the graft-versus-leukemia effect (5). RIC was developed to reduce transplant-related mortality and allow transplantation in patients who would not be eligible for transplantation otherwise due to comorbidities and/or old age (5, 7, 8). The major adverse outcomes after allo-HSCT are relapse, graft-versus-host disease (GVHD), organ toxicity and infections. Early adverse outcomes are acute GVHD (aGVHD) and organ toxicity, whilst chronic GVHD (cGVHD), relapse and secondary cancers, are late adverse outcomes of the treatment. Furthermore, immunomodulatory agents used to prevent or treat GVHD further suppresses the immune system and is associated with a higher risk of infectious complications (5).

1.2.1 Survival after allo-HSCT

About 50,000 hematopoietic cell transplantations are performed annually worldwide. More than 40,000 transplants were done in Europe and more than 8,000 in the US. Of those about 40% were allogeneic and 60% were autologous (patient's own stem cells reinfused) (9-12).

MAC was the established treatment before allo-HSCT in Norway when we planned and initiated our project in 2010. RIC was used as experimental treatment in a minority of patients from the 2000s (6). Over the past two decades there has been a shift from MAC to RIC in transplantation practice. The number of allo-HSCT in adults performed at Oslo University Hospital in 2019 was 121, and of these 31 was performed after MAC. The utilization of RIC has led to a rise in allo-HSCT in elderly patients and those with comorbidity. Improvements in donor selection and many positive changes in supportive

and pretreatment care (e.g. antiviral and antifungal strategies, microbiologic diagnostics, intensive care) have contributed to an increased survival after allo-HSCT. Implementation of RIC has reduced the toxicity problems associated with HSCT. Whilst organ toxicity causes treatment failure after MAC, GVHD and relapse are major causes of mortality after RIC (13). However, the use of anti-thymocyte globulin has reduced lethal GVHD (14, 15). Over the past three decades treatment-related mortality (mortality of all causes except for relapse) was reduced from 20 to 10% and similarly for patients who received MAC versus RIC (8, 13).

Patients' selection and the optimal time to perform allo-HSCT depend on risk stratification of the underlying disease, comorbidity and transplant-related risk. In general, allo-HSCT is indicated for those in risk of relapse with chemotherapy alone. Risk classification of the underlying disease relies on morphology, cytogenetic and molecular abnormalities, and disease stage (4, 16-19). The hematopoietic cell transplantation comorbidity index (HCT-CI) is commonly used for pre-transplant risk assessment of individual patients. The HCT-CI tool comprises 17 categories of organ dysfunction which were found to influence non-relapse mortality (20). These are arrhythmia, cardiac disease, inflammatory bowel disease, diabetes, cerebrovascular disease, psychiatric disturbance, hepatic disease, obesity, infection, rheumatologic disease, peptic ulcer, renal disorders, pulmonary disorders, prior solid tumor, heart valve disease and hepatic disease. Positive findings are collapsed into three risk groups: low (score 0), intermediate (score 1-2), high risk (score ≥ 3) (20). European bone marrow transplantation risk score (EBMT score) was developed to predict pre-transplant-specific risk factors. EBMT score consists of five variables; donor type, disease phase, recipient age, donor/recipient sex combination, and interval from diagnosis to transplantation. Patients are categorized as low-risk (score 0-1)

intermediate-risk (score 2-3) high-risk (score 4-5) and very high-risk group (score >5 (21). Acute myeloid leukemia (AML) is the most common indication for allo-HSCT and includes a number of subtypes largely based on cytogenetic abnormalities and aberrations. The decision to go for transplantation or not depends on the risk of relapse of the underlying AML upon conventional treatment and also on the risk of transplant-related mortality. Relapse-free survival (published 2017) of AML after allo-HSCT with MAC was 67%, whilst with RIC 47% (22). In comparison the relapse rate (published 2010) with chemotherapy alone was 30% in the favorable risk group, and 50% in the intermediate risk group (16). Survival with and without transplantation for each of the diseases is difficult to interpret since randomized, prospective studies are lacking and most studies rely upon historical cohorts from registries.

1.3 Nutritional problems encountered in HSCT

Recipients of allo-HSCT suffer from gastrointestinal dysfunctions including mucositis, vomiting, anorexia and diarrhea leading to nutritional inadequacy (23, 24). These side-effects of the treatment are caused by infections, toxicity of medication or preexisting gastrointestinal disease and GVHD (25-28). Other side-effects associated with compromised nutritional intake are loss or change in smell and taste perception (29, 30). Loss or changes in smell and taste perception are frequent in allo-HSCT, and the etiology are multifactorial. Diagnosis, side effects of cancer therapy (e.g. weight loss, dry mouth), gender, and age impact measurements (29, 31). Oral energy intake declines a few days after hospital admission and is usually not recovered at discharge from hospital (32, 33). High incidence of post-transplant weight loss is common in recipients of allo-HSCT, and some patients may even be malnourished. Those who are malnourished prior to allo-HSCT are at increased risk of being malnourished during the course of transplantation compared to well-nourished patients. A study reported a

weight loss of $\geq 5\%$ in 56% of patients, and the prevalence of malnutrition increased from 28 to 45% from hospital admission to discharge (34). At admission for allo-HSCT, most patients (77%) were classified as well-nourished, 18% as moderately malnourished and few (10%) as severely malnourished. The prevalence of a weight loss of 5-10 and $>10\%$ during the last 6 months before admission for allo-HSCT were 11 and 13%, respectively (35). Another study (n=145) reported a weight loss of $<5\%$ (normal group n=53), 5-10% (mild malnutrition group n=47) and $\geq 10\%$ (severe malnutrition group n=45) during three months of follow-up (36).

Weight loss may result in loss of both lean (fat-free) body mass and fat mass. One study reported loss of both fat-free mass and fat mass 30 days after allo-HSCT (35), whilst a study from the 1980s reported an increase in extracellular fluid and loss of body cell mass 30 days post-HSCT (37). The prevalence of weight loss and malnutrition varies across studies, possibly due to the use of different nutritional assessment tools, cut-off values for nutritional markers and the time points for measurements. Furthermore, comparisons of the results from different studies are complicated due to heterogeneous treatment modalities (e.g. autologous HSCT, RIC, MAC) known to impact on nutritional status, small sample sizes and non-specific clinical parameters. In line with this, capillary leak and fluid retention may mask weight loss and impact on measured body weight and body composition (38-40). Malnutrition can cause adverse effects on body composition, functional and clinical outcomes, and impaired immune system functions (36, 41, 42). Proper nutritional support could improve treatment outcomes and quality of life (24). However, there is conflicting evidence of what is adequate nutritional support during the course of allo-HSCT (24).

1.4 The role of nutritional support in recipients of allo-HSCT

1.4.1 Timing of initiating nutritional support

When to initiate nutritional support traditionally relies on clinical and/or biochemical tools (e.g. impaired oral intake, weight loss, low levels of biochemical indices of nutritional status). Furthermore, traditional clinical and laboratory parameters are non-specific in the course of HSCT due to capillary leakage, fluid retention, and acute phase proteins (43-45). Thus, they cannot be used to assess when to initiate nutritional support. Currently, there is no consensus if nutritional support should be initiated routinely to prevent malnutrition or initiated when signs and symptoms of malnutrition occur (pre-emptive or on-demand). Furthermore, it is unknown whether there is a benefit of nutritional intervention in the early HSCT-phase (46, 47). Irrespective of nutritional status, MAC is *per se* an indication for nutritional support, and total parenteral nutrition (TPN) is frequently delivered to prevent malnutrition when it is impossible to eat due to oral mucositis (23, 48).

1.4.2 Energy requirement

European guidelines recommend 25-30 kcal/kg and 1.5-2 g/protein/kg body weight per day for cancer patients (24) and 30 kcal/kg/day are usually used as a general rule of thumb in practice. However, body weight is influenced by capillary leak and fluid retention, therefore an estimate expressed by kcal/kg may be inaccurate (49). Furthermore, illness severity and body composition impact on energy and protein requirement. Indirect calorimetry is a more accurate method for determine energy requirements than using a pre-set kcal/kg value in critically ill patients. This method measures variations in energy expenditure dependent on disease, illness severity and nutritional status and may prevent over- and underfeeding (49, 50). Although data on energy expenditure are lacking, it is generally assumed that allo-HSCT recipients have

increased energy and protein needs. Loss of epithelial cells, diarrhea, catabolic effects on skeletal muscle, sepsis and aGVHD may increase energy needs and aggravate weight loss (51-53). However, a high energy requirement may be difficult to achieve due to capillary leak and fluid retention and drug-induced liver toxicity with impaired lipid metabolism. Capillary leak appears frequently and has an early onset, whilst impaired lipid metabolism is less frequent in the initial phase. Furthermore, glucose intolerance due to sepsis and usage of glucocorticoids for aGVHD may limit the dose of nutritional support, in part due to the risk of treatment-induced hyperglycemia (52, 54).

1.4.3 Route of nutritional support

Dietary interventions in recipients of allo-HSCT mainly focusing on food restrictions, aim to reduce the risk of bacterial contamination and subsequent bacteremia. Most centers (93%) use neutropenic diet from day +5 until engraftment for recipients of allo-HSCT after MAC. This diet consists mainly of cooked food (55). A low-microbial diet is used in Norway. This diet eliminates high-risk foods, e.g. unpeeled fresh fruit, raw vegetables and unpasteurized dairy products. Vegetables and berries have to be boiled and meat cooked well done (45, 56, 57). The use of neutropenic dietary restrictions is based on best practice and has never been evaluated in controlled clinical trials (56).

A few studies from 1989 to 2003 focused on food service needs to support oral intake when loss of appetite, nausea, vomiting, taste and smell alterations and sore mouth reduced dietary intake. These studies highlighted avoidance of specific food components such as fiber-rich food, red meat, citrus fruits and acidic food. Furthermore, fresh, mild food with soft texture served in small portions and available at all times, were recommended to reduce dependence on medical nutrition (i.e. oral nutritional supplements, enteral tube feeding and parenteral nutrition) (45, 58-61). Dietary intervention has improved oral energy intake and quality of life (QoL) in selected

patients (62, 63). During allo-HSCT, oral energy intake alone has not been considered possible. However, clinical studies on how to increase oral intake are sparse, and most dietary interventions to reduce dependence of medical nutrition are based upon expert opinion (45, 59, 60). Symptoms limiting oral intake (e.g. anorexia, nausea, sore mouth) and proper food (e.g. taste, texture, portion size) are best captured by patient self-report. In our previous observational study we examined the patient's perspective of taste and smell of food when having poor appetite and nausea (30). Patients also reported about sensation of texture of food when sore mouth and appreciation of portion size together with a feeling of early satiety. Patient's experience of proper food and their needs for support to maintain and increase food intake were compared with an institutional food guideline. Patients' choices of food quality i.e. mild taste, soft texture, small helpings, and in between meals were mainly in accordance with the guideline. The patients had less variation in dishes within these qualities compared with the guideline. The patients' experiences of nurses' support were mainly in accordance with the guideline. However, the patients requested increased focus on support from the nurses on maintaining and increasing oral intake (30).

TPN has traditionally been used as first-line nutritional support in allo-HSCT (23, 48). Parenteral nutrition (PN) is effective in regards to monitor given nutrients (52), but has been associated with hyperglycemia (62, 63), increased subclavian vein thrombosis, catheter-line infections (64), volume overload and hepatic dysfunction (65), delayed platelet engraftment (66), and delayed resumption of oral intake (67). Despite that several studies have reported adverse effects of PN, administration of PN was shown to be superior to an electrolyte-enriched solution. A randomized controlled trial (RCT) from 2013 (63) and one from 1987 compared TPN with an electrolyte-enriched solution (47). Both studies reported improved body weight (47, 63), and the latter found

improved overall survival (47). Moreover, a RCT from the 1987 reported an increase in fat mass and extracellular fluid 28 days after allo-HSCT in patients receiving TPN compared to patients receiving an enteral feeding program (44).

It is a commonly held view that enteral nutrition (EN) maintains the gut barrier function and thereby reduces the risk of translocation of enteric bacteria in critical ill patients (68). During the last decade there have been some attempts on tube feeding, and several observational studies have compared the effect of tube feeding versus PN on clinical outcomes. Two observational studies found no effect of EN combined with PN on body weight compared to PN alone (69, 70). One of these studies was a prospective study after MAC (69), and the other was a retrospective study of recipients of MAC and RIC (70). Three months' overall survival was better in the EN group compared to PN alone in one of these two studies (69). Energy and protein intakes were not reported in these two studies and gut nourishment was not defined. In contrast, a small recent RCT could not confirm these results (71). A retrospective study categorized patients in three groups dependent on route of nutritional support and adequacy of energy and protein intake. Adequate EN was associated with reduced 100 days mortality and improved long term survival and gut GVHD compared to PN (72). Moreover, several days with oral intake and tube feeding have been associated with reduced aGVHD in several observational studies in comparison to PN (69, 72-74). In line with this, some studies compared medical and surgical patients with enteral fasting and patients without enteral fasting and found alterations in gut barrier function in the enteral fasting group (75-77). Others could not confirm the association between the use of TPN (i.e. enteral fasting) and changes in barrier function and bacterial translocation (78-83). Notably, the parenteral solution used differed across studies, making it difficult to interpret the results.

European guidelines in contrast to US guidelines recommend recipients of allo-HSCT to receive tube feeding in the peritransplant period, except when there is reasonable doubt as to the adequacy of gastrointestinal functioning (24, 84). However, tube feeding is rarely used routinely in clinical practice (48), possible due to the risk of dysphagia, abdominal pain, diarrhea, ileus, nausea, bleeding and vomiting, which may lead to tube displacement or dislodgement (85). Some data indicate that in general the tube is tolerated, but there is little data on how the tolerance is assessed (69, 70). Moreover, it is often not possible to full-nourish patients by tube. Most patients need a parenteral supplementation (69-71). Some small studies claimed that the time-point of tube placement and start of feeding are crucial for successful feeding (85). Early tube placement (day -7 of the transplantation), allows the patient to be familiar with the tube before the onset of side effects. However, forceful vomiting during the conditioning was associated with vomited tubes. Nasojejunal tube placement day +3 to +5 of transplantation and before pancytopenia and mucositis developed, was recommended to secure enteral feeding (86). Trophic feeding (10–20 mL/h or 10–20 kcal/h or up to 500 kcal/day) has been theorized to provide a sufficient amount of EN to maintain gut integrity while decreasing incidence of EN intolerance in critically ill adults (87). If this beneficial effect of low enteral feeding could be achieved in recipients of allo-HSCT after MAC, is unknown. Furthermore, limitations in study designs do not allow firm conclusions for recommendations regarding the optimal enteral components (oral and by tube feeding) related to treatment-induced mucosal injury and gut GVHD (23). Moreover, a fiber-free formula has been used to increase the tolerance for tube feeding (69, 71, 86), while a retrospective case-control analysis found that oral fibre supplementation (plus glutamine and probiotics) in allo-HSCT was associated with fewer days of diarrhoea and reduced early mortality (88). There is insufficient evidence

for routinely supplementation of glutamine (essential amino acid essential for e.g. enterocytes) (24). Furthermore if administration of prebiotic (non-digestible carbohydrates support the growth of beneficial bacteria) and probiotics (nutritional supplements containing microorganisms) could have a beneficial effect on gut integrity, has yet to be determined (89). Studies comparing different nutritional support in the course of HSCT including MAC are outlined in Table 1. In summary, due to lack of data, there is currently no agreement concerning the time to start, the dosing of the nutritional support, and if enteral feeding should be preferred over parenteral feeding. Furthermore, it is unknown whether nutritional support in the early phase can improve nutritional status and clinical outcomes in recipients of allo-HSCT.

Table 1. Overview of studies comparing different nutritional support in the course of HSCT in adults including MAC

1 st author, year of publication and ref.no.	Number of patients	Study design	Patients and time point of data collection	Type of intervention	Outcomes
Beckerson et al., 2019 ⁷¹	484	Observational, retrospective	MAC, RIC, during hospitalization	Patients were categorized into 3 groups: 1) Adequate EN (oral or ≥ 4 days of tube feeding), 2) adequate PN (≥ 4 days with PN plus EN, 3) inadequate nutrition (oral or by EN and/or PN) from day 0 to neutrophil engraftment.	Day 100: Increased non-relapse mortality with inadequate nutrition and adequate PN compared to adequate EN. Increased aGVHD in patients received PN compared to adequate EN. Adequate PN and inadequate nutrition associated with reduced 5-year survival
Andersen et al., 2019 ⁷⁰	23	RCT, intervention	MAC, RIC day +30 and 100	30-35 kcal/kg/day, 1.2-1.5 g protein/kg/day by EN (oral alone or ≥ 5 days of tube feeding of a polymeric non-fiber formula or plus PN of a triple chamber bag) vs PN (only PN or < 5 days with tube feeding), from day +1 until stool sample collection	No difference in microbial composition at day +30 or aGVHD day 100
Iyama et al., 2014 ⁸⁷	22	Case-control	RIC and MAC, up to day +100	Oral glutamine, fiber and oligosaccharide from day -7 to +28 plus TPN (not specified) vs. matched historical controls receiving TPN	Reduction in diarrhea, mucositis, weight loss and better survival at day +100
Guize et al., 2014 ⁶⁹	56	Retrospective, observational	RIC and MAC, during hospitalization	30-35 kcal/kg/day, 1.2-1.5 g protein/kg/day EN (tube feeding with a standard polymeric formula alone or plus PN lipids representing 35% of total calories, glucose/lipid ratio 58/42) vs PN (PN alone or plus tube feeding) from day +1 and usually stopped at day +30	Less days with fever, reduced need for antifungal therapy, lower rate of catheter replacement, less transfer to intensive care units in the EN group within day +100
Mousavi et al., 2013 ⁶²	59	RCT, intervention	RIC, MAC and autologous, day +1 and discharge	Individual adjusted PN (25 kcal/kg/day, 1.4 g/protein/kg/day of 2,500-3,500 mL consisting of 10% glucose, amino acids, fat emulsion, electrolytes, vitamins, trace elements) dependent on oral intake and serum albumin level vs conventional PN (5% glucose 2,000 mL, 10% amino acid 500 mL, vitamins, electrolytes, twice a week) from day +1 to discharge.	Improved body weight in the individually adjusted PN group. No difference in engraftment. Several days with febrilia and use of antibiotic

Table 1. Overview of studies comparing different nutritional support in the course of HSCT in adults including MAC

1st author, year of publication and ref.no.	Number of patients	Study design	Patients and time point of data collection	Type of intervention	Outcomes
Seguy et al., 2012 ⁶⁸	121	Prospective, observational	MAC, during hospitalization	30-35 kcal/kg/day by EN (tube feeding alone with a standard polymeric formula or plus PN; three chamber bag formula supplemented with micronutrients) vs PN (PN of 1200, 1400 or 2400 kcal alone or oral only). Time for initiating and stop of nutritional support was not given	Improved 3 months' overall survival, shortened time to neutrophil engraftment (not platelet engraftment) and less severe aGVHD in the EN group. No difference in body weight 3 months post HSCT
Sommacal et al., 2012 ⁴³	91	Intervention prospectively, retrospective controls	MAC and RIC, during hospitalization	Before and after implementation of criteria for insertion and discontinuation of PN (intervention) vs. no criteria PN (control). Intervention started at admission for allo-HSCT	Shorter time on PN in the intervention group. No differences in weight loss, severity of aGVHD, infection rate, or neutropenia. No differences in hospital death or death at 6 and 12 months
Weisdorf et al., 1987 ⁴⁴	136	RCT, intervention	MAC and autologous, one week before to 4 weeks after HSCT. Including children	Energy requirement determined by Harris Benedict equations. Prophylactic TPN (fat emulsion, electrolytes, minerals, vitamins, trace elements, and zinc and increased protein if needed) from 1 week prior to 4 weeks after HSCT vs electrolyte-enriched solution (5% dextrose, electrolytes, minerals, trace elements, vitamins)	Improved overall survival, longer time to relapse and longer disease-free survival in the TPN group
Szeluga et al., 1987 ⁴¹	57	RCT, intervention	MAC and autologous, day -1 to +28	Standard TPN (35 kcal/kg/day, 1.4 g protein/kg/day, 25% dextrose, amino acids, electrolytes, vitamins, trace minerals) vs. an individualized feeding program (counseling, high protein snacks i.v. amino acids and/or tube feeding) from day-1 until discharge	Increase in fat mass and extracellular fluid in the TPN group. No differences in bacteremia, catheter insertion, recovery of white blood cells and neutrophils and survival

1.5 Quality of life

Quality of life (QoL) is often used interchangeably with health-related QoL, as done here. QoL includes the impact on illness and treatment on functioning and well-being. In clinical trial research, QoL refers to patients' appraisals of their current level of functioning and satisfaction with it compared to what they perceive to be ideal. QoL and other patients reported outcomes are increasingly used as an independent outcome in clinical trials, introducing a more patient-centered approach. QoL questionnaires used within clinical trials characterize and measure patient's subjective perception of health status when receiving health care. Questionnaires include multiple aspects and domains of QoL (e.g. physical functioning, emotional distress, social well-being), exhibit scales, single items and patients' overall QoL perception (i.e. global) and are both generic and specific (certain populations or interventions). The instruments used to measure QoL often include 30 or more questions (90, 91).

Patients treated with allo-HSCT report poorer QoL up to 1-year post-HSCT compared with those given chemotherapy alone and those ≥ 60 year and those relapsed. However, advanced disease and severe side effect of treatment are associated with poor QoL and disease stage may be a confounder in studies comparing QoL in those with and without HSCT (92). On the longer-term QoL gradually improve and returns to near baseline level 1-4 year post-HSCT in those without cGVHD (92, 93). Physical functioning is still compromised up to 3 months' post-HSCT, and patients' global QoL is also impaired (94, 95). Nausea, vomiting, sore mouth, taste changes, loss of appetite, and fatigue are frequent after allo-HSCT and associated with impaired oral intake and QoL (94-96). A prospective, longitudinal study found an association between physical well-being and higher body mass index (BMI), and poorer physical and social well-being were associated with weight loss 6 months' post-HSCT (97). Symptomatic adverse event

(e.g. nausea, vomiting, sore mouth, taste changes, loss of appetite) and fatigue are more intense up to 3 months after MAC compared to RIC (94, 95). Risk factors for poor QoL are previous treatment, pre-transplant pain, chronic disease self-efficacy (one's ability to manage their disease symptoms) and complications of transplantation (97-99). Since most studies assessing QoL have included patients receiving MAC, RIC and autologous transplantation and used different questionnaires, interpretation of the results across studies is difficult (100). In summary, weight loss and malnutrition in recipients of HSCT are associated with poor QoL outcomes, and nutritional intervention has improved QoL in selected patients groups suffering from poor oral intake and weight loss (101, 102).

1.6 Oral mucositis

Mucositis is a part of mucosal barrier injury and includes the entire gastrointestinal mucosa (103, 104). The regimens in MAC have a direct cytotoxic effect on rapidly dividing cells (i.e. gastrointestinal epithelium cells), leading to mucositis. Oral mucositis usually appears when the blood leucocyte concentrations reach nadir between 7-10 days post-HSCT and recovers at the time of engraftment, usually 2-3 weeks after HSCT (51, 105). Oral mucositis is reported as the worst symptom affecting QoL by patients and is associated with several days of treatment with opioids, PN and antibiotics (106, 107). Oral mucositis is multifactorial and affects between 47% and 100% of the patients (108). Risk factors for development are malignant blood disease, neutropenia, treatment with chemotherapy that has a direct cytotoxic effect on the epithelial cells (e.g. melphalan, cyclophosphamide, busulphan, total body irradiation), old age, malnutrition, impaired kidney function, poor mouth status and smoking (including smokeless tobacco) (104, 108-110). Furthermore, total body irradiation-based regimen and BMI ≥ 25 kg/m², or carriers of methylenetetrahydrofolate reductase 677 TT genotype were

associated with higher risk of oral mucositis development. The use of multivitamins before transplantation had a potential protective effect on the development of oral mucositis (111). Additionally, intestinal microbiota may play a role in mucositis development (112). MAC has in comparison to RIC been associated with increased toxicity and severe oral mucositis (113-116). Surprisingly, the intensity of the conditioning regimen was not associated with severity of oral mucositis in a meta-analysis using Word Health Organization Oral Toxicity Scale and National Cancer Institute Common Toxicity criteria for Adverse Events scales (108).

Mucosal barrier injury together with profound neutropenia predispose for local invasion by microorganisms and systemic life-threatening infections (112). Much effort has been done in research to minimize oral mucositis, however RCTs on risk factors and treatment options for oral mucositis are lacking. In addition to oral hygiene protocol, laser therapy, antioxidant agent and growth factor have been recommended in prevention and treatment of oral mucositis in cancer patients in general (117-119). However, there are insufficient evidence to recommend cytokines and growth factors in hematological malignancies and bone marrow transplantation (120). Malnutrition is possibly a risk factor for oral mucositis development. However, whether nutritional intervention during the course of allo-HSCT can reduce the incidence and severity of mucositis is unknown. Former studies have reported no difference in mucositis in patients receiving PN versus EN (69, 70).

1.7 Acute graft-versus-host disease

Acute GVHD occurs when immunocompetent T cells in the graft recognize recipient tissues as foreign (121). The first sign and symptoms of aGVHD usually appear when the donor cells have engrafted and started to produce blood cells, usually 2-4 weeks after transplantation. Organs usually involved in aGVHD are the skin, liver and the

gastro- intestinal tract. Signs of aGVHD include a maculopapular rash, jaundice, nausea, vomiting, anorexia, watery or bloody diarrhea, and crampy abdominal pain (122). The diagnosis of acute GVHD relies on the assessment of target organs by means of clinical and laboratory analyses and biopsy. The severity is graded clinically by the extent of the involvement of the three main target organs, the skin (the site of the most frequent and often the earliest clinical manifestation), the gastrointestinal tract (the second most common site), and the liver. Acute GVHD is graded as mild (grade 1), moderate (grade 2), severe (grade 3) and very severe (grade 4) (122). The prevalence of aGVHD is 30-50%. Risk factors include the degree of HLA mismatch, unrelated donor, female donor to a male recipient (sex-mismatch), the use of peripheral blood stem cell grafts, and the intensity of the conditioning regimen (121). Gastrointestinal toxicity from the preparative regimen has been associated with an increased risk of GVHD (25) and poor oral intake with severe acute GVHD (73). Lethal GVHD is associated with decreased microbial diversity (123).

1.8 Infections, engraftment and transplant related mortality

Infections are frequent during the course of allo-HSCT. Reactivations of latent pathogens such as invasive fungal disease and virus (e.g. cytomegalovirus (CMV)) are common secondary to GVHD treatment. Bacteremia, in particular with enteric bacteria, contributes to morbidity and mortality in an immunosuppressed host (124). CMV infection is diagnosed by isolation of the virus or detection of viral proteins or nucleic acid in any body fluid or tissue specimen (125). Fungal disease is classified as “proven” verified by histological analysis or culture of a specimen of tissue taken from a site of disease; “probable” verified by serological analysis and CT scan, or “possible” by serological analysis only (126). Bacteremia is defined as a positive blood culture.

Diagnosis of pneumonia requires either a combination of new pulmonary infiltrates on chest X-ray or a CT scan and symptoms of respiratory infection.

Engraftment is defined as the first of three consecutive days with a blood concentration of neutrophil granulocytes $\geq 0.2 \times 10^9/l$ and platelet engraftment as spontaneous increase in blood concentration of platelets $\geq 20 \times 10^9/l$. Transplant-related mortality is death from any cause except from relapse. PN has in comparison with EN been associated with increased transplant-related mortality (69). Furthermore, malnutrition is associated with delayed time of engraftment, increased infectious complications, and increased morbidity and mortality (42, 127-129). The results may be biased as the sickest patients are often those who do not tolerate enteral feeding. Furthermore the impact of nutritional status prior to allo-HSCT on clinical outcomes post-HSCT is unknown. In summary, whether nutritional support can reduce the rate of infectious complications, shorten the time to engraftment and improve survival, is unknown.

1.9 Interruption of gut microbiota homeostasis

The human gut harbors diverse microorganisms which play major roles in the host's life. The microorganisms provide essential nutrients such as short chain fatty acids to the host produced by food digestion. Furthermore, the gut microbiota serves as a barrier to pathogens and regulates the local and systemic immunity (130). The role of gut microbiota for clinical outcomes after allo-HSCT has received increased attention during the last decade. Major changes in microbial composition, both loss of diversity and domination of single taxa, are common in recipients of allo-HSCT (131). Several single center studies suggest that microbiota may be relevant for clinical outcome (89, 132-134). The diversity of the gut microbiota throughout the course of allo-HSCT has been correlated with survival and outcome of aGVHD (123, 135, 136). One study reported that high diversity of the intestinal microbiota at the time of engraftment was

associated with improved overall survival (137). Furthermore, high microbial diversity in the days between allo-HSCT and development of aGVHD has been associated with reduced GVHD-related mortality (123). Single taxa (e.g. the genus *Blautia* and *Enterococcus*) have been associated with reduced lethal GVHD and improved overall survival (123, 138). Since most studies have assessed microbiota after transplantation there has been some uncertainty about the impact of pre-transplant microbiota on clinical outcomes. Furthermore, previous studies have small sample sizes and included patients with conditioning regimen known to have different impact on gut microbiota. Additionally, critical factors for microbial composition, e.g. geographic variations in the human microbial communities, differences in antibiotic usage and nutritional support, may question whether results from small single center studies are generalizable. However, recently one multicenter study found an association between high microbial diversity both before allo-HSCT and until engraftment, and increased survival and better outcome of aGVHD (131). Furthermore, the stability of the gut microbiota throughout the treatment has been associated with improved outcome (139).

Short-chain fatty acids (SCFAs) are end-products of bacterial fermentation of colonic food residues, may directly influence the mucosal immune system (140), and may have a role in mediating microbiota-derived signaling between the gut and brain (141).

Studies investigating the correlation between SCFAs and gut microbiota and clinical outcomes after allo-HSCT are sparse. Lower levels of butyrate and propionate are reported after transplantation in children developing aGVHD (142) and butyric acid may modulate intestinal epithelial cell damage and mitigate GVHD in mice (143, 144).

Damage of epithelial surfaces could represent another link between gut microbiota composition and clinical outcome. In line with this, several studies have investigated the relations between circulating markers of gut barrier function and clinical outcome. A

decrease in intestinal fatty acid binding protein (I-FABP) has been reported as a biomarker of enterocyte turnover, but with no predictive value (103, 145). In a study higher level of lipopolysaccharide binding protein (LBP) at day +7 and +14 post-HSCT indicated higher probability of aGVHD (146). Furthermore, the soluble cluster of differentiation molecule (sCD14, a marker of monocyte activation) has been associated with the progression of neutropenic fever to septic shock (147).

Interventions aimed to stabilize gut microbiota have received increased attention. Diet is a critical determinant of microbial diversity and enteral feeding may, as opposed to parenteral feeding, have a beneficial effect on gut microbiota after HSCT. A study reported an association between low *Blautia* abundance and increased GVHD. This study also reported a correlation between several days with parenteral feeding and low *Blautia* (123). In line with this, a study reported prompt post-HSCT recovery of microbial diversity and SCFAs in children receiving tube feeding compared to children with PN (148). In contrast, no association was found between diversity and number of days on PN in another study (137). Furthermore, tube feeding and several days with oral intake have been associated with reduced aGVHD in two observational studies, both in comparison to PN (69, 73). Energy and protein intake were not reported in these two studies and a detailed description of nutrient content was lacking. Microbiota produce SCFAs throughout fermentation of fiber, but a fiber-free enteral formula has been used in recipients of allo-HSCT (86, 149). Similarly, a recent study used a fiber-free formula to optimize EN tolerance and found no difference in microbial diversity between patients receiving predominantly EN versus PN (71). In contrast, a retrospective case-control analysis suggested that oral fiber supplementation (plus glutamine and probiotics) in allo-HSCT was associated with fewer days of diarrhea and reduced early mortality (88). In conclusion, previous studies are mainly observational, and the results

may be influenced by selection bias (i.e. the sickest patients were those who did not tolerated enteral feeding), thus they do not allow firm conclusions to be drawn.

Examples of studies of gut microbiota in the course of HSCT including MAC are outlined in Table 2.

Table 2. Overview of studies of gut microbiota during the course of HSCT including MAC					
1 st author, year of publication and ref.no.	Number of patients	Study design	Patients and time point of data collection	Outcomes	
Peled et al., 2020 ¹²⁸	1362	Observational, prospective multicenter	MAC, RIC, weekly fecal samples from ≤30 days before allo-HSCT until engraftment	Loss of diversity and domination of single taxa. Lower diversity both before and after allo-HSCT was associated with poor 1-year survival	
Kusakabe et al., 2020 ¹³⁶	24	Observational, prospective	Autologous, MAC, RIC, day -8, daily fecal samples from start conditioning until day +30	Skewed microbiota associated with complication and 2-year mortality after allo-HSCT	
Han et al., 2019 ¹³³	141	Observational, prospective	MAC, RIC, day 0 and 15	Lower diversity in the aGVHD group compared with non-aGVHD group	
Stein-Thoeringer et al., 2019 ¹³⁵	1325	Observational, prospective	MAC, RIC, after day -30	Increased <i>Enterococcus</i> was associated with GVHD and mortality 20 months post-HSCT	
Jenq et al., 2015 ¹²⁰	64	Observational, prospective	MAC, RIC, +8 to +16, median day +12	Increased abundance of <i>Blautia</i> genus was associated with reduced lethal GVHD and improved 5-year overall survival	
Taur et al. 2014 ¹³⁴	80	Observational, prospective	MAC, RIC, within 7 days following HSCT	Low microbial diversity associated with 3 year mortality	
Taur et al., 2012 ¹²¹	94	Observational, prospective	MAC, RIC, from before transplant until day 35	Disruption of diversity and stability. Domination of bacteria associated with bacteremia	

1.10 Rationale for the current thesis

Recipients of allo-HSCT have reduced QoL before, during and until one year after the treatment. Data show that gastrointestinal side effects of the treatment are associated with impaired energy intake, poor nutritional status and reduced QoL. Nutritional intervention may increase energy intake, improve nutritional status and QoL. However, nutritional status is measured with a few non-specific methods, and there is no consensus on the optimal nutritional support of recipients of allo-HSCT. In our previous study patients reported a limited number of tolerable food items available and individualized small helpings on a regular basis, and they missed focus on nutritional care (30). Studies comparing EN versus PN have shown that tube feeding alone is not feasible in all patients. Finally, we were not able to identify evidence based recommendation for energy and protein requirements, time point for initiating medical nutrition or the superior route of nutritional support, and a search in Clinical Trials did not reveal any ongoing RCTs on this subject (as of May 2010, when we started our project). Thus the main aim of this study was to investigate the effects of optimized energy and protein intake on QoL and clinical outcome in recipients of MAC within a RCT.

2 Aim and objectives

2.1 Aim

The aim of this study was to improve global QoL and clinical outcomes among recipients of allo-HSCT by providing optimized energy and protein intake during transplantation. To achieve this, the following study objectives were defined.

2.2 Study objective

The specific objectives of the study were to:

1. In a RCT, examine if an intervention comprising optimized energy and protein intake preferably by the enteral route, would improve QoL 3 months after HSCT (main aim).
2. Test the effects of the intervention on oral mucositis and aGVHD 3 months after allo-HSCT.
3. Test the effects of the intervention on anthropometrical measures, infectious complications, engraftment and transplant-related mortality 3 months after allo-HSCT.
4. Test the effects of the intervention on 1-year survival, body weight and body composition.
5. Test the effects of the intervention on gut microbial composition, SCFAs and markers of gut barrier function at baseline and 3 weeks post-HSCT.

3 Methodology

3.1 Study design

In Paper I, we performed an open, two-armed randomized controlled trial. Patients were randomly assigned using a 1:1 ratio (block sizes of 10) to receive optimized energy and protein preferably by the enteral route (intervention) or conventional nutritional support (control). Recording dietary intake could increase awareness of energy content in food and thereby lead to false increase intake. Therefore we did not record dietary intake in the control group. Instead we compared total energy intake between the intervention group and a reference group (serving as a proxy for the control group). We compared global QoL, oral mucositis, aGVHD, nutritional status, infectious complications, engraftment and mortality between the two study arms 3 months post-HSCT. Analyses were performed according to intention-to-treat.

Paper II was a follow-up of the RCT described in Paper I. We used a longitudinal study design to assess body weight, body composition and survival at 1-year follow-up.

Paper III describes microbial diversity, fecal short chain fatty acids and markers of gut barrier function in a subset of patients included in the RCT described in Paper II.

3.2 Study populations and selection of participants

We conducted the RCT at the Department of Haematology, Oslo University Hospital, Rikshospitalet. Oslo University Hospital is the only hospital in Norway performing allo-HSCT with unrelated donors. About 50 allo-HSCT were performed annually with MAC when commencing the RCT in 2010. Since then allo-HSCT after RIC has evolved and has outnumbered allo-HSCT following MAC.

Inclusion criteria for the studies in Paper I-III were patients aged ≥ 18 year diagnosed with a hematological malignancy and offered allo-HSCT with MAC. Participants were

invited 1 to 3 months before admission for allo-HSCT. We used the same inclusion criteria for the reference group that contributed to estimate total energy intake before commencing the RCT. The reference group was invited to participate the day they arrived for the treatment.

3.3 Treatment procedures

Patients arrived at hospital about one week before transplantation (Figure 2).

Figure 2. Overview over the early transplantation phase. Patients arrived one day before commencing the conditioning regimen (usually day -8). The conditioning regimen was administered from day -7 to -1. GVHD prophylaxis was given from day -1. Stem-cell was given day 0 via a central venous catheter. Protective isolation and a diet to protect against foodborne infections were initiated day +5. Supportive treatment e.g., antimicrobial treatment, blood and platelet transfusion, TPN were administered from day +5 and until recovery. Length of rehabilitation depends on the presence of complications.

All patients in the RCT received a conditioning regimen that consisted of busulphan and cyclophosphamide or total body irradiation combined with cyclophosphamide. Hydration with 5% glucose was a part of the conditioning regimen. We used HLA-identical related or unrelated donors. Allografts were mobilized peripheral blood stem cells or bone marrow cells. At our institution i.v. gentamicin and penicillin are the preferred first line treatment for neutropenic fever in patients undergoing allo-HSCT. When fever lasts >3 days we change to i.v. ceftazidime or piperacillin-tazobactam. We used meropenem in combination with antifungal treatment when fever persisted (>7 days) (150). Antibiotic prophylaxis against *Pneumocystis jirovecii* and partial gut decontamination were trimethoprim-sulfamethoxazole. We used cyclosporine and methotrexate for aGVHD prophylaxis.

3.4 Calculation of sample size in the RCT

We used the European Organization for Research and Treatment of Cancer Core Quality-of-Life Questionnaire (EORTC QLQ-C30) to assess QoL. Sample-size calculation was based upon an expected change by 15 points in the global QoL score (primary end-point) as this has been considered clinically relevant (151-156) and consistent with our prior results in Norwegian patients with acute myeloid leukemia where mean global QoL was 60 (156). Furthermore, reference data on health-related QoL in a sample of the Norwegian general population was between 75-80 points (157). In total 88 patients (44 in each group) were required to achieve 80% power with a two-sided significant level of 5%.

3.5 Nutritional intervention in the RCT

The targeted minimum energy intake was 30 kcal/kg/day and protein intake of 1.5-2.0 g protein/kg/day (53, 158) and are in line with current guidelines (24). We determined the targeted energy requirement by correcting for resting energy expenditure (49, 50). Resting energy expenditure was measured by indirect calorimetry (Vmax Spectra 229, SensorMedics, Yorba Linda, California) (50) with an added physical activity level factor of 1.4 (159). Patients received routine hospital food from a central kitchen and were encouraged to take high-energy and lactose-reduced snacks denoted “Lite og godt”© and oral supplements. The idea of the “Lite og godt” diet arose in collaboration between the hospital kitchen and the Division of Surgery, Inflammatory Diseases and Transplantation, Oslo University Hospital. This diet was modified according to our previous observations of patients’ food choices during the peritransplant period (30). The “Lite og godt” diet consisted of 16 dishes of small energy enriched portions, available at all time points. Pictures of the dishes were presented in a brochure

(Appendix 1). We performed dietary counseling on a daily basis and evaluated the selected meal menu.

First-line nutritional support was via the enteral route (23). A nasojejunal (NJ) tube (Flocare Bengmark Ch8, Nutricia, Schiphol, The Netherlands or Freka Endolumina 10 Fr, Fresenius Kabi, Bad Homburg, Germany) was placed using guide-wire-assisted techniques with fluoroscopic assistance. If guide-wire-assisted placement was not successful we converted to endoscopic guidance. The targeted tube placement was the tip past ligament of Treitz (86, 160). NJ tubes were fixed with tape at the cheek to reduce loss of tubes when vomiting, thus allowing the tube to come out the nose during vomiting and when vomiting has ceased, will pass spontaneously again (86). The NJ tube was placed within day +5, before pancytopenia and mucositis developed (86). Loss of NJ tube was replaced until two times with a nasogastric (NG) tube (Flocare 12 FR, Nutricia).

We used an enteral fiber-free formula with hydrolyzed medium triglyceride fat (Nutrison Advanced Peptisorb, Nutricia) (86, 149). The feeding commenced at 15 mL/hour and the feeding rate increased with 15 mL/24 hours (maximum 100 mL/hour), depending on tolerance (86, 149). EN ran continuously (over 24 hour) via an enteral feeding pump. If patients were intolerant to the tube or tube feeding (i.e. the targeted energy requirement was not achieved) we supplemented with PN or changed to PN which met estimated nutritional requirement in conjugation with oral intake. The nurses provided the nutritional intervention based on a predefined algorithm (Appendix 2) and monitored the energy intake from each administration route. The intervention started when commencing the conditioning regimen and lasted until hospital discharge. Dietary advice and oral supplements were provided at the outpatient clinic.

3.6 Standard care in the RCT – control group

The patients in the control group were treated according to institutional guidelines. Patients received the conventional hospital menu and TPN was first line treatment for nutritional support, initiated and dosed by the attending physician. TPN was administered through a central venous catheter using a central PN solution in a triple chamber bag including an amino acid solution with electrolytes, glucose solution and lipid emulsion (Olimel, Oliclinomel (Baxter, Illinois) or SmofKabiven (Fresenius Kabi). This formula included supplementation with trace elements (Tracel®) together with water and fat-soluble vitamins (Solu-vit® and Vitalipd®) and administered at a rate required to meet 1100, 1600 or 2200 kcal/day. We did not routinely use glutamine supplementation. Estimation of energy and protein requirement for the individual patients, daily recording of food intake and placement of a NJ/NG feeding tube were not used as a routine.

3.7 Assessment tools and instruments

3.7.1 Recording energy and protein intake in the RCT

Patients, relatives and/or nurses recorded energy intake in the intervention group on a dietary form developed for the study (Appendix 3). We used a dietary form modified from Department of Clinical Nutrition St. Olavs Hospital, Trondheim. This dietary form was updated according to changes in the hospital menu. Energy and protein contents were calculated using the software package Aivo 2000 (AIVO AB, Stockholm, Sweden). Energy from PN was given at the medical record. Amount of energy administered via tube-feeding was given at a chart developed by Section of Clinical Nutrition, Department of Clinical Service, Oslo University Hospital and modified for this study (Appendix 4). The rate of tube-feeding and symptoms interfered with tube feeding (e.g. nausea, vomiting, diarrhea, abdominal pain) was recorded in the latter

chart. The amount of protein intake from food, tube feeding and PN were calculated the day after. Before the regular 3 months follow-up consultation patients in both groups were asked to record food and drink intake for a single day. To increase the accuracy of the food intake interviews were carried out.

3.7.2 Reporting quality of life

Patients self-reported QoL by the EORTC QLQ-C30 questionnaire (161) (Appendix 5). EORTC QLQ-C30 is a cancer-specific 30-item questionnaire consisting of multi-item scales and single-item measures on symptoms and level of function. This questionnaire is used to describe QoL in hematological malignancies and recipients of allo-HSCT worldwide (162, 163) and has previously been used in the Norwegian allo-HSCT population (164) and in the general population (157). These scales include five functional scales (physical, role, emotional, cognitive and social function), three symptom scales (fatigue, nausea/vomiting and pain) and six single items (dyspnea, insomnia, appetite loss, constipation, diarrhea and financial problems) (165). Global QoL combines two item-global overall health and QoL scale from 1 (very poor) to 7 (excellent) and transformed to a 0-100 continuous scale. Higher scores on the functional and global QoL scales indicate better functioning, whereas worsening on the symptom and single item scales. We assessed QoL outcomes at baseline, 3 and 6 weeks and 3 months post-HSCT.

3.7.3 Oral mucositis assessment

We scored oral mucositis with the 0-4 WHO Toxicity Scale (104, 166) (Appendix 6) from onset of oral mucositis until score zero in three consecutive days. WHO Toxicity Scale consist of objective and functional assessment, is easy to use and is known to be the most comprehensive tool for measure oral mucositis in clinical practice and research (104, 108).

3.7.4 Acute graft-versus-host disease assessment

Acute GVHD was diagnosed and graded according to the International Bone Marrow Transplant Registry criteria and we reported the highest grade (122) (Appendix 7).

3.7.5 Measurement of anthropometry, body composition and nutritional status

Anthropometric measurement consisted of upper arm circumference, upper-arm skinfold thickness, handgrip strength and body composition. Height was measured with a wall-mounted stadiometer with an accuracy of 0.1 cm (Soehnle Professional, Backnang, Baden-Württemberg, Germany). Weight was measured with Tanita BC-418 MA Body Composition Analyzer (Tanita Corp, Tokyo, Japan) and read to the nearest 0.1 kg, with the patient wearing light clothes and no footwear. A correction factor of -1 kg was used to adjust for the weight of light clothing. Fat free mass and fat mass were measured with the latter instrument, read to the nearest 0.1 kg and divided by the body surface area (m^2) for fat free mass index. We considered fat-free mass index $<17 \text{ kg/m}^2$ for men and $<15 \text{ kg/m}^2$ for women as low (167) and body fat mass index $<2 \text{ kg/m}^2$ for men and $<4 \text{ kg/m}^2$ for women (168).

Triceps skinfold (TSF) thickness was measured on the non-dominant arm using a Harpenden Skinfold Caliper (West Sussex). Mid-arm circumference was measured on the dominant arm with a non-stretchable tape on the midpoint of the arm, with the measuring tape between the shoulder (acromion) and the elbow (olecranon) while the person was bending the arm 90° . The mid-arm muscle circumference was calculated using the following formula: mid-arm circumference - $(3.14 \times \text{TSF} / 10)$ (169). All measurements were performed by two trained nurses at inclusion and then repeated at 3 and 6 weeks and 3 months. Nutritional status was evaluated by the Patient Generated Subjective Global Assessment tool (PG-SGA). We scored patient as well nourished, moderate or suspected malnutrition and severely malnourished based on six

components: 1) Weight history and 2) food intake, 3) patient self-reported symptoms interfere with food intake (e.g. loss of appetite, nausea, vomiting, diarrhea, pain, mouth sores), 4) physical activity, 5) diseases impact on energy requirement, 5) metabolic demand and 6) physical examination of fat stores, muscle status and fluid status (170, 171) (Appendix 8). Weight history before baseline was self-reported.

3.7.6 Assessments of infection and other clinical outcomes

Infectious complications were diseases due to virus, invasive fungal disease, bacteremia, pneumonia or fever of unknown etiology. CMV infection was defined as recommended and we used routine PCR-based techniques for detecting CMV in blood (125). Fungal disease was classified according to recommendations (126) (see section 1.8). Bacteremia was defined as the first positive blood culture during a 10-day period. Repeated positive blood cultures obtained >10 days after the first were considered as new episodes. Diagnosis of pneumonia required detection of new pulmonary infiltrates on X-ray or CT scan and symptoms of respiratory infections. Fever was defined as a rectal temperature ≥ 38 °C. Empirical use of antibiotic was reported as number of days. We registered all outcomes from first event until death or hospital discharge. Engraftment was reported as recovery of neutrophil granulocytes and defined as the first of three consecutive days of a blood concentration $\geq 0.2 \times 10^9/l$ and platelet engraftment as a blood platelet concentration $\geq 20 \times 10^9/l$ without platelet transfusion. Transplant-related mortality within 3 months was death of any cause except for relapse (Paper I). Relapse was defined as bone marrow blasts >5% and non-relapse mortality as death from any cause except from relapse (Paper II). One year mortality was defined as death from any cause (Paper III).

3.7.7 Assessment of gut microbiota, fecal short chain fatty acids and markers of gut barrier function

We collected fecal samples throughout the last five randomization blocks. Samples were stored in a standard Fecal Container (Thomas Scientific, Swedesboro, NJ) and immediately frozen at -80°C or immediately stored at 4°C and frozen within 24 hours at -80°C. Blood samples were immediately frozen and stored at -80°C. Both fecal samples and blood samples were collected at baseline and 3 weeks post-HSCT.

Gut microbiota was measured and interpreted by collaborators at the Norwegian PSC Research Center and Research Institute of Internal Medicine, Division of Surgery, Inflammatory Diseases and Transplantation, Oslo University Hospital. Microbial DNA were extracted from fecal samples using PSP Spin Stool DNA Kit (Stratec Molecular GmbH) and sequenced on the Illumina MiSeq platform targeting the V3-V4 region of the 16s rRNA (REF PMID 24558975). Reads containing Illumina Universal Adapters or PhiX were discarded using bbdut version 37.55 (BBTools, <https://jgi.doe.gov/data-and-tools/bbtools>), and the remaining reads were demultiplexed using je version 1.2 (172). Indices, heterogeneity spacers and primers were trimmed with cutadapt version 1.14 (173) and the paired end reads were subsequently quality trimmed and merged using bbmerge version 37.55 (174). The merged contigs were quality filtered using default values in Quantitative Insights Into Microbial Ecology (QIIME) version 1.9.1 (175). Closed reference observed operational taxonomic units (OTUs) mapping to the Silva database (176) (version 128, reference OTUs pre-clustered at 97% sequence similarity) was performed using SortMeRNA version 2.0 (177) through QIIME. OTUs with a number of sequences <0.005% of the total number of mapped sequences were discarded as recommended (178). The samples were rarefied (subsampled) to an OTU count of 15,004 per sample, and all further analyses were performed on this rarefied

dataset.

Fecal concentrations of SCFAs were measured and interpreted by collaborators at the Unger-Vetlesen Institute, Lovisenberg Diaconal Hospital, Oslo. SCFAs were analyzed using vacuum distillation followed by gas chromatography as previously described (179). Levels of the following SCFAs were assessed: acetic acid, propionic acid, iso-butyric acid, butyric acid, iso-valeric acid, valeric acid and total SCFAs. The plasma levels of the gut-leakage markers were measured and interpreted by collaborators at the Center for Clinical Heart Research, Department of Cardiology, Oslo University Hospital, Ullevål, Oslo. Intestinal fatty acid binding protein (I-FABP), lipopolysaccharide binding protein (LBP) and soluble cluster of differentiation (sCD14) were measured by ELISAs (Hycult Biotech, Uden, The Netherlands and R&D Systems Europe, Oxfordshire, UK, respectively). The inter-assay coefficients of variation were 5.8%, 9.8% and 7.4%, respectively.

3.8 Data analysis

Paper I: Mann-Whitney U test was used to identify the significant difference in energy intake between the intervention group and the reference group for the control group. Analysis of covariance was used to test the effect of group on global QoL from baseline to 3 months. A linear mixed model for repeated measure was used to compare global QoL and subscale scores between groups at the various time points. We used chi-square test and estimated relative risk of secondary outcomes (oral mucositis and aGVHD). Chi-square test, Fisher exact test and Mann-Whitney *U* test were performed to determine possible relationship between the two study group and various clinical outcomes.

Paper II: Cox regression model was used to study the effects of treatment group (intervention vs control), weight, fat-free mass index and body fat mass index on death,

relapse and non-relapse mortality. We used the standard competing risk framework for relapse and non-relapse mortality. Kaplan-Meier method was used to compare the overall survival between the intervention and control group. We adjusted for treatment group, gender and age in the multivariable analysis. Additionally, we performed a sensitivity analysis for death, adjusting for discharge (no/yes), disease status (standard risk and high risk, Hematopoietic Cell Transplantation-specific comorbidity Index (low risk and intermediate/high risk) and European Group for Blood and Marrow Transplantation score (low and intermediate/high). Baseline body weight, fat-free mass index and body mass index were analyzed as continuous variables and weight change before baseline as a categorical variable. A linear mixed model for repeated measures was used to test the interaction between group and time for body weight, fat-free mass index, body fat mass index and total body water during 1-year follow-up. Chi-square or Fisher exact test was used to study association between the use of glucocorticoids and weight change.

Paper III: Mann-Whitney U test was performed to determine associations between gut microbiota composition, SCFAs, markers of gut function, survival, aGVHD and groups. We performed Wilcoxon signed rank test to determine changes in gut microbiota composition, SCFAs, markers of gut function. Correlation analysis was performed to determine possible relationship between gut microbiota composition, SCFAs and markers of gut function. Permutational multivariate analysis of variance was used to compare changes in beta diversity between groups. Benjamini-Hochberg method was used to detect false discovery rate when multiple testing.

All data (Paper I-III) were analyzed using SPSS software version 25.0 and 26.0 IBM SPSS INC. (IBM Corp., Armonk, NY) (180, 181) and R package, version 3.5.2 and 3.6.1 (182, 183).

3.9 Research approvals

The study was approved by The Regional Committee for Medical and Health Research Ethics South East Norway and the Data Protection Officer, Oslo University Hospital. Reference number was #S-09136c 2009/2115. The study was also registered at ClinicalTrials.gov, ID NCT01181076. All participants gave their written consent to participate in these studies. We informed the participants that they were free to withdraw from the study at any time. An independent data monitoring committee assessed safety aspect after hospital discharge for the first 40 patients (20 in each study group), and again after the next 40 patients in the RCT. No safety concerns were identified.

4 Summary of results

4.1 Paper I

The main purpose was to assess the effect of the intervention on global QoL, oral mucositis, and aGVHD 3 months post-HSCT. Other outcomes were nutritional status, infectious complications, engraftment time and treatment-related mortality. We randomized patients to the intervention (n=57) or control (n=60) group. Global QoL and subscale scores were calculated from the QoL-forms. Oral mucositis was assessed by WHO oral toxicity scale and aGVHD diagnosed according to the International Bone Marrow Transplant Registry criteria. We used the PG-SGA form for reporting nutritional status and measured body composition. Infectious complication, engraftment and treatment-related mortality were assessed by consensus criteria. The intervention group received a median intake of 32 kcal/kg body weight and 1.1 g protein/kg body weight per day. Furthermore, these intakes were significantly higher compared to a reference group for the control group. We did not find any significant differences in mean global QoL score between the two study groups. Similarly, with the other QoL subscale scores there were no significant differences between the intervention and control group, except for less constipation in the intervention group (p=0.04). The risk of oral mucositis and aGVHD was the same in each group. The number of patients being well-nourished, moderately or suspected malnourished or severely malnourish at 3 months were 28, 11 and 2, respectively, in the intervention group, and 28, 16 and 4 respectively, in the control group. Body composition did not differ between groups. Similarly, infectious complications, engraftment time and treatment-related mortality were the same in each group. At 3 months follow-up the median (min-max) energy intake was 30 (8-65) kcal/kg and 27 (9-75) kcal/kg in the intervention and control group, respectively. Corresponding to 1.1 (0.3-2.4) g/protein/kg and 1.0 (0.3-2.4)

g/protein/kg in the intervention and control group, respectively. In conclusion, the nutritional intervention did not improve global QoL or early clinical outcome in recipients of allo-HSCT.

4.2 Paper II

The main purpose of this 1-year follow-up study was to assess the effect of the nutritional intervention on survival, changes in weight and body composition and the effect of changes on in weight and body composition on survival. Body weight and body composition were measured at baseline, 3 and 6 weeks, and 3, 6, 9 and 12 months post-HSCT. We did not find any significant differences in 1-year survival between the two study groups. Changes in body weight and body composition were the same in each group. We pooled the groups and found that weight gain (i.e. fluid retention) was significantly associated with increased 1-year mortality.

4.3 Paper III

We assessed if gut microbiota composition, microbial function (as measured by SCFAs) and markers of gut barrier function (I-FABP, LBP and sCD14) were associated with overall survival, aGVHD and altered by the nutritional intervention. This was a follow-up study on a sub-cohort (n=47) with fecal samples from the RCT. Markers of gut barrier function were assessed in the total cohort (n=117). Fecal samples and markers of gut barrier function were collected at baseline and 3 weeks post-HSCT. Microbial composition and fecal SCFAs were significantly depleted from baseline to 3 weeks, whereas I-FABP decreased, LBP increased and sCD14 remained stable from baseline to 3 weeks. Lower microbial diversity was significantly associated with increased 1-year mortality, but not with aGVHD. Baseline diversity was not associated with clinical outcomes. We observed minor associations between SCFAs and markers of gut barrier

function and clinical outcome. The nutritional intervention was not associated with microbial composition, SCFAs or the markers of gut barrier function.

5 General discussion

5.1 Summary of main findings

The results of the nutritional intervention revealed no effect on global QoL or any of the clinical outcomes oral mucositis, aGVHD, nutritional status, infectious complications, engraftment and mortality 3 months post-HSCT (Paper I). There was no significant difference in 1-year mortality or body composition between groups during 1 year follow-up. When we pooled the groups, weight gain (interpreted as fluid retention) was significant associated with 1-year mortality (Paper II).

The nutritional intervention had no effect on gut microbiota, SCFAs or markers of gut barrier function. One-year mortality in the pooled groups was significantly higher in patients with lower bacterial diversity at 3 weeks. There were minor associations between gut microbiota, SCFAs and markers of gut barrier function. The SCFAs and markers of gut barrier function were not associated with clinical outcomes (Paper III).

5.2 Quality of life

QoL and patient reported outcomes are increasingly evaluated in cancer RCTs to provide information about treatment risks, benefits, and tolerability (91, 184-186). However, QoL measurement has been criticized for not representing what is experienced by the individual patient and in groups of patients. Impact of confounders and statistical challenges are other limitations (187, 188). Lack of a uniform definition of QoL and wide variation in the use of questionnaires may lead to difficulties in interpreting and comparing results across studies (100, 189). Increasing use of scientifically developed questionnaires validated in specific patient populations and cultural settings has proven its usefulness (190). However, a method for determining the clinical meaningfulness of changes in scores is still needed (190). Patients undergoing

allo-HSCT suffer from a significant symptom burden of the treatment and reduced psychosocial and physical functioning. Their symptoms and functioning are core factors in the daily evaluation of supportive care in clinical practice. Individually tailored nutritional support could counteract the effects of adverse outcomes of the treatment and maintain or improve functioning. We have shown that global QoL was still impaired 3 months after allo-HSCT, similar to other studies (94, 95). We also found that global QoL score and subscale scores (except for dyspnea, constipation and financial difficulties) were lowest 3 weeks after transplantation and thereby increased, but were not back to baseline level 3 months after allo-HSCT. Scores on dyspnea, constipation and financial difficulties were stable throughout the follow-up. The intervention did not change the global QoL (Paper I). This could be attributed to the impact of symptom severity and impaired function on global QoL. Several lines of research show that nutritional status is of importance for functioning and QoL. Absence of effect of the intervention on nutritional status might be the most likely explanation for no effect of the intervention on QoL. In support of this, the number of patients classified as well-nourished, moderately or suspected malnourished or severe malnourished were the same in both study groups. Oral function (i.e. chewing and swallowing) is less affected by day 100 (191). However, low energy level (fatigue) and dysfunction of factors involved in food regulation (e.g. smell, taste, appetite) may persist in the recovery phase and affect the desire to eat and result in lack of interest in social family dining of outpatients. Those with lower energy level experienced more taste disorders, more food aversion and more weight loss (31, 191). Thus, 3 months after transplantation might be too early to assess the effect of a nutritional intervention on global QoL. In line with this, reportedly, at six months post-HSCT, physical well-being was associated with

higher BMI whereas poor physical and social well-being was associated with weight loss (97).

We adapted the best knowledge from previous studies and designed a patient-centered intervention for the individual patient. The study was performed per protocol and there was satisfactory compliance to the protocol. We monitored energy and protein intake each day, and the results of our study revealed the need for individual tailored nutritional support. We do not believe that a high energy intake in the control group (similar to the intervention group) could explain similar scores in global QoL in the two groups. Energy intake was not monitored in the controls, and if they had a high energy intake, it is likely that global QoL in both groups should be better than what is reported by others. We did not achieve our targeted protein intake of 1.5-2.0 g/kg body weight/day (52), possibly because such a high protein intake is challenging without glutamine supplementation. Routine use of glutamine supplementation was not recommended when we commenced the study or later (23, 24). Furthermore, we achieved a median protein intake similar to what is recommended by others (1.1/kg body weight/day) (24). However, the intervention might have been given for a too short time and of a much shorter duration than the side effect of the transplantation procedure. In support of this, we found a gradual improvement in body weight between 6 and 9 months post-HSCT. In addition, some patients had an extremely low energy intake a single day before the regular 3 months' visit. In line with this, several studies have documented the need for close monitoring of nutritional status and the need for dietary counselling after discharge from hospital (32, 33, 192).

5.3 Clinical outcomes

During prolonged energy and protein deficit (starvation) the body metabolizes fat stores and conserves structural proteins (e.g. skeletal muscle, internal organs). Whereas during

disease and stress reduced energy and protein intake may be associated with sustainable metabolism of protein and rapid muscle wasting. Nutritional deficit and malnutrition cause adverse effects on tissues, body composition and possibly on biological and physical functioning. Even short term nutritional deficit may cause impaired function (193, 194). Inadequate nutrition may result in impaired immune function and infections, poor wound healing, loss of lean body mass, atrophy of the gut lining, decreased intestinal secretions and changes in gut flora. The latter may further reduce gut function and the ability to absorb nutrients from food. Cyto-reductive therapy pause renewal of cells, and a good nutritional status may counteract some of the adverse events of the treatment (195).

5.3.1 Oral mucositis

Optimized nutritional support may improve immune function and cell renewal and thereby reduce mucositis (195). The prevalence of oral mucositis varies between studies, probably due to the use of different diagnostic criteria, conditioning regimens, and type of transplantation procedure (allo- and autologous HSCT, MAC, RIC). We revealed no effect of the intervention on oral mucositis (Paper I), thus supporting studies comparing oral mucositis in patients undergoing parenteral feeding versus enteral feeding (69, 70), that oral mucositis during the course of allo-HSCT cannot be reversed by nutritional support. Nutritional strategies should emphasize on dietary advice and food selection for coping with mouth soreness, and changes in smell and taste perceptions (“mucositis” of the nose and tongue) (31, 196).

5.3.2 Acute graft-versus-host disease

We observed no differences in aGVHD between the two study groups. Oral intake and tube feeding were associated with less aGVHD in comparison to parenteral feeding in observational studies (69, 72, 73), but these results were not confirmed in another

observational study and a RCT (70, 71). Several immunological mechanisms are involved in GVHD. Donor T cells interact with activated host and donor antigen presenting cells. Cytokines, chemokines, cellular receptors and immune cells modulate T cell/antigen presenting cells leading to initiation and maintenance of GVHD. Preventing and treatment of GVHD affect both non-specific immunosuppressive and anti-inflammatory mechanisms (197). The role of enteral feeding *per se* and specific nutrients in preventing or mediating GVHD is unclear. Specific fecal bacteria have been associated with reduced death-rate from GVHD and improved overall survival (123, 138). However, if e.g. fiber known to have a beneficial effect on commensal bacteria could mediate GVHD is unknown. No study existed on probiotics, prebiotic and dietary fiber in 2013 (198) and we were not able to identify recent studies. Expert opinions recommend 0–per oral and TPN in the case of severe diarrhea (>1 L/day of feces). When diarrhea <500 mL/day an empirical derived step wise oral diet with limited amounts of fats, fiber, lactose, acidic food-stuff and gastrointestinal irritants was recommended (198).

5.3.3 Body composition, nutritional status, infection, engraftment and survival

We showed no effect of the intervention on body weight and body composition during 1-year follow-up. Weight loss plateaued and was transient for most patients 6 months post-HSCT. Weight gain interpreted as fluid retention was associated with increased 1-year mortality, indicating that the sickest patients gained weight, and this weight gain was a complication of the treatment (Paper II). Weight gain is possibly due to capillary leak syndrome (leakage of intravascular fluids into interstitial space). Capillary leakage has an early onset, and is a result of endothelial injury (“mucositis” in the blood vessels) caused by the allo-HSCT procedure (40). Accumulations of fluids into interstitial space with no response of diuretic therapy possibly lead to renal, pulmonary or cardiac

dysfunction. Subsequently multiorgan failure occur, requiring intensive care treatment and is associated with poor prognosis (39). Furthermore, the number of patients classified as malnourished at 3 months was the same in both study groups and mainly in line with previous studies (35). Variations in the prevalence of malnutrition between studies are possibly due to the use of different nutritional assessment tools, time points for measuring, heterogeneous patient-groups and the largely use of cross-sectional study design. Furthermore, weight gain is in fact accumulations of fluids, therefore body composition should be measured.

Optimized nutritional support may improve immune function and reduce number of infections (195). The number of infectious complications was the same in both study groups, which is in line with one retrospective study (70), but in contrast to a prospective, not randomized study showing lower infection-related mortality in an EN group compared with a PN group (69). We revealed no effect of the intervention on time to engraftment. Shorter time to engraftment was associated with enteral feeding in contrast to a parenteral feeding in one study (69), but not in others (70, 73). Furthermore, malnutrition was associated with delayed engraftment (129). Three months transplant-related mortality was the same in the two study groups and in line with what is expected (6, 8, 13). Less treatment-related mortality was found in patients receiving EN in comparison with PN in one study (69), in contrast to others (70, 73). Studies assessing the effect of nutritional support on clinical outcomes are mainly cross-sectional or cohort-studies and one therefore cannot make causal relationship. Our study was not powered to perform subgroup analyses of nutritional status and clinical outcomes.

5.4 Microbial diversity, short chain fatty acids, markers of gut barrier function and clinical outcomes

The human gut harbors trillions of microorganisms. These microorganisms are involved in fermentation of nutrients and play an important role for gut functioning and the immune system. Diet is a determinant of gut microbiota composition. Furthermore, specific nutrients are associated with variation in the gut microbiome. In general we distinguish between bacteria specialized in fermentation of carbohydrate versus bacteria involved in protein fermentation. Diet rich in fiber is associated with increased bacterial diversity (130). Additionally, foodborne microorganisms (bacteria, fungi and virus) colonize the gut. SCFAs are end-products of fiber fermentation. The SCFAs butyrate is the most important energy source for the epithelia cells in the lining of the gut and has an important role in immune regulation (199). Our long-term food habits influences gut microbiota. It is not clear how rapidly the human gut responds upon changes in the composition of the diet (200). Changes in microbial composition have been detected within 24 hours of initiating a high-fat/low-fiber versus low-fat/high-fiber diet (201). However, these changes may be transient (202).

We observed a major depletion of gut microbiota in both study groups (Paper III), similar to others (131). We found the same reduction in SCFAs and altered markers of gut barrier function from baseline to 3 weeks (Paper III). Similarly has a reduction of SCFAs been reported in children (142) and in mice (143). I-FABP decreased, similar to the reports of others (103, 145), whereas LBP increased from baseline to 3 weeks, in line with others (146). Serum CD14 remained stable. We revealed that the nutritional intervention did not modify microbial composition, SCFAs or markers of gut barrier function. This is similar to a RCT comparing EN with PN (71), and in contrast to an observational study of children receiving EN (148). In contrast to the parenteral route, the enteral route is thought to have a beneficial effect on intestinal flora (75, 76).

Trophic feeding (enteral feeding of ~500 kcal/day) aims to maintain gut integrity in patient suffering from EN intolerance (87). If this beneficial effect is achievable in patients with conditioning-induced loss of gastrointestinal mucosal mass, is unknown. The main aim of our intervention was to provide the targeted energy and protein requirements for the individual patient and thereby counteract the challenges in previous studies that mostly aimed to compare EN with PN. In such studies the concept of gut nourishment was largely not defined. Furthermore, half of the patients predominantly allocated to EN, ended up with PN or EN in combination with PN, thus leading to difficulties with interpretation of the results (69-72). Clearly, the sickest patients are often those with most severe toxicity and aGVHD, and they do not tolerate enteral feeding and the results are likely to be explained by group assignment bias.

Dietary fibers and prebiotics (non-digestible carbohydrates) are known to have a protective advantage for commensal bacteria that are capable of metabolizing these substrates (130, 200), and the beneficial effects of enteral feeding may be related to the prebiotic of fiber from selected fruits and vegetables (88, 203). We used a fiber-free enteral formula in line with others (71, 86, 149). Furthermore, we lack data on fiber intake by the oral route. Patients may avoid fiber-rich food periodically during the course of transplantation (30, 58). It is therefore likely to assume that fiber intake in both the intervention and control groups were too low to have a beneficial effect on the microbiota.

We observed low microbial diversity and less abundant *Blautia* at 3 weeks, but not at baseline in those who died during 1 year follow-up in the pooled cohort of both study groups, whereas aGVHD was not associated with microbial diversity. Microbial diversity both before admission to hospital for allo-HSCT and during the course of the treatment was associated with both survival and aGVHD in a multicenter study (131).

Fecal total SCFAs and markers of gut barrier function correlated with diversity and *Blautia* genus at 3 weeks, but were less strongly associated with clinical outcomes.

Due to the complex mechanisms involved in immune regulation and cell renewal during the course of allo-HSCT, it seems less likely that optimized energy and protein intake could alleviate side effects of the treatment such as mucositis, aGVHD, shorten the time to engraftment or modify microbial diversity. This raises the question whether it is worth patient struggling to achieve an adequate energy and protein intake, and undergo tube feeding during the early phase due to the poor tolerance. Mild weight loss due to insufficient energy and protein intake during the early phase may be acceptable. The composition of the diet may modify microbiota and affect disease development and progression (204, 205). Design of tailored diets (personalized food) has in comparison to traditional nourishing (breast milk) shown to change the abundances of targeted bacteria and improve growth, neurodevelopment, and immune function in several malnourished children in randomized, double-blind controlled study of undernourished children (206).

While we are waiting for further understanding of the role of the microbiome in the outcome of allo-HSCT, patients should encourage selecting fiber-rich food periodically when they are capable to ingest such food substrates. Furthermore, much could be gained to prevent malnutrition by providing nutritional support in those undergoing several cycles of chemotherapy before transplantation and those with chronic GVHD at nutritional risk (41, 207, 208).

5.5 Future directions in nutritional support

Based on the results in this thesis and our previous experience (30) we suggest the time has come for revision of nutritional guideline(s) in allo-HSCT. A revised guideline

should focus on recommendation for food selection with respect to smell, taste, temperature and texture and linked to periods (i.e. before transplantation, during the neutropenia, after engraftment) of the treatment. The food service systems should be designed for providing palatable enriched mid-meals available at all time points. Dishes containing fresh fruit and vegetables added high-energy ingredients and oral nutritional supplements could increase energy and protein intake. Pictures of the menu should be available for the patients. Insertion of feeding tubes should not be recommended routinely. Supplementation of individual adjusted pharmacy based PN could be the best option to ensure sufficient energy and protein intake in those who are unable to eat. Furthermore, nutritional and food care should receive increased status in the high technical environment that allo-HSCT is part of.

5.6 Methodological considerations

The randomized design constitutes a major strength. In general, randomization should eliminate group assignment bias, specifically selection bias and confounding (209). The intervention included a detailed and daily monitoring of energy and protein intake. The intervention included QoL in nutritional measurement and was the first RCT with global QoL as the main end point. In addition, we used a validated, commonly used QoL questionnaire within this setting. We have detailed measurement of energy- and protein intake and body composition. A strength was the longitudinally measurement of body composition. Furthermore, the homogenous patient group (all had a malignant blood disease and received MAC) reduces the risk of clinical effects to be masked by heterogeneity in the transplantation procedure. The study had a long follow-up time, and the rates of loss of follow-up were as expected in critically ill patients.

This study has several methodological weaknesses. We lack data on the specific nutrients provided. Compliance to tube feeding possibly affects the results of the study.

We did not analyze variations of dose and number of days of tube feeding. The use of a fiber-free enteral formula may partly explain lack of effect of the intervention. We did not collect fecal samples from the initially included patients, as the possible role of gut microbiota in allo-HSCT was not on the agenda in this type of research when we started the study in 2010. Additionally, we lack data from other habitats (e.g. mouth, nose). Patients randomized to both study arms were treated in the same ward, therefore we cannot exclude any “contaminating” effects of the intervention being adopted by patients in the control group. To counteract for this, we registered dietary intake in a reference group (serving as a proxy for the control group) before commencing the trial, and before the nurses were trained in the intervention. We ensured that the patients in this reference group did not differ from the control group in clinical characteristics. However, we cannot exclude that the similar outcomes in both study groups could be explained by similar energy and protein intake. Patients included in the RCT self-reported energy and protein intake a single day before the regular 3 month visit. Reporting dietary intake a single day might be biased by capturing food not consumed on a regular basis (210). If dietary intake before the visit was unusual, we asked patients about dietary intake a normal day. We did not include biomarkers for dietary intake e.g. plasma concentration of vitamin C. Similarly, we did not measure nitrogen in urine for protein, antioxidants or albumin, although several of these biomarkers may be unspecific within this patient population. Furthermore, we did not analyze biomarkers for nutritional status (e.g. transferrin, pre-albumin). Variable compliance to tube feeding could possibly have affected the results of the study.

6 Conclusion

In our randomized nutritional intervention trial, optimized energy and protein intake preferably by the enteral route was not superior to conventional nutritional support for global QoL, oral mucositis, aGVHD, body weight and body composition, nutritional status, infectious complications, time to engraftment, and mortality 3 months post-HSCT. Global QoL score was not back to baseline level 3 months after transplantation. Body weight and body composition were the same in both study groups during 1-year follow up. In the pooled cohort of both study groups, weight gain was associated with 1-year mortality. A major depletion of microbial diversity and SCFAs and altered markers of gut barrier function were observed in both treatment arms from baseline to 3 weeks. Lower microbial diversity was observed in those who died during 1 year follow-up compared with survivors. Fecal SCFAs and markers of gut barrier function were not associated with clinical outcomes.

7 Future research

More studies are needed to identify the impact of weight loss and poor nutritional status on QoL and clinical outcomes in recipients of allo-HSCT. Also, there is a need for large RTCs to identify energy requirement, the role of dose, route of delivery and the impact of early or on-demand nutritional support for individual patients. To gain deeper insight into changes in smell and taste perceptions during allo-HSCT and the impact on QoL, changes in chemosensory function should be tested. Safety and efficacy of a diet aim to steer microbiota in a healthy direction should be designed and tested within the framework of a RCT. Risk groups that could benefit from different nutritional intervention (diet and medical nutrition) should be identified and nutritional support should be tailored for subgroups analysis of QoL and clinical outcomes. Long-term

RCTs are needed to determine appropriate and suitable nutritional support after hospital discharge. Nutritional intervention combined with physical exercise activities may prevent loss of muscle mass, impaired function and QoL in cancer and critically ill patients.

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9 List of errata

Page 9, line 19, correction. Original reference (62): Sheean PM, Braunschweig CA. Exploring the clinical characteristics of parenteral nutrition recipients admitted for initial hematopoietic stem cell transplantation. *JAmDietAssoc.* 2007;107(8):1398-403. Corrected reference: Ravasco P, Monteiro-Grillo I, Camilo ME. Does nutrition influence quality of life in cancer patients undergoing radiotherapy? *RadiotherOncol.* 2003;67(2):213-20.

Page 9, line 19, correction. Original reference (63): Mousavi M, Hayatshahi A, Sarayani A, Hadjibabaie M, Javadi M, Torkamandi H, et al. Impact of clinical pharmacist-based parenteral nutrition service for bone marrow transplantation patients: a randomized clinical trial. *Support Care Cancer.* 2013;21(12):3441-8. Corrected reference: Ravasco P, Monteiro-Grillo I, Vidal PM, Camilo ME. Dietary counseling improves patient outcomes: a prospective, randomized, controlled trial in colorectal cancer patients undergoing radiotherapy. *JClinOncol.* 2005;23(7):1431-8.

Page 32, line 14, correction. Original reference (165): Ottery FD. Definition of standardized nutritional assessment and interventional pathways in oncology. *Nutrition.* 1996;12(1 Suppl):S15-9. Corrected reference: Aaronson NK, Ahmedzai S, Bergman B, Bullinger M, Cull A, Duez NJ, et al. The European Organization for Research and Treatment of Cancer QLQ-C30: a quality-of-life instrument for use in international clinical trials in oncology. *J Natl Cancer Inst.* 1993;85(5):365-76.

10 Appendices

Appendix 1: The “Lite og godt” menu

Næringsdrikker

Vi kan lide hjemmelagte smoothies.

Ret nr. 17: *Smoothie* med mælk og bær

Mælk er en kilde til glukoze. Den er i sig selv eller bare opvarmet, og kan den færdig og sødmevirkende som oftest gøres lidt tilføjet i bordskålen. Gæst vil gerne se husholdningen ligger smukke og lækre som kan duke sig igen i det helt alene forberedt. Det kan være den søde duften af de bær eller som fylder her, og bær med lidt korn, eller som en ristet brød og varm kaffe om morgenen. Alle disse duftene fortæller om helse som hver av os har med sig.

Laura Fontey

Lite og godt

Små matretter for å vekke din appetitt

Husk det er en stor del av å spise og det er det som er det viktigste.

Et tilbud til pasientene i ernæringsgruppen i studien individualisert ernæring til utøvere av egen skoleutdanning pasienter. Sjekket for blodglukose.

Utvalgt av: Sykehuset Marianne Trondheim i samarbeid med studiet. Ole-Andreas Røysland, Helsevitenskapelig fakultet, januar 2019.

Beregnet av: Ole-Andreas Røysland, Ole-Andreas Røysland.

Innledning

I denne leksjonen presenteres små appetittretter. Retter som kan brukes til å vekke din appetitt. Lægen og sykepleieren legger om du trenger ekstra næring, drikke i sammenheng med i kombinasjon med i blodet. Det er det viktig at du betror til at tarmer til næringstilførsel og jern med kroppen trenger næring for å bygge seg opp i forbindelse med behandlingen.

OMELETT • FISK	KYLLING • PASTA	SMØRBRØD • KJØTTBOLLER • SUPPER	OST • DESSERT
<p>Ret nr. 1: Omelette med fisk og grønnsaker</p>	<p>Ret nr. 2: Kylling med pasta og grønnsaker</p>	<p>Ret nr. 3: Smørbrød med kjøttboller og suppe</p>	<p>Ret nr. 4: Ost og dessert</p>
<p>Ret nr. 5: Omelette med fisk og grønnsaker</p>	<p>Ret nr. 6: Kylling med pasta og grønnsaker</p>	<p>Ret nr. 7: Smørbrød med kjøttboller og suppe</p>	<p>Ret nr. 8: Ost og dessert</p>
<p>Ret nr. 9: Omelette med fisk og grønnsaker</p>	<p>Ret nr. 10: Kylling med pasta og grønnsaker</p>	<p>Ret nr. 11: Smørbrød med kjøttboller og suppe</p>	<p>Ret nr. 12: Ost og dessert</p>

Små hyggelige måltider

Vi vil anbefale små og hyggelige måltider. Mange blir merke av noen få minutter. Små porsjoner er mer appetittvekkende. Små porsjoner gjør det også mulig å spise opp alt.

Spis gjerne med to- tre timer mellom måltidene. Spis det du liker. Litt mat er bedre enn ikke noe mat. Det viktigste er å spise.

For å få til å spise mer kan du bruke måltidene utvortes dagene

Småskjema

Et annet tips er å spise i småskjema. Dette for at du ikke forbereder maten med spikeren når du blir syk. Småskjema sett, stort, kaldt og brettet ender seg ofte ved sykdom. Noen opplever at smaken blir borte. Mange pasienter ber om at maten kan smake borsalt, metall eller søtt. Prioriter mat som lukter godt. Mange opplever at romtemperert mat smaker bedre. Bruk gjerne musikk eller TV som avledning når du skal spise. Husk at godt humør er viktig.

Rutine

Prøv at alt som kan hjelpe deg. Start gjerne dagen med kaffe og te. Kaffet gir en liten boost, og det er godt mat kan anbefales. Ligg gjerne ned på sengen og slapp av etter måltidet. Ha gjerne en kveldsmiddag med kaffe og te.

Du må selv gjøre en innsats for å få i deg mat. Si i fra til sykepleieren din om hva du vil spise og drikke.

Appendix 2:

Prosedyre for ernæringsstudien (NASQ), intervensjonsgruppen Avdeling for blodsykdommer, OUS

Utføres av	Oppgave
	Nedleggelse av sonde
Lege	<ul style="list-style-type: none"> Henviser pasienten til Gastro undersøkelsenhet for nedleggelse av Endolumina FREKA Easy In ernæringssonde skop veiledet i den første del av tynntarmen, innen tre til fem dager etter transplantasjon. Hvis sonden faller ut henvises pasienten til å legge ny ernæringssonde Flocare Pur 12FR, inntil to ganger i ventrikkelen av gastroenterolog på pasientrommet. Platetall ≥ 20 ved nedleggelse av sonde. Vurderer høyere platetall der det er indikasjon for dette. Henvisningen merkes: "Pasienten inngår i ernæringsstudien" og spesifiser hvilken sonde som er ønsket nedlagt." Pasienten faster i fire timer før nedleggelse av sonden. Bekrefter rett posisjon til nasogastrisk sonde før bruk ved hjelp av røntgen oversikt øvre abdomen.
	Ordinasjon
Sykepleier	<ul style="list-style-type: none"> Ordinerer kaloribehov: 30-40 kcal/kg/døgn i Kurve F1A, side 4. Ordinerer ernæringsløsningene: <ul style="list-style-type: none"> Nutrison Advanced Peptisorb 1 kcal/ml sondeløsning enteralt i F1A, side 4 og SmofKabiven 1,1 kcal/ml infusjonsvæske med standard tilsetning (i.h.t. bestillingsskjema til Sykehusapoteket) eller evt. annen I.V. ernæring. Ordinerer Afipran 10-20 mg po/I.V. f.o.m morgenen før nedleggelse av ernæringssonde og så lenge sonden er nedlagt.
Lege	<ul style="list-style-type: none"> Signerer for ordinert energibehov. Signerer for: <ul style="list-style-type: none"> Nutrison Advanced Peptisorb 1 kcal/ml sondeløsning enteralt.og SmofKabiven 1,1 kcal/ml infusjonsvæske med standard tilsetning (i.h.t. bestillingsskjema til Sykehusapoteket) eller evt. annen I.V. ernæring. Afipran 10 mg x 4 po/I.V.
	Administrering av ernæring
Sykepleier	<ul style="list-style-type: none"> Starter med dietten "Lite og godt" dag minus syv. Hvis 30 kcal/kg/døgn ikke nås per oralt, så suppler med intravenøs ernæring inntil 30 kcal/kg/døgn i påvente av nedleggelse av ernæringssonde. Hos pasienter som får hydrering og SmofKabiven kan hastigheten på hydreringen reduseres slik at total ml per time (hydrering og SmofKabiven) er 250 ml/t. Når sonden er lagt ned fordeles ernæringsbehovet til pasienten på dietten "Lite og godt", Nutrison Advanced Peptisorb (se plan under) og SmofKabiven. Registrerer fortløpende inntak av ernæring/antall ml sondeløsning i form av: <ul style="list-style-type: none"> Mat og drikke: NASQ Kostregistrering; Sondeløsning: Arbeidsskjema til Nutrison Advanced Peptisorb 1 kcal/ml sondeløsning. Vurderer kontinuerlig inntak av kcal fra hver av administrasjonsmåtene, slik at behovet på minimum 30-35 kcal/kg/døgn nås for hver pasient. Summerer antall ml som er inntatt per døgn av h.h.v. Nutrison Advanced Peptisorb og SmofKabiven i kurve F1A, i tillegg til annen flytende kost og drikke kl. 07. Kaloriinntak per os summeres i kvadranten på en åpen rad under "Vekt." Totalt kaloriinntak (per oralt, I.V. og sondeløsning) summeres på raden "Sum antall kalorier gitt" på Kurve F1A, s. 4.
	Administrering av sondeernæring som langsom kontinuerlig tilførsel
Sykepleier	<ul style="list-style-type: none"> Sørger for at pasienten har hevet hodeende; 30-45 grader, ved administrering av sondeløsningen p.g.a fare for aspirering i flatt leie. Stopper tilførselen under transport og ved eksterne undersøkelser. Bruker ren teknikk ved håndtering av ernæringsløsning og infusjonssett. Skifter ernæringsløsning og sett hver 24. time. Bytter sett ved lengre pause i infusjon. Kontrollerer posisjon på nasojejunal sonde ved at sonden ikke har sklidd ut.

Kristin Skaarud Avdeling for blodsykdommer

Utføres av	Oppgave
	<ul style="list-style-type: none"> • Kontrollerer posisjon på ventrikkelsonde ved å sette ned 10-20 ml luft i sonden, lytter samtidig etter boblelyder over epigastriet (like under brystbeinspissen). Aspirerer luften som er satt ned. • Opptrappingsplan for Nutrison Advanced Peptisorb 1 kcal/ml som kontinuerlig tilførsel: Første dag med sondeernæring: 20 ml/t, totalt mengde/døgn 500 ml. Øker med 15 ml/t kl 07, hver 24. time inntil ordinert kcal behov er nådd. Maksimum 100 ml/t, hos pasienter som tolererer det. <ul style="list-style-type: none"> ○ Andre døgn med sondeernæring 35 ml/t, total mengde/døgn 840 ml. ○ Tredje døgn med sondeernæring 50 ml/t, total mengde/døgn 1200 ml. ○ Fjerde døgn med sondeernæring 65 ml/t, total mengde/døgn 1560 ml. ○ Femte døgn med sondeernæring 80 ml/t, total mengde/døgn 1920 ml. ○ Sjette døgn og påfølgende døgn med sondeernæring 100 ml/t, total mengde/døgn 2400 ml. • Supplerer evt. med SmofKabiven inntil ordinert kaloribehov er nådd. • Tillegg ved ventrikkelsonde: <ul style="list-style-type: none"> ○ Aspirer hver 4. time to ganger. Hvis maveinnhold er ≤ 200 ml, sett tilbake inntil 200 ml og fortsett å aspirere hver 6. time 2 ganger og hver 8. time to ganger. Deretter hver 12. time. ○ Hvis ≥ 200 ml maveinnhold, sett tilbake 200 ml av aspiratet, reduser tilførselen av Nutrison Advanced Peptisorb til 10-20 ml og fortsett å aspirere hver 4. time inntil aspiratet er ≤ 200 ml. ○ Hvis aspiratet er ≤ 200 ml øk med 15 ml/24 time i henhold til opptrappingsplanen og fortsett å aspirere som beskrevet i kulepunkt en. Hvis aspiratet er ≥ 200 fortsett på kulepunkt to. • Hvis volumiøs diaré og/eller kvalme relatert til administrering av sondeernæring, reduser tilførselen med sondeernæring inntil diareen og/eller kvalmen avtar og i minimum ett døgn og supplerer med I.V. ernæring inntil ordinert kcal behov er nådd.
	Forebygge tildetning av sonden
Sykepleier	<ul style="list-style-type: none"> • Sondens skylles med 20-30 ml sterilt vann kl 08, 16 og 23. Sonde som ikke er i bruk skylles én gang per døgn, kl 08. • Gir ikke legemidler i ernæringssonden.
	Observasjon
Sykepleier	<ul style="list-style-type: none"> • Registrerer dato og klokkeslett, antall ml sondeløsning som gis og evt. årsaker til at oppsatt plan ikke følges (oppkast, diaré, slimhinneblødning, tap av sonde og ny sonde) i Arbeidsskjema til Nutrison Advanced Peptisorb 1 kcal/ml sondeløsning og i vaktrapporten/pasientstatus.

Appendix 3:

Mat/drikke	Kcal/ enhet	Antall/ mengde	Tot antall/ mengde	Sum Kcal
Brød/kjeks				
Brød grovt/kneip 1 skive/35g	90			
Brød, glutenfritt brød 1 skive/35g	67			
Baguette, grov/fin 60g	169			
Boboli Calzone 120g	288			
Brytebrød 1stk/60g	141			
Focaccia 1 stk/34g	80			
Kjeks, Marie, Riskjeks 1 stk	30			
Kjeks, Kornmo, Start 1 stk/13g	60			
Kjeks, Kaptein, Ritz 1 stk 4/4g	20			
Knekkebrød 1 stk/13g	44			
Loff 1 skive/28 g	70			
Lompe 1stk/26g	47			
Maryland Cookies, Bixit 1stk/15g	74			
Polarbrød 1 stk/38g	110			
Pølsebrød 1stk/25g	68			
Rundstykke, fint, grovt 1 stk 50 g	117			
Pålegg				
Meierismør 1 pakke/12 g	90			
Vitamargarin 1 pakke/10 g	72			
Egg, kokt 1 stk/67g	95			
Eggerøre 50 g	87			
Makrell i tomat, kaviar porsjon/22/12 g	39			
Nugatti, sjokolade 1 porsjon/23 g	124			
Hvitost 1 skive fra pakke/15 g	53			
Brunost G35 1 skive fra pakke/15 g	70			
Kremost naturell 1 porsjon/20 g	60			
Kremost krydder, urter 1 porsjon/20 g	56			
Baconost 1 porsjon/20 g	45			
Kjøttpålegg, magert/kokt skinke 1 stk/10 g	12			
Kjøttpålegg, fet/servelat 1 stk/10g	22			
Leverpostei 22 g	59			
Majonessalat 20 g	86			
Salami 1 stk/8 g	34			
Snøfrisk 1 porsjon/12g	31			
Syltetøy 1 porsjon/22 g	35			
Grøt/supper/yoghurt/småretter/ 1 porsjon				
Aspargesuppe 1,5 dl	138			
Cornflakes/honnikorn uten melk 2 dl/32g	119			
Havregrøt, kokt på H-melk 2 dl	192			
Havresuppe, kokt på vann 2 dl	40			
Nudler porsjon/85g tørrvekt/370g tilberedt	373			
Rett i koppen 2 dl	80			
Risgrøt 2 dl	170			
Rømmegrøt, smørgrøt 2 dl	570			
Semulegrøt 2 dl	480			
Skinkeomelett 130g	131			
Suppe (pose) 2 dl	100			
Toast 1 stk	325			
Yoghurt 1 beger/150 g	140			
Yoghurt med müsli 1 beger/194 g	246			
Tilbehør (grønnsaker, salat, berikning) /1 porsjon				
Pasta 150g	86			
Potet 38g/stk	30			
Poteter, stekte amaldine 150g	136			
Poteter, båter 150g	241			
Potetmos 150g	188			
Pommes frites (gatekjøkken) 100g	330			
Fløtegratinerte poteter 150g	225			
Ris 150g	167			
Brokkolitorper 40g	11			
Couscous 250g	425			

KOSTREGISTRERING Ernæringsstudien, NASQ intervensjonsgruppen, Avdeling for blodsykdommer

Utfylt dag/måned/år:

Klistrelapp kan settes her

Energibehov Kcal

Målenheter

1 ss toppet = 15 ml
1 skål = 200 ml
1 glass = 150 ml
1 kopp = 150 ml

En hel porsjon skrives som: 1
Trekvart porsjon skrives som 0,75
En halv porsjon skrives som: 0,5
En kvart porsjon skrives som: 0,25
Alt som er mindre skrives som: 1 ss

Ferdig utfylt skjema arkiveres i
oppholdsmappen. Har du spørsmål, ta
kontakt med Kristin Skaarud.

Kristin Skaarud Avdeling for blodsykdommer

1

Mat/drikke	Kcal/ enhet	Antall/ mengde	Tot antall/ mengde	Sum Kcal
Erter, gulrot	80g	38		
Ertepure	110g	175		
Grønnsaksblanding	80g	26		
Grønn salatblanding	80g	10		
Gulrot, mini	40g	13		
Gulrotstuing	80g	149		
Gulrot, broccoli, blomkål	80g	22		
Kålstuing	150g	150		
Stekt løk	34g	83		
Erter og gulrot	80g	38		
Urtdressing	0,5dl	103		
Baconskive (bacondryss 20g=75kcal)	7g	43		
Kaffe/fløte	10ml	12		
Kremfløte/kremtopp	1ss/20g	40		
Remulade	1 pk/12g	74		
Rotmos	100g	55		
Rommedressing/Thousand Island	22g/stk	50		
Sukker	2 biter/1 pose/2 ts/4 g	15		
Surkål	100g	50		
Majones, olje, smør	1 pk/12 g/ 1 ss	80/100		
Kjøtt/fisk/ 1 porsjon				
Biff stroganoff	100g/100g	314		
Chili con carne	250g	344		
Fiskesympfonigrøt	100g/150g	252		
Fiskegrateng, smeltet smør	1 stk 200g/12g	330		
Fiskekaker	3 stk/120g	148		
Fiskerullade, urtesaus	120g/100g	278		
Grillpølse	stk/60g	141		
Hamburger m/tilbehør (McDonald)	100g	505		
Kalkunfilet, ferskensaus	100g/100g	143		
Kalkungrøt	100g/150g	302		
Kalvestek, brun fløtesaus	100g/ 1dl	318		
Karbonader, fløtesaus	2stk 140g/1dl	380		
Kjøttboller, tomat-saus	6stk 150g/100g	338		
Kjøttkaker	3stk/150g/100g	397		
Kjøtt-saus, pasta	200g/150g	368		
Kyllingfilet, bearnais-saus	100g/100g	277		
Laks, hollandais-saus	100g/1dl	402		
Lakseburger	120/1 dl	309		
Lasagne	200g	262		
Oksegrøt	150g	170		
Pannekaker	1stk/60g/stk	127		
Pasta, ostesaus, skinke	150g/100g/30g	590		
Pizza	slice/100g	220		
Svinebøt, røkt, sennepssaus	2skiver/100g/100g	306		
Seigbiff	1stk 140g	151		
Torsk, dampet, sitronsau	1 stk/120g/100g	223		
Wienerpølse	1stk/65g	153		
Annet/kommentarer (spesifiser hvis du har spist/drukket noe annet)				
Dessert/snacks/1 porsjon				
Blåbærkake	76g	265		
Bringebermousse	40g	85		
Bringeberfromasj	80g	178		
Brownies	60g	253		
Eplekake	80g	269		
Fløteis	kule/32g	63		
Frukt	1 stk, middels (banan 100kcal)	80		
Gele, vaniljesau		135		
Gulrotmuffins	84g	258		
Grønn eplemousse	59g	94		
Hermetisk fersken, pære	1 stk/250g	88		

Kristin Skaarud Avdeling for blodsykdommer

KOSTREGISTRERING Ernæringsstudien, NASQ intervensjonsgruppen, Avdeling for blodsykdommer

Utfylt dag/måned/år:

Klistrelapp kan settes her

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Målenheter

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En halv porsjon skrives som: 0,5
En kvart porsjon skrives som: 0,25
Alt som er mindre skrives som: 1 ss

Ferdig utfylt skjema arkiveres i oppholdsmappen. Har du spørsmål, ta kontakt med Kristin Skaarud.

Mat/drikke	Kcal/ enhet	Antall/ mengde	Tot antall/ mengde	Sum Kcal
Hvetebolle	66g	211		
Jordbær/rabarbradessert	50g	76		
Karamellpudding, karamellsaus	125g/30g	268		
Karamell fromasj m/sjokoladesaus	50g	140		
Lefse, smurt	1stk/65g	157		
Mandelpudding, jordbærtopping	125g/0,3dl	212		
Mango/jordbær mousse	55g	99		
Marsipanstang med valnøtt	1stk/80g	250		
Nøtter	0,5 dl/30 g	180		
Potetchips	en håndfull/1dl/15 g	80		
Pære/hasselnøtt fromasj	55g	137		
Rabarbra, jordbærdessert	1stk/55g	94		
Riskrem m/bringebersaus, Piano	108g	157		
Rislunsj	150g	204		
Rullekake	1 stk/50g	135		
Saftis	64g	74		
Semulekrem med kiresbær	1stk/55g	106		
Semulepudding med bringebærsaus	77g	164		
Sjokolade	1rute 5g	25		
Sjokoladepudding, vaniljesaus, Piano	25g/0,5dl	228		
Sjokolademousse	30g	56		
Smågodt, blandet uten sjokolade	9stk/25g	88		
Tiramisu	71g	235		
Vaffelhjerte	1 stk/30g	65		
Vanilje panacotta, blodappelsinsaus	1 stk/55g	99		
Drikker				
H-melk, kefir, kultur, sjoko	150 ml	100		
Rett i koppen sjokoladedrikk	34 g/porsjon	131		
L-melk, Cultura, Biola (naturell)	150 ml	70		
Biola med frukt	150 ml	90		
S-melk, kultur	150 ml	50		
Iste	150ml	50		
Juice, saft, brus, vorterøl, øl	150 ml	65		
Kaffe, te vann, lightbrus, buljong	150 ml	0/0		
Milkshake	150ml	115		
Smoothie, blåbær/skogsbær	150 ml	110		
Lite og godt dietten (energirik)				
Blomkålsuppe	2 dl	331		
Fiskeboller, hvit saus	50g/77g	287		
Gronnsakmos med smørsaus	70g/50 dl	307		
Havregrot	2 dl	414		
Kjøttboller med brun fløtesaus	51g/50g	250		
Kyllingbryst med urtesaus	67g/67g	292		
Kyllingfrikase	270g	204		
Kyllingsuppe	2dl	294		
Laks med hollandaisesaus	50g/1dl	196		
Lefse med salami	117g	345		
Omelett med ost og skinke	75g	178		
Ost, frukt og kjeks	89g	294		
Pasta med ostesaus og skinke	1dl/75g	378		
Riskrem	150g	231		
Smørbrød med egg og bacon	110g	314		
Smørbrød med ost og skinke	74g	235		
Toast	1 stk	485		
Tomatsuppe	2 dl	351		
Næringstilskudd/ berikning				
Nutridrink	200 ml	300		
Resource addera	200 ml	170		
Fresubin 2Kcal/ml	200 ml	400		
Energi og proteinpulver Resource Meritene ss/12,5g		48		
Energipulver Resource, nøytral smak	1 ss	28		
Calogen/Fresubin shot (150 kcal)	30 ml	135		
Sum energiinntak				

Kristin Skaarud Avdeling for blodsykdommer

KOSTREGISTRERING Ernæringsstudien, NASQ intervensjonsgruppen, Avdeling for blodsykdommer

Utfylt dag/måned/år:

Klistrelapp kan settes her

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En halv porsjon skrives som: 0,5
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Ferdig utfylt skjema arkiveres i oppholdsmappen. Har du spørsmål, ta kontakt med Kristin Skaarud.

Appendix 4:

Ernæringsstudien, intervensjonsgruppen, Avdeling for blødsykdommer

Klistrelapp

Lege signerer for SmoffKabiven 1.1 kcal/ml og Nutrison Advanced Peptisorb 1 kcal/ml. Sykepleier kontrollerer, tilbereder og dokumenterer i henhold til vanlige rutiner på nivå 1 og 2 prosedyrer i gjeldende kurveark. Se skjema for: Oppgaver og ansvar i forbindelse med ernæring til intervensjonsgruppen. NASQ. Avdeling for blødsykdommer.

Dd.mnd.år oppstart sondeernæring:

Opptreppingsplan for Nutrison Advanced Peptisorb 1 kcal/ml, 1000 ml		Kun ved ventrikkelsonde (ikke ved nasojejunalsonde):		Merknader:																						
Døgn	ml/t	Tot ml																								
1	20	500																								
Øker med 15 ml/t, hver 24. time inntil ordinert kcal behov er nådd. Maksimum 100 ml/t hos pasienter som tolererer det. Hvis voluminøs diaré, oppkast og/eller kvalme reduser antall ml/time inntil symptomene avtar og i minimum ett døgn til.																										
2	35	840																								
3	50	1200																								
4	65	1560																								
5	80	1920																								
6. og påfølgende døgn	100	2400																								
Arbeidsskjema til Peptisorb 1 kcal/ml, 1000 ml sondeløsning som kontinuerlig infusjon																										
Klokkeslett		06-07	07-08	08-09	09-10	10-11	11-12	12-13	13-14	14-15	15-16	16-17	17-18	18-19	19-20	20-21	21-22	22-23	23-24	00-01	01-02	02-03	03-04	04-05	05-06	Tot.ml
Dato/kl	ml/t																									
	Merkn.																									
Dato/kl	ml/t																									
	Merkn.																									
Dato/kl	ml/t																									
	Merkn.																									
Dato/kl	ml/t																									
	Merkn.																									
Dato/kl	ml/t																									
	Merkn.																									
Dato/kl	ml/t																									
	Merkn.																									
Dato/kl	ml/t																									
	Merkn.																									

Appendix 5: EORTC QLQ-C30, quality of life questionnaire

NASQ
EORTC QLQ-C30

Skjema nr.

Pas.ID:

Utfyllt dato: . .

Dag Måned År

Vi er interessert i forhold vedrørende deg og din helse. Vær så vennlig å besvare hvert spørsmål ved å sette et kryss x i den boksen som best beskriver din tilstand. Det er ingen «riktige» eller «gale» svar. Alle opplysningene vil bli behandlet konfidensielt.

	Ikke i det hele tatt	Litt	En del	Svært mye
1. Har du vanskeligheter med å utføre anstrengende aktiviteter, slik som å bære en tung handlekurv eller en koffert?	☐	☐	☐	☐
2. Har du vanskeligheter med å gå en <u>lang</u> tur?	☐	☐	☐	☐
3. Har du vanskeligheter med å gå en <u>kort</u> tur utendørs?	☐	☐	☐	☐
4. Er du nødt til å ligge til sengs eller sitte i en stol i løpet av dagen?	☐	☐	☐	☐
5. Trenger du hjelp til å spise, kle på deg, vaske deg eller gå på toalettet?	☐	☐	☐	☐
<u>I løpet av den siste uka:</u>				
6. Har du hatt redusert evne til å arbeide eller utføre andre daglige aktiviteter?	☐	☐	☐	☐
7. Har du hatt redusert evne til å utføre dine hobbyer eller andre fritidsaktiviteter?	☐	☐	☐	☐
8. Har du vært tung i pusten?	☐	☐	☐	☐
9. Har du hatt smerter?	☐	☐	☐	☐
10. Har du hatt behov for å hvile?	☐	☐	☐	☐
11. Har du hatt søvnproblemer?	☐	☐	☐	☐
12. Har du følt deg slapp?	☐	☐	☐	☐
13. Har du hatt dårlig matlyst?	☐	☐	☐	☐
14. Har du vært kvalm?	☐	☐	☐	☐

Bla om til neste side

61925

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Svært
mye

[illegible]

☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7

Svært dårlig Helt utmerket

☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7

Svært dårligHelt utmerket

Appendix 6:

Oslo universitetssykehus HF

Postadresse:
Trondheimsveien 235
0514 Oslo

Sentralbord:
02770

Org.nr:
NO 993 467 049 MVA

www.oslo-universitetssykehus.no

Vår ref./saksbeh./dir.tlf.: Kristin Skaarud/73657

Dato: August 2010

Vurdering av oral mukositt hos pasienter i ernæringsstudien

Spør først pasienten om han/hun har smerter, sår i munnen, problemer med å spise og om pasienten kan spise fast eller flytende føde. Bruk numerisk skala 0-10 til å skåre for smerte i munnen både i ro og ved bevegelse. Grader deretter mukositt ved bruk av Verdens Helseorganisasjons Oral Toksisitet Skala. Områder i munnen som skal vurderes er innsiden av øvre og nedre leppe, innsiden av høyre og venstre kinn, ganen, under tungen, og på høyre og venstre side av tungen i.h.t følgende gradering

Grad 0: Ingen.

Grad 1: Ømhet og rødme.

Grad 2: Rødme og sår, pasienten kan svelge fast føde.

Grad 3: Sår, pasienten kan ikke svelge fast føde (trenger flytende diett).

Grad 4: Næringsinntak ikke mulig. Trenger intravenøs eller sondeernæring. (Oversatt og bearbeidet fra World Health Organization, 1979, WHO Handbook for Reporting Results of Cancer Treatment).

Hvis du er usikker på om du skal gi skår "0" eller "1", registrer som laveste skår.

Graderingen gjøres daglig innen kl 11. Utstyr til bruk i graderingen er lommelykt, spatel, engangshanske.

Registrer smerteskår ved bevegelse og i ro ved hjelp av Numerisk Rating Scale (NRS) 0-10, og skår på mukositt på Kurve F1, nederst på side 2, i.h.t følgende eksempel

	23/8	24/8	25/8	26/8	27/8	28/8	29/8
Mukositt grad/smerte skår i ro-bevegelse	0/0-0	0/0-0	0/1-2	1/3-4	1/3-4	2/4-5	2/4-5

Registreringen avsluttes når det er skåret 0/0-0 i tre påfølgende dager.

Kristin Skaarud

Appendix 7:

Modified Glucksberg criteria			
Stage	Skin	Liver (bilirubin; μmol/l)	Gut (stool output; ml/day)
1	Maculopapular rash <25%	34-50	500–999 and/or persistent nausea
2	Maculopapular rash	51-102	1000-1500
3	Maculopapular rash	103-255	>1500
4	Generalized erythroderma plus bullous formation	256	Severe abdominal pain with or without ileus
Grade			
I	Stage 1–2	None	None
II	Stage 3 or	Stage 1 or	Stage 1
III	-	Stage 2–3 or	Stage 2–4
IV	Stage 4 or	Stage 4	-

Appendix 8:

NASQ Vurdering av ernæringsstatus Patient-Generated Subjective Global Assessment (PG-SGA)		Pas.ID:
		Skjema nr.
<p><i>Spørsmål 1-4 fylles ut av pasienten.</i></p> <div style="display: flex; justify-content: space-between;"> <div style="width: 60%;"> <p>1. Vekt</p> <div style="display: flex; justify-content: space-around;"> <div style="width: 45%;"> Jeg er ca: , cm </div> <div style="width: 45%;"> Jeg veier ca: , kg </div> </div> <div style="display: flex; justify-content: space-around; margin-top: 5px;"> <div style="width: 45%;"> For ett år siden veide jeg ca: , kg </div> <div style="width: 45%;"> For 6 måneder siden veide jeg ca: , kg </div> </div> <p>De siste 2 ukene har vekten min: ☐ 1. Minsket ☐ 2. Vært uforandret ☐ 3. Økt</p> <p>Boks 1: </p> </div> <div style="width: 35%;"> <p>Utfyllt dato:</p> <div style="display: flex; justify-content: space-around; align-items: center;"> <div style="border: 1px solid black; width: 20px; height: 20px; text-align: center;"> </div> <div style="border: 1px solid black; width: 20px; height: 20px; text-align: center;"> </div> <div style="border: 1px solid black; width: 20px; height: 20px; text-align: center;"> </div> <div style="border: 1px solid black; width: 20px; height: 20px; text-align: center;"> </div> <div style="border: 1px solid black; width: 20px; height: 20px; text-align: center;"> </div> <div style="border: 1px solid black; width: 20px; height: 20px; text-align: center;"> </div> </div> <div style="display: flex; justify-content: space-around; font-size: 8px; margin-top: 2px;"> Dag Måned År </div> </div> </div>		
<p>2. Mat og drikke (ett valg)</p> <p>Sammenliknet med mitt normale, har matinntaket mitt siste måneden vært:</p> <p>☐ 1. Som vanlig</p> <p>☐ 2. Mer enn vanlig</p> <p>☐ 3. Mindre enn vanlig :</p> <p style="margin-left: 20px;">Jeg spiser og drikker nå: (angi ett eller flere alternativer)</p> <p style="margin-left: 20px;">☐ Vanlig mat, men mindre mengde enn vanlig</p> <p style="margin-left: 20px;">☐ Litt fast føde</p> <p style="margin-left: 20px;">☐ Kun flytende</p> <p style="margin-left: 20px;">☐ Kun næringsdrikker</p> <p style="margin-left: 20px;">☐ Veldig lite av alt</p> <p style="margin-left: 20px;">☐ Kun sondeernæring eller intravenøs ernæring</p> <p>Boks 2: </p>	<p>3. Symptomer</p> <p>De siste ukene har jeg hatt følgende problem som har hindret meg fra å spise tilstrekkelig (angi ett eller flere alternativer)</p> <div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> ☐ Ingen problem ☐ Kvalme ☐ Diaré ☐ Vondt i munnen ☐ Smerter _____ ☐ Maten smaker annerledes eller ingenting: ☐ Annet: <div style="border: 1px solid black; width: 100px; height: 15px; margin-top: 5px;"></div> </div> <div style="width: 45%;"> ☐ Ingen appetitt, ikke lyst til å spise: ☐ Brekninger ☐ Forstoppelse ☐ Munntørhet ☐ Plaget av lukter </div> </div> <p>Boks 3: </p>	
<p>4. Fysisk aktivitet (ett valg)</p> <p>Den siste måneden vil jeg beskrive aktiviteten min som:</p> <p>☐ 1. Normal, ingen begrensinger</p> <p>☐ 2. Ikke normal, men er oppe og er i noe aktivitet</p> <p>☐ 3. Ikke vært i form, men vært oppe mer enn halve dagen</p> <p>☐ 4. Vært i litt aktivitet, tilbringer det meste av dagen i sengen eller i stol</p> <p>☐ 5. Ligget for det meste i sengen</p> <div style="display: flex; justify-content: flex-end; align-items: center; margin-top: 10px;"> <p>Sammenlagte skår 1-4:</p> <div style="border: 1px solid black; width: 30px; height: 20px; text-align: center; margin: 0 5px;"> </div> A </div> <p>Boks 4: </p>		

54759

Pas.ID:

Skjema nr.

5. Sykdommen og dens sammenheng med næringsbehov (se retningslinjer punkt 2)

Alle relevante diagnoser:

Primær sykdom, stadium (hvis kjent): ☐ I ☐ II ☐ III ☐ IV

Andre:

Skår retningslinjer punkt 2 B

Alder:

6. Metabolsk påvirkning (se retningslinjer punkt 3)

Skår retningslinjer punkt 3 C

7. Fysisk påvirkning (0 = ingen, 1= lav, 2= moderat, 3= høy)

Tap av underhudsfett

Tap av muskelmasse

Ødem

Ascites

Skår retningslinjer punkt 4 D

Global vurdering (se retningslinjer)

1 - Velemært (SGA-A):

2 - Noe/mistenkt underernært (SGA-A):

3 - Alvorlig underernært (SGA-C):

Totalt PG-SGA skår
(Total numerisk skår fra A+B+C+D over)

11 Papers I-III

Paper I

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Randomized Controlled Trial

Effects of individualized nutrition after allogeneic hematopoietic stem cell transplantation following myeloablative conditioning; a randomized controlled trial

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SUMMARY

Background & aims: Reduced quality of life (QoL) is prevalent after allogeneic hematopoietic stem cell transplantation (allo-HSCT). In this randomized trial we examined the effect of individualized nutritional support during hospitalization for allo-HSCT. Primary outcome was change in global QoL three months post-HSCT with oral mucositis (OM) and acute graft-versus-host disease (aGVHD) as main secondary outcomes. **Methods:** Whereas the intervention group received recommended minimum daily intakes of 126 kJ/kg and 1.5–2.0 g protein/kg as food, supplements, enteral or parenteral nutrition, the controls received routine feeding. QoL was self-reported using the EORTC QLQ-C30 questionnaire.

Results: Between August, 2010 and February, 2016, we randomized 59 and 60 patients to intervention and control, respectively; 40 and 48 being eligible for analysis of QoL. There was no difference between the two groups in mean global QoL after three months (-3.10 , 95% CI -11.90 – 5.69 ; $P = 0.49$). Nor were there any differences in OM grades 3 or 4 (RR (vs grades 0–2), 1.11, 95% CI 0.59–2.11 and 0.95, 95% CI 0.72–1.25, respectively; $P = 0.78$), or aGVHD grades 3 or 4 (RR (vs grades 0–2) 0.44, 95% CI 0.12–1.60; and 0.65, 95% CI 0.20–2.20, respectively; $P = 0.37$).

Conclusion: Individualized nutritional support with recommended energy and protein intakes during hospitalization had no effect on QoL, OM or aGVHD three months after allo-HSCT compared to routine nutrition.

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1. Introduction

Weight loss and malnutrition are frequent following allogeneic hematopoietic stem cell transplantation (allo-HSCT) [1]. The patients typically experience nausea, vomiting, sore mouth, taste changes, loss of appetite and fatigue [2]. These symptoms are more intense after myeloablative conditioning (MAC) compared to

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reduced-intensity conditioning (RIC), and they are associated with impaired QoL [2,3]. Nutritional support may alleviate these symptoms and thus improve QoL, however, the evidence for such an effect is weak [4]. One randomized controlled trial (RCT) reported improved survival in allo-HSCT recipients receiving total parenteral nutrition (TPN) compared with an electrolyte-enriched solution in allo-HSCT recipients [5]. However, there is no conclusive evidence of the use of TPN versus parenteral nutrition (PN) or enteral nutrition (EN) on other outcomes [6–9]. Furthermore, a significant association between severe acute graft-versus-host disease (aGVHD) and poor oral intake has been reported [10]. As EN is thought to preserve the integrity of the gut mucosa and reducing infections, EN is recommended when the gut resumes normal function [4,11].

Up to three months after MAC, allo-HSCT patients score high on nutrition-related symptoms known to impair QoL [2,3]. Importantly, no evidence-based recommendations exist on when and how to best provide nutritional support, and there are no RCTs with a tailored nutritional intervention to allo-HSCT patients with QoL as the main outcome. The primary aim of our study was therefore to examine if individualized nutritional support could change global QoL three months after allo-HSCT compared to routine nutritional support. Main secondary outcomes were occurrence and duration of oral mucositis (OM) grades 3 and 4 and occurrence of aGVHD grades 3 and 4.

2. Methods

Patients ≥ 18 years admitted for allo-HSCT with MAC at Oslo University Hospital for a hematological malignancy were eligible. Exclusion criteria were previous allo-HSCT and inability to consent and/or to follow the trial protocol. The study was approved by the Regional Committee for Medical and Health Research Ethics South East Norway (#S-09136c 2009/2115) and the Data Protection Supervisor, Oslo University Hospital and registered at [ClinicalTrials.gov](https://www.clinicaltrials.gov), ID NCT01181076. All patients provided written, informed consent.

2.1. Procedures

Eligible patients were informed about the study at their last visit before allo-HSCT and recruited upon admission for transplantation. A computer-generated 1:1 block randomization (block size 10; www.randomization.com) was used.

All patients received MAC with either (i) busulphan and cyclophosphamide; or (ii) total body irradiation and cyclophosphamide. GVHD prophylaxis was cyclosporine and methotrexate. From day –7 to day –1 before the transplantation, patients received hydration with 5% glucose.

The study period started when commencing conditioning and continued until hospital discharge. For the intervention group the aim was a minimum daily energy intake of 126 kJ (30 kcal)/kg body weight and protein intake of 1.5–2.0 g protein/kg body weight [12,13]. The target energy intake was validated by measuring resting energy expenditure using indirect calorimetry [14]. An activity factor of 1.4 was used to calculate total energy expenditure [15]. At inclusion the patients received routine hospital food and were encouraged to eat energy-enriched and lactose-reduced snacks and oral supplements on a daily basis. A nasoenteric tube (Flocare Bengmark Ch 8, Nutricia, Schiphol, The Netherlands or Freka Endolumina 10 Fr, Fresenius Kabi, Bad Homburg, Germany) was inserted between days +3 and +5 [16,17]. Nutrison Advanced Peptisorb (Nutricia) was used for enteral feeding [16,17]. The enteral solution consisted of hydrolyzed medium triglyceride fat without fiber, lactose or gluten, and the feeding started at 15 ml/h

and was increased with 15 ml/24 h (maximum 100 ml/h), depending on tolerance. Those unable to meet the energy target by the oral or enteral route received the supplementary PN Olimel, OliClimel (Baxter, Illinois) or SmofKabiven (Fresenius Kabi) added micronutrients (10 ml Tracel), and A, B, C, D, E and K-vitamins (Soluivit mixed in 10 ml of Vitalipid Adult). The nurses provided the nutritional supplements based on a predefined algorithm and monitored daily oral, enteral and parenteral energy intake. During hospitalization and outpatient follow-up, the oral energy and protein contents were calculated using the software package Aivo 2000 (AIVO AB, Stockholm, Sweden). After discharge, nutritional advice and oral supplements were provided at the regular outpatient visits.

The control group received routine practice. Energy and protein requirements were not calculated, dietary intake not recorded and enteral feeding was not used. TPN (Olimel or SmofKabiven 1100, 1600 or 2200 kcal/day) was delivered at the discretion of the treating physician to patients unable to eat due to OM. Oral intake was not monitored to avoid increased attention on nutritional intakes among the controls, since participants from both the intervention and control group stayed in the same ward. To obtain proxy-estimates from the controls we therefore included data from an independent reference group ($n = 13$) consecutively recruited and receiving the same nutrition as the control group (Supplemental Panel 1). The patients in both the intervention and control group registered their oral intake one day before the three-month visit.

2.2. Safety monitoring

An independent data monitoring committee evaluated patient safety after hospital discharge for the first 40 patients (20 in each study group), and again after the next 40 patients. No safety concerns were identified.

2.3. Assessments of study outcomes

The primary outcome was change in global QoL from baseline to three months post-transplantation assessed by the EORTC QLQ-C30 [18], a cancer-specific 30-item self-report questionnaire consisting of multi-item scales and single-item measures on symptoms and level of functioning. The global QoL score combines two items; overall health and QoL, rated from 1 (*very poor*) to 7 (*excellent*) that are transformed to a 0–100 continuous scale. Global QoL was completed at inclusion, at three- and six weeks, and three months post-transplantation.

OM was scored with the 0–4 WHO Toxicity Scale [19,20] from onset of OM until score zero on three consecutive days. Acute GVHD grades 3 and 4 were diagnosed according to the modified Glucksberg criteria [21].

Weight was measured with the Tanita BC-418 MA Body Composition Analyzer (Tanita Corp, Tokyo, Japan) read to the nearest 0.1 kg, with the patient wearing light clothes and no footwear. One kg was subtracted to adjust for the weight of clothing. Weight change from baseline to three months were categorized as $<5\%$, $5\text{--}10\%$ and $>10\%$. Nutritional status was categorized as well-nourished, moderately malnourished or suspected malnutrition or severely malnourished with the Patient-Generated Subjective Global Assessment tool (PG-SGA) [22]. Fat-free mass index and fat mass index were calculated as fat-free mass and fat mass (kg)/height (m)². Weight, fat-free mass index and fat mass index were determined at baseline and then repeated at 3 and 6 weeks and at 3 months.

Infectious complications were defined as disease due to virus, invasive fungal disease, bacteremia, pneumonia or empirical use of IV antimicrobial treatment. Cytomegalovirus infection was defined

according to Ljungman et al. [23] and fungal disease was classified according to De Pauw et al. [24]. Bacteremia was defined as the first positive blood culture during a 10-day time period. Repeated positive blood cultures obtained >10 days after the first were considered new episodes. Diagnosis of pneumonia required detection of new pulmonary infiltrates on X-ray or CT scan and symptoms of respiratory infection. Fever was defined as a rectal temperature $\geq 38^\circ\text{C}$. All outcomes were registered from any first event until death or discharge from first hospital stay. Neutrophil engraftment was defined as the first of three consecutive days with neutrophil granulocytes $\geq 0.2 \times 10^9/\text{l}$ and platelet engraftment as platelets $\geq 20 \times 10^9/\text{l}$ without platelet transfusions. Transplant-related mortality was death of any cause except relapse, before three months.

2.4. Statistical analyses

Sample-size calculation was based on an expected change by 15 points in the global QoL score which was considered clinically relevant [25–30] and consistent with our prior results in Norwegian patients with acute myeloid leukemia where global QoL was 60 (SD) [29,30]. In total, 88 patients (44 in each group) were required to achieve 80% power with a two-sided significance level of 5%.

Results are presented as means (95% confidence intervals [CIs] or SDs), medians and ranges or frequencies (percentages). We used Mann–Whitney *U* test to test differences between groups in energy and protein intake and length of hospital stay. Analysis of covariance was used to compare differences between the two study groups in global QoL scores at three months adjusting for baseline score [31,32]. Additionally, the global QoL and subscale scores at all-time points (day –8, three and six weeks and three months) were analyzed with a linear mixed model for repeated measures. Subscale scores were dichotomized (score 0 = 0 and scores > 0 = 1) and analyzed by a logistic regression model with general estimating equations when lack of normality was found. We tested for interaction between group and time. Analyses of OM and aGVHD and other secondary outcomes were performed on an intention-to-treat basis. For secondary outcomes we used chi-squared test and estimated relative risks. Nutritional status, infectious complications and transplant-related mortality were analyzed by chi-squared test or Fishers exact test. Weight, fat-free mass index and fat mass index were analyzed with a linear mixed model for repeated measures. Time to engraftment was analyzed by Mann–Whitney *U* test. A *P*-value <0.05 was considered statistically significant. Analyses were performed using IBM-SPSS 26 (IBM Corp., Armonk, NY).

3. Results

From 2010 to 2016 we assessed 173 patients for eligibility. Of these, 119 (69%) consented and were randomly assigned to the intervention (*n* = 59) or the control (*n* = 60) group. The median length of hospital stay was 37 (20–104) days in the intervention group and 39 (22–108) days in the control group. None of the patients withdrew, but two patients in the intervention group were excluded from further analyses (Fig. 1). Eighty-eight patients completed the three months' follow-up of QoL (intervention: *n* = 40; control: *n* = 48) while 117 were included in the intention-to-treat analysis of secondary outcomes. Clinical and demographic characteristics are shown in Table 1 and Supplemental Tables 1 and 2.

3.1. Energy and protein intake

Energy and protein intakes are shown in Table 2. In the intervention group a gastrointestinal tube was inserted in 55 patients (two refused) and EN commenced in 49 patients (six wanted to remove the tube before commencing EN). The tubes stayed in

position a median of 12 (1–50) days. All patients received PN. The median number of days with oral intake, EN, PN and glucose were 27 (6–98), 13 (1–49), 24 (1–78) and 28 (17–64), respectively.

Fifty-nine of 60 control patients received TPN. One patient lacked data on amount and number of days with TPN whereas two received EN as part of intensive care treatment. The median number of days with EN, TPN and glucose was 30 (8–52), 18 (1–84) and 29 (1–98), respectively. In the reference group, median number of days with oral, TPN and glucose were 24 (10–57), 15 (5–27) and 24 (14–35), respectively. There were no significant differences between the controls and the reference group in energy or protein intakes derived from EN, TPN and glucose (Table 2).

At three months, energy and protein intakes were available from 72 patients (36 in each study group). The median daily energy intake was 126.0 (134.8–271.6) kJ/kg in the intervention group and 111.8 (162.8–314.4) kJ/kg in the control group (*P* = 0.43). The corresponding daily protein intakes were 1.1 (0.3–2.4) g/kg and 1.0 (0.3–2.4) g/kg (*P* = 0.51), respectively.

3.2. Quality of life

We found no significant differences between the two study groups in the global QoL scores at three months, nor in the subscale scores, except for constipation (Table 3). In both groups significant changes over time was found in global QoL scores and all subscale scores except for dyspnea, constipation and financial difficulties. The global QoL scores were lowest three weeks after transplantation and then improved, though not back to baseline levels. No significant interaction effects were found between group and time for any of the QoL scores (*P*-values 0.08–0.89), except for fatigue (*P* = 0.016) with lower scores for the intervention group three weeks after transplantation (Supplemental Table 3).

3.3. Secondary outcomes

There were no significant differences between the two study groups in the number of patients with OM grades 3 or 4 or the median number of days with OM grades 3 or 4. The median duration for OM grade 4 was six days in both groups. No significant differences were found between the two study groups regarding the number of patients with aGVHD grades 3 or 4 (Table 4) and infectious complications (*P* = 0.23–1.00, Supplemental Table 4). We found no significant differences between the intervention, control and reference groups in body weight. In the three groups significant changes over time was found in body weight. Body weight was decreased six weeks after transplantation and was lowest three months after transplantation. Moreover, there was no significant difference between the intervention and control group in fat-free mass index and fat mass index. In both groups significant changes over time were found in fat-free mass index and fat mass index. Loss of body weight resulted in loss of fat mass (Table 5). No significant interaction effects were found between groups and time for body weight, fat-free mass index or fat mass index (*P*-values 0.08–0.77). From baseline to three months the number of patients experiencing a weight change <5%, 5–10% or >10%, were 22, 11 and 11 in the intervention group, respectively, and 21, 10 and 21 in the control group, respectively (*P* = 0.28). The number of patients categorized as well-nourished, moderately or suspected malnourished or severely malnourished at three months, were 28, 11 and 2, respectively, in the intervention group, and 28, 16 and 4 respectively, in the control group (*P* = 0.54). The median (range) number of days to neutrophil engraftment was 15 (11–31) in the intervention group and 16 (10–30) among the controls (*P* = 0.63). Fifty (87.7%) patients in the intervention and fifty-three (88.3%) in the control group were available for analysis of days to platelet

Fig. 1. Flow diagram showing the inclusion process.

engraftment and the median (range) days were 17 (26) and 14 (53), respectively ($P = 1.0$). Seven patients in each group had an increased platelet transfusion need, and were excluded from analysis. Nine patients in the intervention group and five controls died before three months ($P = 0.26$).

4. Discussion

In this RCT, an individualized nutritional intervention with recommended daily intakes of energy and protein had no superior effect on global QoL three months post-transplant compared to routine nutritional practice. Furthermore, no effects were found on other QoL-outcomes, OM or aGVHD.

This is the first RCT with an individualized nutritional intervention and QoL as the primary endpoint and direct comparisons with previous studies are thus of limited value. One explanation as to why the intervention had no effect on global QoL in our study could be that three months is too short for potentially significant differences to become apparent. Given the aggressive treatment of allo-HSCT after MAC, our results show that most of the scales and single items reflecting physical impairments may still be compromised at three months. This may be reflected in the patient's overall QoL perceptions. In line with our results, no significant improvement has been reported in global QoL three months after transplantation [2,3]. However, six months post-HSCT an association between physical well-being and higher BMI, and conversely between poorer physical and social well-being and weight loss, were reported in a prospective, longitudinal study [33].

Another potential explanation could be lack of differences between the two study groups in nutritional status at study-end. Patients may have lost weight after discharge. The similar energy intake in both groups at three months despite nutritional counseling in the intervention group upon discharge supports this notion.

Few studies have examined the effect of nutritional intervention on OM or aGVHD. There were no significant differences between the two study groups in frequency or duration of severe OM or severe aGVHD. One prospective [34] and one retrospective observational study [35] compared the effect of EN versus PN on clinical outcomes 100 days post-HSCT with either MAC [34] or both MAC and RIC [35]. Although neither study found any effects on OM, fewer patients with aGVHD was found in the EN compared to the PN group in one of these studies [34], in contrast to the other [35]. Notably, the actual energy and protein intakes in these two studies were not reported, and about half of the patients in the EN groups received additional PN. Interestingly, a retrospective study of allo-HSCT patients following MAC reported a correlation between increased number of days with no oral intake (i.e. before the diagnosis of aGVHD) and the incidence of severe aGVHD [10].

We found no significant differences in infectious complications between the two study groups. This is partly in line with a previous report [34]. Whether nutritional support influences time to engraftment is not known. In line with a study comparing EN vs PN [35], we found no significant difference in the time to neutrophil engraftment, while earlier neutrophil engraftment has been reported when comparing EN vs PN [34].

Table 1

Baseline characteristics.

Characteristic	Intervention (n = 57)	Control (n = 60)
Age yr – median (range)	45 (19–65)	41 (18–62)
Female – no. (%)	20 (35)	25 (42)
AML – no. (%)	36 (63)	31 (51)
High risk first remission	23	22
After relapse, beginning of first relapse and in second remission	10	9
First remission standard risk	3	–
ALL – no. (%)	6 (10)	10 (17)
First remission high risk	3	7
Early first relapse, second remission	3	3
CML – no. (%)	2 (4)	7 (12)
Chronic phase	–	1
Accelerated phase	2	6
CMML – no. (%)	3 (5)	3 (5)
MDS – no. (%)	6 (11)	5 (8)
Other – no. (%) ^a	4 (7)	4 (7)
Donor – no. (%)		
HLA-identical sibling	17 (30)	13 (22)
HLA-identical unrelated	40 (70)	47 (78)
Stem-cell source – no. (%)		
Bone marrow	25 (44)	27 (45)
Peripheral-blood hematopoietic cells	32 (56)	33 (55)
Sex mismatch ^b – no. (%)	17 (30)	10 (17)
Positive CMV serology – no. (%)		
Donor	27 (47)	24 (40)
Recipients	45 (79)	43 (72)
Conditioning – no. (%)		
Busulphan + Cyclophosphamide	56 (98)	56 (93)
TBI + Cyclophosphamide	1 (2)	4 (7)
HCTI - CI risk groups – no. (%) ^a		
Low risk	42 (74)	45 (75)
Intermediate risk	8 (14)	10 (17)
High risk	7 (12)	5 (8)
EBMT score – no. (%) ^a		
0–3	33 (58)	36 (60)
4	14 (24)	14 (23)
5–7	10 (18)	10 (17)
Performance status ECOG – no. (%)		
0	55 (96)	54 (90)
1	2 (4)	6 (10)
BMI – no. (%)		
Underweight	2 (4)	4 (7)
Normal weight	31 (54)	27 (45)
Overweight	17 (30)	26 (43)
Moderately obese	4 (7)	3 (5)
Severely obese	3 (5)	0 (0)

Abbreviations: AML = Acute myeloid leukemia; ALL = Acute lymphocytic leukemia; CML = Chronic myeloid leukemia; CMML = Chronic myelomonocytic leukemia; MDS = Myelodysplastic syndrome; CMV = Cytomegalovirus; TBI = Total body irradiation; HCTI-CI = Hematopoietic Cell Transplantation-specific comorbidity index [37]; EBMT score = European Group for Blood and Marrow Transplantation score [38]; ECOG = Eastern Cooperative Oncology Group.

^a An expanded list of baseline values for other diagnosis, EBMT score and HCTI-CI score is provided in [Supplemental Table 1](#).

^b Sex mismatch was defined as female donor to male recipients.

We cannot fully exclude the possibility of an unintentional increased focus on nutrition among control patients and staff leading to increased intakes. However, the total energy intake in the intervention group was significantly higher than in the reference group while the amount of energy derived from

medical nutrition did not differ significantly between the control and the reference group. It is therefore reasonable to assume that the oral intake among the controls did not exceed that of the reference group. These intake data therefore argue against a similar total intake of energy in the intervention and control

Table 2

Daily intake of energy and protein from the day the patient commenced the conditioning regime until hospital discharge.

Group	n	Days of hospital stay	Energy intake (kJ/kg body weight)			Protein intake (g/kg body weight)		
			Total	Oral	Medical Nutrition ^a	Total	Oral	Medical Nutrition ^a
Intervention	57	37 (20–104)	131.9 (58.2–178.7) ^c	52.7 (12.1–126.0)	101.7 (43.5–167.0)	1.1 (0.5–1.5) ^c	0.39 (0.10–0.91)	1.00 (0.52–1.48)
Control	60	39 (22–108)	–	–	74.9 (5.0–147.8) ^d	–	–	0.98 (0.30–1.64) ^d
Reference group	13	32 (22–64) ^b	99.2 (50.2–139.8)	48.6 (15.5–95.0)	64.5 (23.4–137.3)	0.6 (0.4–1.0)	0.27 (0.08–0.61)	0.98 (0.57–1.35)

Values are medians (range).

^a Sum of glucose, enteral and parenteral nutrition.

^b Number of days energy and protein intake were registered.

^c Intervention group compared to the reference group, $P = <0.001$.

^d Control group compared to the reference group: energy intake $P = 0.12$, protein intake $P = 0.89$.

Table 3

Comparison of quality of life scores from baseline to three months.

Outcome	Intervention (n = 40)		Control (n = 48)		Intervention versus control at 3 months ^a	
	Baseline	3 months	Baseline	3 months	Difference	P-value
	Mean (SD)		Mean (SD)		Mean (95% CI)	
Global quality of life	70.4 (17.6)	58.8 (19.2)	69.6 (22.0)	55.4 (23.3)	−3.10 (−11.90 to 5.69)	0.49
Physical functioning	78.3 (14.8)	63.5 (23.6)	80.3 (20.2)	66.6 (23.6)	−2.20 (−7.23 to 11.57)	0.65
Role functioning	51.3 (27.3)	42.9 (29.2)	57.3 (36.5)	39.9 (32.4)	−4.61 (−17.39 to 8.17)	0.48
Emotional functioning	82.7 (15.8)	79.4 (16.6)	83.3 (19.1)	81.5 (21.4)	−1.84 (−5.81 to 9.50)	0.63
Cognitive functioning	80.8 (21.5)	81.7 (18.8)	82.6 (24.8)	80.9 (23.8)	−2.01 (−10.09 to 6.07)	0.62
Social functioning	51.3 (29.1)	48.8 (25.7)	47.2 (30.2)	47.9 (32.9)	0.31 (−9.52 to 10.15)	0.95
Fatigue	41.4 (21.9)	49.2 (25.8)	35.5 (24.4)	53.0 (29.5)	6.70 (−4.28 to 17.67)	0.23
Nausea/vomiting	7.1 (15.0)	16.3 (21.2)	11.1 (16.6)	20.5 (24.1)	11.50 (−1.67 to 24.68)	0.86
Pain	11.3 (19.8)	22.1 (28.3)	9.4 (20.3)	22.2 (32.5)	0.55 (−12.47 to 13.57)	0.93
Dyspnea	24.2 (27.2)	20.0 (23.6)	21.5 (31.1)	24.1 (27.5)	4.97 (−4.96 to 14.91)	0.32
Insomnia	25.0 (28.0)	32.5 (33.3)	22.0 (28.9)	23.6 (27.5)	−7.89 (−20.80 to 5.03)	0.23
Appetite loss	20.8 (25.8)	35.8 (34.9)	16.3 (23.9)	36.8 (34.5)	0.64 (−14.36 to 15.65)	0.93
Constipation	11.7 (22.1)	6.8 (13.6)	14.6 (25.6)	17.0 (25.9)	9.06 (0.67–17.45)	0.04
Diarrhea	15.0 (18.4)	32.5 (35.0)	9.0 (16.5)	33.3 (37.7)	2.48 (−13.23 to 18.19)	0.75
Financial difficulties	16.7 (26.1)	17.5 (29.2)	17.4 (33.0)	18.1 (31.5)	0.17 (−10.74 to 11.07)	0.98

^a Difference between the intervention and control group at 3 months adjusted for baseline by analysis of covariance.**Table 4**

Severe oral mucositis and acute GVHD.

Outcome	Intervention (n = 57)	Control (n = 60)	Relative risk (95% CI)	P-value
Oral mucositis – no. (%)				0.78
Grades 0–2	15 (26)	15 (25)	Reference	
Grade 3	12 (21)	10 (17)	1.11 (0.59–2.11)	
Grade 4	30 (53)	35 (58)	0.95 (0.72–1.25)	
Acute GVHD – no. (%)				0.37
Grades 0–2	50 (88)	47 (78)	Reference	
Grade 3	3 (5)	7 (12)	0.44 (0.12–1.60)	
Grade 4	4 (7)	6 (10)	0.65 (0.20–2.20)	

group. Furthermore, a low protein intake in the intervention group may potentially explain lack of differences between the two study groups, even if a median protein intake of 1 g/kg/day was achieved in the intervention group, corresponding to the lowest recommended protein intake when we designed the study in 2009 [13].

We chose to include only allo-HCST patients treated with MAC since their nutritional problems due to drug-induced toxicity are more severe [36] and their QoL outcomes more impaired than after RIC [2]. The nutritional intervention was individualized based on assessment of resting energy expenditure using indirect

calorimetry and day-to-day monitoring of food and nutrient intake to ensure accuracy of energy and protein intake. Moreover, the study outcomes were based on validated scoring methods. Furthermore, the lack of intervention effect is probably not explained by non-adherence to the protocol since the targeted minimum of energy and protein intakes per day was achieved for most patients in the intervention group during the hospital stay. A limitation is that our trial was not designed to analyze sub-groups, e.g. single diagnoses or route of nutritional support.

Our trial showed that individualized nutrition targeting recommended daily intakes of energy and protein during

Table 5

Body weight, fat-free mass index and fat mass index during the study period.

Outcome	Intervention group		Control group		Reference group		P-value*	
	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)	Group effect	Time effect
Body weight								
Baseline	57	77.7 (16.2)	60	75.9 (15.2)	13	73.8 (15.5)		
3 weeks	52	77.2 (15.3)	59	75.0 (14.4)	12	73.5 (18.0)		
6 weeks	50	73.4 (14.1)	58	71.5 (13.8)	11	70.9 (18.6)		
3 months	44	72.8 (14.6)	52	70.6 (14.1)	7	70.4 (18.2)	0.32	<0.001
Fat-free mass index								
Baseline	56	18.5 (3.1)	59	18.2 (2.5)	—	—		
3 weeks	48	19.2 (3.1)	55	19.1 (3.1)	—	—		
6 weeks	38	18.2 (2.9)	42	18.3 (2.9)	—	—		
3 months	39	18.5 (3.0)	48	18.5 (3.0)	—	—	0.59	<0.001
Fat mass index								
Baseline	56	6.2 (3.0)	59	6.2 (3.0)	—	—		
3 weeks	48	5.6 (3.1)	55	5.0 (3.0)	—	—		
6 weeks	38	5.2 (2.8)	42	4.6 (3.1)	—	—		
3 months	39	4.7 (2.7)	48	4.1 (2.7)	—	—	0.52	<0.001

hospitalization had no effect on global QoL or QoL subscales three months after allo-HSCT. Moreover, we found no effect of the intervention on nutritional status, OM, aGVHD, infectious complications, time to engraftment or transplant-related mortality. Whether nutritional support in the post-transplant- and rehabilitation phase could improve outcomes, warrants further testing.

Statement of authorship

KJS designed the study, implemented the intervention, collected and analyzed the data, and drafted the manuscript. MJH designed the study, contributed to data interpretation and specifically supervised the analysis of the QoL results. AB and KEAL contributed to data interpretation and specifically contributed to the design of the intervention. MBV contributed to the interpretation of the data and the statistical analysis. AMG implemented the intervention and contributed to data interpretation. SD and LB contributed to data analyses and interpretation. GET and POI designed the study and analyzed and interpreted the data. All authors prepared and approved the final manuscript.

Conflict of interest

The authors declare no conflict of interests.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.clnesp.2018.08.002>.

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Supplementary material

Skaarud et al.: Effects of individualized nutrition after allogeneic haematopoietic stem cell transplantation following myeloablative conditioning; a randomized controlled trial

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1.0 Reference group

We considered the possibility that a daily registration of the dietary intake among the controls would increase their energy- and protein intakes. To gain proxy data on energy and protein intakes among the controls we collected prospectively oral energy and protein intakes in a separate reference group with similar characteristics as those in the control group and before commencing the trial. Panel S1 shows the characteristics of this reference group.

Panel S1. Characteristics of the reference group receiving routine nutrition support

Characteristic	n=13
Age – yr median (range)	42.9 (18–59)
Sex – no. (%)	
Female	9 (69)
Male	4 (31)
Diagnosis – no. (%)	
AML	8 (62)
CMML	1 (7)
MDS	4 (31)
Conditioning – no. (%)	
Busulphan + Cyclophosphamide	13 (100)

Abbreviations: AML = Acute myeloid leukemia; CMML = Chronic myelomonocytic leukemia; MDS = Myelodysplastic syndrome.

2.0 An expanded list of baseline values for other diagnosis, EBMT score and HCTI-CI score

Supplemental table S1. Diagnoses and morbidity scores at baseline by allocated treatment group

Characteristic	Intervention (n= 57)	Control (n= 60)
Other diagnoses		
ACML – no. (%)	1 (2)	-
APL – no. (%)	1 (2)	-
ABL – no. (%)	-	1 (2)
AUL – no. (%)	-	1 (2)
MCL – no. (%)	-	1 (2)
MF – no. (%)	2 (4)	-
MS – no. (%)	-	1 (2)
HCTI – CI scores – no. (%) [*]		
0	42 (73)	45 (74)
1	5 (9)	9 (15)
2	3 (5)	1 (2)
3	4 (7)	4 (7)
4	1 (2)	1 (2)
5	1 (2)	-
6	-	-
7	-	-
8	1 (2)	-
EBMT scores – no. (%)		
0	2 (4)	2 (3)
1	3 (5)	4 (7)
2	11 (19)	13 (22)
3	17 (30)	17 (28)
4	14 (25)	14 (23)
5	6 (11)	8 (14)
6	3 (5)	2 (3)
7	1 (2)	-

Abbreviations: ACML = Atypical chronic myeloid leukemia; APL = Acute promyelocytic leukemia; ABL = Acute basophilic leukemia; AUL = Acute undifferentiated leukemia; MCL = Mast cell leukemia; MF = Myelofibrosis; MS = Myeloid sarcoma; HCTI-CI index = Hematopoietic Cell Transplantation-specific comorbidity index; EBMT score = European Group for Blood and Marrow Transplantation score.

3.0 Baseline sociodemographic data

Supplemental table S2. Baseline sociodemographic data of the two study groups

Characteristics	Intervention (n= 57)	Control (n= 60)
Marital status – no. (%)		
Married/partner	40 (70)	38 (63)
Other	17 (30)	22 (37)
Have children – no. (%)	43 (75)	37 (62)
Living with others – no. (%)	51 (89)	48 (80)
Education – no. (%)		
Elementary school up to 10 years	4 (7)	7 (12)
High School	27 (47)	34 (56)
University/college	26 (46)	19 (32)
Employment – no. (%)		
Fulltime	9 (16)	14 (23)
Part-time work	2 (4)	3 (5)
Studying	3 (5)	2 (3)
Receiving social welfare benefit	43 (75)	41 (68)

4.0 Quality of life scores

Supplemental table S3. Quality of life scores during the study period

	Intervention		Control		P-value*	
	n	Mean (SD)	n	Mean (SD)	Group effect	Time effect
Global quality of life						
Baseline	40	70.4 (17.6)	48	69.6 (22.0)	0.32	<0.001
3 weeks	39	41.9 (16.9)	47	34.8 (19.6)		
6 weeks	37	48.2 (20.0)	43	50.0 (20.8)		
3 months	40	58.8 (19.2)	48	55.4 (23.3)		
Physical functioning						
Baseline	40	78.3 (14.8)	48	80.3 (20.2)	0.91	<0.001
3 weeks	39	56.6 (23.3)	47	47.9 (23.9)		
6 weeks	39	55.7 (25.3)	46	59.4 (20.5)		
3 months	40	63.5 (23.6)	48	66.6 (23.6)		
Role functioning						
Baseline	40	51.3 (27.3)	48	57.3 (36.5)	0.80	<0.001
3 weeks	37	22.5 (27.0)	46	17.8 (23.4)		
6 weeks	39	32.5 (25.9)	46	33.7 (29.3)		
3 months	40	42.9 (29.2)	48	39.9 (32.4)		
Emotional functioning						
Baseline	40	82.7 (15.8)	48	83.3 (19.0)	0.86	0.002
3 weeks	39	75.9 (19.5)	47	74.2 (21.1)		
6 weeks	38	80.1 (19.0)	44	80.4 (21.7)		
3 months	40	79.4 (16.6)	48	81.5 (21.4)		
Cognitive functioning						
Baseline	40	80.8 (21.5)	47	82.6 (24.8)	0.75	<0.001
3 weeks	39	72.2 (21.1)	47	61.3 (30.7)		
6 weeks	38	76.8 (18.8)	44	75.4 (22.0)		
3 months	40	81.7 (18.8)	48	80.9 (16.7)		
Social functioning						
Baseline	40	51.3 (29.1)	48	47.2 (30.2)	0.71	<0.001
3 weeks	38	27.2 (22.7)	47	28.7 (27.7)		
6 weeks	38	38.6 (23.9)	44	45.1 (28.9)		
3 months	40	48.8 (25.7)	48	47.9 (32.9)		
Fatigue						
Baseline	40	41.4 (21.9)	48	35.5 (24.4)	0.37	<0.001
3 weeks	39	63.2 (25.1)	48	76.2 (21.3)		
6 weeks	39	55.3 (22.9)	46	58.9 (28.8)		
3 months	40	49.2 (25.8)	48	53.0 (29.5)		
Nausea and vomiting						
Baseline	40	7.1 (15.0)	48	11.1 (16.6)	0.19	<0.001
3 weeks	39	43.2 (28.0)	48	47.6 (27.9)		
6 weeks	39	29.1 (27.8)	46	28.3 (27.2)		
3 months	40	16.3 (21.2)	48	20.5 (24.1)		

Supplemental table S3. Quality of life scores during the study period

	Intervention		Control		P-value*	
	n	Mean (SD)	n	Mean (SD)	Group effect	Time effect
Pain						
Baseline	40	11.3 (19.8)	48	9.4 (20.3)	0.96	<0.001
3 weeks	39	34.6 (27.1)	48	49.7 (32.5)		
6 weeks	39	19.7 (26.2)	46	19.9 (24.2)		
3 months	40	22.1 (28.3)	48	22.2 (32.5)		
Dyspnea						
Baseline	40	24.2 (27.2)	48	21.5 (31.1)	0.72	0.10
3 weeks	38	23.7 (28.9)	47	36.9 (32.0)		
6 weeks	39	26.5 (31.7)	46	27.5 (30.9)		
3 months	40	20.0 (23.6)	47	24.1 (27.5)		
Insomnia						
Baseline	40	25.0 (28.0)	47	22.0 (29.0)	0.27	<0.001
3 weeks	38	47.4 (30.6)	47	48.9 (33.9)		
6 weeks	39	42.7 (30.5)	46	34.8 (34.4)		
3 months	40	32.5 (33.3)	48	23.6 (27.5)		
Appetite loss						
Baseline	40	20.8 (25.8)	47	16.3 (23.9)	1.00	<0.001
3 weeks	38	76.3 (27.8)	47	84.4 (24.9)		
6 weeks	38	55.3 (36.6)	46	50.7 (35.7)		
3 months	40	35.8 (34.9)	48	36.8 (34.5)		
Constipation						
Baseline	40	11.7 (22.1)	48	14.6 (25.6)	0.013	0.52
3 weeks	39	6.0 (15.0)	45	22.2 (35.5)		
6 weeks	38	4.4 (11.4)	44	15.9 (25.4)		
3 months	39	6.8 (13.6)	47	17.0 (25.9)		
Diarrhea						
Baseline	40	15.0 (18.4)	48	9.0 (16.5)	0.24	<0.001
3 weeks	39	54.7 (33.8)	46	62.3 (34.9)		
6 weeks	38	37.7 (30.2)	44	31.1 (29.1)		
3 months	40	32.5 (35.0)	48	33.3 (37.7)		
Financial difficulties						
Baseline	40	16.7 (26.1)	48	17.4 (33.0)	0.61	0.43
3 weeks	38	16.7 (29.8)	46	15.9 (28.8)		
6 weeks	38	18.4 (27.6)	44	16.7 (28.3)		
3 months	40	17.5 (29.2)	48	18.1 (31.5)		

*P-values are derived from linear mixed model analysis for repeated measures for global quality of life scores and all subscale scores; except for pain, dyspnea, constipation and financial difficulties that were dichotomized (score 0 = 0 and scores > 0 = 1) and analyzed by a logistic regression model with general estimating equations.

6.0 Infectious complications

Supplemental table S4. Infectious complications

Outcome	Intervention (n=57)	Control (n= 60)	P-value*
Episodes of bacteremia – no. (%)			
0	37 (65)	43 (72)	0.59
1	16 (28)	15 (25)	
2	3 (5)	2 (3)	
3	1 (2)	0	
Fungal disease – no. (%) ¹			
None	50 (96)	53 (96)	1.00
Proven	1 (2)	1 (2)	
Probably	0	1 (2)	
Possible	1 (2)	0	
Virus infections – no. (%)			
Yes – no. (%)	6 (11)	11 (18)	0.23
Non-agent specific pneumonia – no. (%)			
Yes – no. (%)	1 (2)	4 (7)	0.37
Empirical use of IV antibiotics			
Yes – no. (%)	37 (65)	43 (72)	0.43
No. of days – median (range)	16 (7–40)	16 (7–72)	
Empirical use of IV antifungal			
Yes – no. (%)	27 (52)	32 (58)	0.52
No. of days – median (range)	13 (6–45)	19 (2–73)	
Fever			
Yes – no.	55 (97)	59 (98)	0.61
No. of days – median (range)	9 (1–28)	9 (1–52)	

*P-values are derived from Fisher's exact test (2 and 3 episodes were collapsed for episodes of bacteremia; fungal disease was dichotomized) or the chi-squared test.

¹Five patients in the intervention group and five patients in the control group had fungal disease at baseline.

Paper II

ARTICLE

Body weight, body composition and survival after 1 year: follow-up of a nutritional intervention trial in allo-HSCT recipients

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Abstract

The role of body weight change in survival among recipients of hematopoietic stem-cell transplantation is controversial. We assessed the effect of optimizing energy and protein intake on 1-year survival, body weight and body composition, and the effect of body weight and body composition on 1-year survival in 117 patients (57 intervention, 60 control) in a randomized controlled trial. Cox regression was used to study effects of the intervention, weight and body composition on death, relapse, and nonrelapse mortality (NRM). We found no significant effect of intervention versus control on death hazard ratio (HR) 1.05, 95% confidence interval (CI) 0.54–2.04, $p = 0.88$), relapse (HR 1.15, 95% CI 0.48–2.27, $p = 0.75$), and NRM (HR 0.95, 95% CI 0.39–2.28, $p = 0.90$). Body weight, fat-free mass index, body fat mass index and total body water changed over time ($p < 0.001$), similarly in both groups ($0.17 \leq p \leq 0.98$). In multivariable analyses adjusted for group, gender and age, HRs and 95% CIs per one kilo increase in weight were 1.03 (1.01–1.06) and 1.04 (1.01–1.08) for death and NRM after 1 year ($p \leq 0.02$), respectively, and 1.08 (1.01–1.15) for relapse after 3 months ($p = 0.02$). In conclusion, weight gain is possibly due to fluid retention and is an indicator of a complication in HSCT, rather than a marker of improved nutritional status.

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Introduction

Upon admission for allogeneic hematopoietic stem-cell transplantation (allo-HSCT) many patients have lost body weight and some may even be malnourished [1, 2]. Weight loss often increases in the peritransplant period [3–6]. A study found increased risk of nonrelapse mortality (NRM) and inferior overall survival in patients with >10% weight loss compared with weight loss <10% [3], while an association between weight loss and survival was not found in another study [5]. One complicating factor when measuring body weight in the early phase of transplantation is the influence of capillary leak and fluid retention [7–9], which may mask weight loss. Therefore, measures of the different body composition compartments i.e. muscle and fat mass and total body water would be more relevant. To the best of our knowledge, no former study has measured body composition longitudinally after allo-HSCT and assessed the impact on survival.

Several studies have assessed the effect of nutritional interventions on body weight and survival in allo-HSCT recipients. A randomized controlled trial (RCT) of old date, compared total parenteral nutrition with an electrolyte-enriched solution [10], and a later RCT compared

individually adjusted parenteral nutrition with an electrolyte-enriched solution [11]. Both studies reported improved body weight [10, 11], and the former found improved overall survival [10]. However, there are no data showing that parenteral nutrition is superior to enteral nutrition in allo-HSCT [12–14]. Furthermore, it is not certain whether nutritional support during hospitalization for allo-HSCT improves survival.

We previously reported results of an RCT investigating optimized energy and protein intake compared to routine hospital procedure for nutritional support on global quality of life and clinical outcomes 3 months post-HSCT. The intervention had no effect on the primary endpoint, global quality of life, or the main secondary outcomes, oral mucositis and acute graft-versus-host disease (aGVHD) [15]. The aim of this 1-year follow-up was to explore: (i) the effect of the intervention on death, relapse and NRM, (ii) changes in weight and body composition, and (iii) the effect of changes in weight and body composition on death, relapse and NRM.

Patients and methods

Patients

In total, 117 eligible patients (intervention: $n = 57$, control: $n = 60$) with a hematological malignancy ≥ 18 years of age, and able to provide informed consent received myeloablative conditioning before allo-HSCT were included in the study [15]. Data were collected from August 2010 to February 2017 at Oslo University Hospital, Norway. The study was approved by The Regional Committee for Medical and Health Research Ethics South East Norway (#S-09136c 2009/2115) and the Data Protection Supervisor, Oslo University Hospital, and registered at ClinicalTrials.gov, ID NCT01181076.

Procedures

The procedures have been described in detail [15]. All patients received either (i) intravenous busulphan (four oral doses/day with target serum concentration of 900 ng/ml from days -7 to -4 prior to the transplantation) and intravenous cyclophosphamide (60 mg/kg body weight on days -3 and -2); or (ii) total body irradiation (1.3 Gy $\times$ 2 on days -8 to -4) combined with intravenous cyclophosphamide (60 mg/kg on days -3 and -2). Allografts were mobilized peripheral-blood stem cells or bone marrow cells from HLA-identical related or unrelated donors. GVHD prophylaxis was cyclosporine and methotrexate.

The nutritional intervention started when conditioning was initiated and continued until hospital discharge, and aimed at a daily energy intake of ≥ 126 kJ/kg body weight

and a protein intake of 1.5–2.0 g/kg body weight [15]. Patients received routine hospital food and were encouraged to take energy-enriched and lactose-reduced snacks and oral supplements daily. A nasogastric tube was inserted within 5 days after transplantation. Those unable to meet the energy target by the oral or enteral route received supplementary parenteral nutrition (PN). Oral, enteral and PN energy intake was monitored on a daily basis. After discharge, nutrition advice, oral nutritional supplements and enrichment powder were provided at the regular outpatient visits. The control group received routine hospital procedure for nutritional support. During hospitalization, the intervention group received significantly more (median (range)) energy (kJ/kg) and protein (g/kg) compared to a reference group for the control group, 131.9 (58.2–178.7), 1.1 (0.5–1.5) and 99.2 (50.2–139.8), 0.6 (0.4–1.0) ($p < 0.001$, $p < 0.001$), respectively [15].

Definition and assessment of study outcomes

Power calculation was based on the trials initial primary endpoint as previously described [15]. Outcome variables in this follow-up study were death, relapse, and NRM 1 year post-HSCT, and body weight, fat-free mass index (FFMI) (including body cell mass, extracellular solids, extracellular water and intracellular water) and body fat mass index (BFMI) during the first year post-HSCT. One year post-HSCT was defined as the day the patients arrived for the regular 1-year visit. Death was defined as death from any cause, relapse was defined as bone marrow blasts $> 5\%$ and NRM as death from any cause except from relapse. Weight was measured by a Tanita scale (BC-418 MA, Tanita Corp., Tokyo, Japan) to the nearest 0.1 kg. A correction factor of 0.1 kg was used to adjust for the weight of light clothing. Body composition was estimated using a single frequency bioimpedance analyzer (Tanita scale), operating at 50 kHz, with eight-point contact electrodes. FFMI and BFMI were calculated as fat-free mass and body fat mass (kg/m^2). Body weight, FFMI and BFMI were assessed at inclusion before conditioning, at 3 and 6 weeks and 3, 6, 9 and 12 months post-HSCT. Weight 6 months before inclusion was self-reported using the Patient-Generated Subjective Global Assessment form [16]. FFMI was considered as low if $< 17 \text{ kg/m}^2$ for men and $< 15 \text{ kg/m}^2$ for women [17]. Based on a previous study BFMI was considered low if $< 2 \text{ kg/m}^2$ for men and $< 4 \text{ kg/m}^2$ for women [18]. The use of glucocorticoids was determined as the number of days for each patient on such treatment.

Statistical analyses

Cox regression was used to study the effects of the treatment group (intervention versus control), weight, FFMI and

BFMI on death, relapse and NRM, using the standard competing risk framework for relapse and NRM [19], while the Kaplan–Meier method was used to compare the probability of overall survival between the intervention and control group. We present hazard ratios (HRs) and 95% confidence intervals (CIs). Two patients had their allograft rejected and were censored at the time of the event when analyzing death, relapse and NRM. Those who rejected the allograft or relapsed had no further observations of weight and body composition. Body weight, FFMI, and BFMI were modeled as time-dependent continuous variables. In the multivariable analysis we adjusted for treatment group, gender, and age (categorized as <44 and ≥44 years). We conducted a sensitivity analysis for death, also adjusting for discharge status (not discharged/discharged from hospital stay) as a time-varying covariate. Additional adjustment for disease status (standard risk and high risk) (see Table 1), Hematopoietic Cell Transplantation-specific comorbidity index (low risk and intermediate/high risk) [20] and European Group for Blood and Marrow Transplantation score (low and intermediate/high) [21] did not change the results. The proportional hazard assumption was not fulfilled in the analysis of relapse, but when introducing a cut-off at 100 days the assumption was met. We also studied baseline body weight, FFMI and BFMI (all continuous) and weight change before baseline as a categorical variable (no weight loss, >0–<5% loss, 5–<10% loss, and ≥10% loss; the first category includes the patients that gained weight). Body weight, FFMI, BFMI, and total body water were analyzed with a linear mixed model for repeated measures using all-time points (baseline, 3 and 6 weeks and 3, 6, 9 and 12 months). We tested for interaction between group and time. Chi-square or Fisher exact test was used to study the use of glucocorticoid (yes, no) during the early phase (baseline to 6 weeks) in relation to weight change (no weight gain/weight loss versus weight gain). A *p* value < 0.05 was considered statistically significant. Analyses were performed with SPSS 26 (IBM Corp., Armonk, NY) and with R package bda, version 3.5.2 (R Core Team, Vienna, Austria).

Results

Characteristics at inclusion of the 117 patients (intervention: *n* = 57, control: *n* = 60) are shown in Table 1 and Supplementary Table S1. In total, 35 (29.9%) patients died during 1-year follow-up (17 in the intervention and 18 in the control group). One-year overall survival was similar in the intervention and control group (*p* = 0.88) (Fig. 1). Twenty (17.1%) patients suffered a relapse (10 in both groups), and NRM included 20 (17.1%) patients (9 and 11, respectively). Five patients who relapsed were still alive at 1 year. Results

Table 1 Clinical characteristics at inclusion (previously published [15])

Characteristics	Intervention (<i>n</i> = 57)	Control (<i>n</i> = 60)
Age yr—median (range)	45 (19–65)	41 (18–62)
Female	20 (35)	25 (42)
AML	36 (63)	31 (51)
High risk first remission	23	22
After relapse, beginning of first relapse and in second remission	10	9
First remission standard risk	3	—
ALL	6 (10)	10 (17)
First remission high risk	3	7
Early first relapse, second remission	3	3
CML	2 (4)	7 (12)
Chronic phase	—	1
Accelerated phase	2	6
CMML	3 (5)	3 (5)
MDS	6 (11)	5 (8)
Other ^a	4 (7)	4 (7)
Donor		
HLA-identical sibling	17 (30)	13 (22)
HLA-identical unrelated	40 (70)	47 (78)
Stem-cell source		
Bone marrow	25 (44)	27 (45)
Peripheral-blood hematopoietic cells	32 (56)	33 (55)
Sex mismatch ^b	17 (30)	10 (17)
Positive CMV serology		
Donor	27 (47)	24 (40)
Recipients	45 (79)	43 (72)
Conditioning		
Busulphan + Cyclophosphamide	56 (98)	56 (93)
TBI + Cyclophosphamide	1 (2)	4 (7)
HCTI—CI risk groups ^a		
Low risk	42 (74)	45 (75)
Intermediate risk	8 (14)	10 (17)
High risk	7 (12)	5 (8)
EBMT score ^a		
0–3	33 (58)	36 (60)
4	14 (24)	14 (23)
5–7	10 (18)	10 (17)
Performance status ECOG		
0	55 (96)	54 (90)
1	2 (4)	6 (10)
BMI		
Underweight	2 (4)	4 (7)
Normal weight	31 (54)	27 (45)
Overweight	17 (30)	26 (43)
Moderately obese	4 (7)	3 (5)
Severely obese	3 (5)	0 (0)

Values are numbers (%) unless otherwise stated

AML acute myeloid leukemia, ALL acute lymphocytic leukemia, CML chronic myeloid leukemia, CMML chronic myelomonocytic leukemia, MDS myelodysplastic syndrome, CMV cytomegalovirus, TBI total body irradiation, HCTI-CI hematopoietic cell transplantation-specific comorbidity index, EBMT score European Group for Blood and Marrow Transplantation score, ECOG Eastern Cooperative Oncology Group

^aAn expanded list of baseline values for other diagnosis, EBMT score and HCTI-CI score is provided in Supplementary Table S1

^bSex mismatch was defined as female donor to male recipients

Fig. 1 Kaplan–Meier plot for 1-year overall survival

of analysis of death, relapse, and NRM within 1-year post-HSCT are shown in Table 2. We found no significant effect of the nutritional intervention on any of the three outcomes ($0.75 \leq p \leq 0.90$, Table 2).

Significant changes were observed in body weight, FFMI, BFMI and total body water in both the intervention and control group during the 1-year follow-up ($p < 0.001$ for all), but with no significant differences between the groups ($0.17 \leq p \leq 0.98$) as shown in Fig. 2a–d and Supplementary Table S2. Also, no significant interaction was found between group and time, i.e. the effect of time was similar in both groups ($0.12 \leq p \leq 0.65$). Mean body weight and BFMI decreased during the first 6 months, and then remained fairly stable throughout the 1-year follow-up (Fig. 2a, c). Mean FFMI and total body water increased initially, while only FFMI decreased before stabilizing (Fig. 2b, d).

Table 2 shows that the effects of weight, FFMI and BFMI on 1-year death, relapse and NRM were quite similar in univariable and multivariable analysis. Here we report multivariable results. For each kg increase in body weight the risk of death increased with 3% ($p = 0.01$) and the risk of NRM with 4% ($p = 0.02$). No significant association was found between weight and the risk of relapse over the whole period ($p = 0.57$); however, due to lack of proportional hazards, follow-up was split. No significant association was found between weight and risk of relapse during the first 100 days ($p = 0.17$), while a significant effect was found after 100 days (6% increased risk per kg increase in weight, $p = 0.02$). FFMI was not significantly associated with death ($p = 0.42$) or NRM ($p = 0.30$). FFMI was not significantly associated with relapse before 100 days ($p = 0.17$), and borderline significant after 100 days ($p = 0.05$). BFMI was significantly associated with death ($p = 0.01$) and NRM ($p = 0.04$), while no association was found with relapse

($p = 0.50$) (Table 2). Adjustment for discharge status did not change the associations between weight, FFMI, BFMI and death ($0.01 \leq p \leq 0.50$) (Supplementary Table S3).

We also assessed the effects of weight, FFMI and BFMI at baseline on death, relapse and NRM 1-year post-HSCT with similar results in univariable and multivariable analyses except for BFMI who changed from nonsignificant in the univariable to significant in the multivariable analysis (Supplementary Table S4a), the multivariable p values are reported here. We found no effects of baseline weight and FFMI ($0.14 \leq p \leq 0.97$), while baseline BFMI was significantly associated with death ($p = 0.04$) and increased NRM ($p = 0.03$), but not with relapse ($p = 0.83$). Moreover, no significant association was found between weight change the last 6 months before allo-HSCT and death, relapse and NRM 1-year post-HSCT ($0.37 \leq p \leq 0.93$) (Supplementary Table S4b).

Twenty-one (36.8%) patients in the intervention and 24 (40.0%) in the control group received glucocorticoid treatment in the early post-transplant period. Median (range) numbers of days with glucocorticoids were 25 (2–78) and 34 (8–88), respectively. We found a significant association between glucocorticoid usage and weight change from baseline to 6 weeks post-transplant; 25.7% among those treated with glucocorticoids and 5.5% among those not treated with glucocorticoids gained weight ($p = 0.004$), but not from baseline to 3 weeks (corresponding percentages were 33.7% and 31.3%, respectively, $p = 0.85$, Supplementary Table S5).

Discussion

The present study yielded three main results. Firstly, we found no significant difference between the intervention

Table 2 Hazard ratios (HRs) and 95% confidence intervals (CIs) for treatment group, body weight, fat-free mass index and body fat mass index, and risk of death, relapse and nonrelapse mortality

			Univariable		Multivariable ^a	
Variables	<i>N</i>	Cases	HR (95% CI)	<i>p</i> value	HR (95% CI)	<i>p</i> value
Death						
Group ^b	117	35	1.05 (0.54–2.04)	0.88		
Weight (kg)	117	35	1.03 (1.00–1.05)	0.02	1.03 (1.01–1.06)	0.01
FFMI (kg/m ²)	115	35	1.05 (0.94–1.17)	0.41	1.07 (0.91–1.26)	0.42
BFMI (kg/m ²)	115	35	1.13 (1.01–1.26)	0.03	1.19 (1.05–1.35)	0.01
Relapse						
Group ^b	117	20	1.15 (0.48–2.27)	0.75		
Weight (kg) ≤ 100 days ^c	117	20	0.98 (0.94–1.03)	0.48	0.96 (0.91–1.02)	0.17
Weight (kg) > 100 days ^c			1.07 (1.02–1.13)	0.01	1.08 (1.01–1.15)	0.02
FFMI (kg/m ²) ≤ 100 days ^c	115	20	0.95 (0.77–1.16)	0.59	0.82 (0.61–1.09)	0.17
FFMI (kg/m ²) > 100 days ^c			1.34 (1.03–1.75)	0.03	1.44 (1.00–2.06)	0.05
BFMI (kg/m ²)	115	20	1.02 (0.86–1.20)	0.83	1.06 (0.89–1.27)	0.50
NRM						
Group ^b	117	20	0.95 (0.39–2.28)	0.90		
Weight (kg)	117	20	1.03 (1.00–1.06)	0.06	1.04 (1.01–1.08)	0.02
FFMI (kg/m ²)	115	20	1.07 (0.92–1.23)	0.40	1.13 (0.90–1.41)	0.30
BFMI (kg/m ²)	115	20	1.12 (0.97–1.29)	0.11	1.18 (1.01–1.39)	0.04

FFMI fat-free mass index, BFMI body fat mass index, NRM nonrelapse mortality

^aAdjusted for treatment group, gender and age^bIntervention versus control^cThe effect changed over time (nonproportional hazards) and follow-up was split into two periods ≤100 and >100 days

group with optimized energy and protein intake and the control group in risk of death, relapse and NRM within 1 year of allo-HSCT. Secondly, body weight and body composition changed significantly during 1-year follow-up, and the changes were similar in both groups. Thirdly, weight gain increased the risk of death and NRM during 1-year follow-up and the risk of relapse after 100 days (i.e. 3 months). We found the same association between weight gain and death when adjusting for discharge status.

There might be several explanations for why optimized energy and protein intake was not superior to routine hospital procedure for nutritional support on 1-year survival. Firstly, the targeted 126 kJ/kg and 1.5–2.0 g protein/kg calculated at baseline may be too low in the intense catabolic phase following allo-HSCT [22]. However, a higher energy intake may be difficult to achieve without a risk of fluid overload in the early post-transplantation period. Secondly, we cannot ignore the possibility of no differences in energy and protein intakes between the intervention group and control group, since oral energy intake was not monitored in the control group to avoid unintended focus on nutrition. However, we monitored energy and protein intakes in a reference group prior to the study and found no significant differences between the controls and the reference group in energy and protein intakes derived from glucose, enteral and parenteral nutrition. Moreover, total

energy and protein intakes were significantly lower in the reference group than in the intervention group [15]. Thirdly, the effect of enteral nutrition (EN) versus PN on survival has been debated. All the intervention patients received a PN supplement in addition to EN to achieve the targeted energy requirement [15]. Similar to the present study, two observational studies found no effect of EN combined with PN on body weight compared to PN alone [23, 24], but 3 months overall survival was better in the EN group compared to PN alone in a study [23]. One of these studies was a prospective study of recipients of allo-HSCT after myeloablative conditioning [23], and the other was a retrospective study of recipients of myeloablative conditioning and reduced intensity conditioning [24]. Energy and protein intakes were not reported in these two studies and since the sickest patients are often those who cannot tolerate EN, their results may be considered as inconclusive.

In the present study, mean body weight decreased during the first year post-HSCT, in line with several other studies [3–6]. Moreover, mean percentage of total body water increased until 3 months and remained high compared to baseline during the 1-year follow-up. Thus, we speculate that weight gain (i.e. fluid retention) may be an indicator of a complication of allo-HSCT, and not a marker of improvement in nutritional status. An initial increase in fat-free mass and a loss of fat mass can be masking an increase

Fig. 2 Mean values and standard errors for: **a** body weight (kg), **b** fat-free mass index (kg/m²), **c** body fat mass index (kg/m²) and **d** total body water (%) during 1-year follow-up

in extracellular water in the early post-transplant period. This is partly in line with a small study showing fluid shifts to the extracellular space along with body cell mass loss till 30 days after allo-HSCT, where after body weight and fat mass remained constant [25]. Moreover, a RCT of old date reported an increase in fat mass and extracellular solids 28 days after allo-HSCT in patients receiving total parenteral nutrition compared to patients receiving an enteral feeding program [14]. In the present study fat-free mass and body water were stable after hospital discharge as opposed to loss of fat mass. This is partly in line with a study showing loss of both fat-free mass and fat mass 30 days after allo-HSCT [4]. Two of the present studies are of old date [14, 25] and differences in changes in body composition between studies may be due to the use of different assessment tools and the time points for measurements.

Weight change before allo-HSCT has been correlated with outcome [1, 2]. We found a positive association between BFMI and risk of death and NRM. In line with this has overweight before transplantation been associated with poor survival in allo-HSCT [20]. In contrast, weight loss before transplantation has been correlated to an increase in relapse rate after transplantation [1], but this was not confirmed in our study.

Weight gain may be a symptom or sign of one of the vascular endothelium syndromes (e.g. sinusoidal obstruction syndrome, capillary leak syndrome, engraftment syndrome) [9] activated by the conditioning regimen, cytokines produced by the injured tissues, endogenous microbial products translocated through damaged mucosal barriers [26], drugs [27, 28] and engraftment [9]. Moreover, fluid overload has been associated with poorer overall survival and increased NRM [8]. Additionally, glucocorticoid therapy is known to have an effect on weight gain and body composition, in particular muscle atrophy, central accumulation of fat mass and increased extracellular fluid volume [29, 30]. Corroborating this, one study found an association between decrease in indirect measurement of fat-free mass, and aGVHD, and glucocorticoid therapy [6]. We interpreted an early increase in FFMI and weight gain as fluid retention. Therefore our findings suggest that the sickest patients gained weight and this weight gain was a complication of HSCT. Former studies suggesting that malnutrition is an independent risk factor of mortality are cross-sectional and thus not able to establish causal relationships. It is questionable if weight loss is an independent risk factor that can be reversed with nutritional support or a result of the underlying disease [31, 32]. It is possible that

weight loss prior to treatment not merely reflects insufficient nutritional intake and malnutrition, but also is a marker of disease severity.

Moreover, a significant association was found between weight gain and glucocorticoid usage from baseline to 6 weeks. However, independent of corticosteroid usage more patients (33.3%) experienced weight gain from baseline to 3 weeks than from baseline to 6 weeks (12.0%). An increase in total body water (i.e. fluid retention) in the early phase may be a symptom or sign of endothelium damage (organ dependent or systemic) due to toxicity of the conditioning regimen. An essential enzyme for metabolizing busulphan is glutathione transferase (GST), and increased toxicity of the conditioning regime may be a result of reduced activity of the GST enzymes [33]. An association between glutathione transferase *GSTA1* and *GSTM1* gene variants and busulphan pharmacokinetics and weight gain and mortality ≤ 30 days post-HSCT have been reported [7]. We can only speculate on genetics as an explanation model for the association between weight gain and death in the present study.

This study has several strengths and limitations. We achieved the recommended energy and protein intake in the intervention group [15]. Moreover, the energy and protein intake were based on the available recommendations, when we designed the study [22, 34] and are in line with current guidelines [13]. Furthermore, the targeted energy requirement was adjusted by resting energy expenditure measured with indirect calorimetry [35].

Dehydration or overhydration overestimate or underestimate fat-free mass or fat mass, respectively [36, 37]. A particular strength of our study was that we performed and interpreted longitudinally estimations of body composition with the same device under the same conditions [36, 37]. However, the main study was not powered for these secondary analyses.

In summary, we found no differences between the intervention and control group in risk of death, relapse, and NRM after 1 year. Moreover, changes in body weight and body composition were significant during 1-year follow-up, but similar in both groups. Weight gain was associated with increased risk of death and NRM during 1-year follow-up and increased risk of relapse after 3 months. In conclusion, weight gain is possibly due to fluid retention and is an indicator of complications in HSCT, rather than a marker of improved nutritional status.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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Supplementary Information

Body weight, body composition and survival after one year: follow-up of a nutritional intervention trial in allo-HSCT recipients

Table S1. Diagnoses and comorbidity scores at baseline by allocated treatment group*

	Intervention (n= 57)	Control (n= 60)
Other diagnoses		
ACML	1 (2)	-
APL	1 (2)	-
ABL	-	1 (2)
AUL	-	1 (2)
MCL	-	1 (2)
MF	2 (4)	-
MS	-	1 (2)
HCTI – CI scores		
0	42 (73)	45 (74)
1	5 (9)	9 (15)
2	3 (5)	1 (2)
3	4 (7)	4 (7)
4	1 (2)	1 (2)
5	1 (2)	-
6	-	-
7	-	-
8	1 (2)	-
EBMT scores		
0	2 (4)	2 (3)
1	3 (5)	4 (7)
2	11 (19)	13 (22)
3	17 (30)	17 (28)
4	14 (25)	14 (23)
5	6 (11)	8 (14)
6	3 (5)	2 (3)
7	1 (2)	-

*Previously published [1]. Values are frequencies (%), *ACML* Atypical chronic myeloid leukemia, *APL* Acute promyelocytic leukemia, *ABL* Acute basophilic leukemia, *AUL* Acute undifferentiated leukemia, *MCL* Mast cell leukemia, *MF* Myelofibrosis, *MS* Myeloid sarcoma, *HCTI-CI* index Hematopoietic Cell Transplantation-specific comorbidity index, *EBMT* score European Group for Blood and Marrow Transplantation score.

Table S2. Body weight, fat-free mass and body fat mass index and total body water during one-year follow-up

		Intervention		Control		P-value*	
		n	Mean (SD)	n	Mean (SD)	Group effect	Time effect
Body weight (kg)							
	Baseline	57	77.7 (16.2)	60	75.9 (15.2)		
	3 weeks	52	77.2 (15.3)	59	75.0 (14.4)		
	6 weeks	50	73.4 (14.1)	58	71.5 (13.8)		
	3 months	44	72.8 (14.6)	52	70.6 (14.1)		
	6 months	39	69.8 (12.5)	44	69.9 (13.9)		
	9 months	37	68.8 (12.8)	40	68.7 (14.1)		
	12 months	37	68.9 (11.9)	38	69.4 (14.5)	0.17	<0.001
Fat-free mass index (kg/m ²)							
	Baseline	56	18.5 (3.1)	59	18.2 (2.5)		
	3 weeks	48	19.2 (3.1)	55	19.1 (3.1)		
	6 weeks	38	18.1 (2.9)	42	18.3 (2.9)		
	3 months	39	18.5 (3.0)	48	18.5 (3.0)		
	6 months	38	18.2 (2.9)	42	18.3 (2.7)		
	9 months	35	17.9 (2.8)	36	18.3 (2.7)		
	12 months	34	18.1 (2.7)	36	18.4 (2.8)	0.56	<0.001
Body fat mass index (kg/m ²)							
	Baseline	56	6.3 (3.1)	59	6.2 (3.0)		
	3 weeks	48	5.6 (3.1)	55	5.0 (3.0)		
	6 weeks	38	5.5 (2.9)	42	4.6 (3.1)		
	3 months	39	4.7 (2.7)	48	4.1 (2.7)		
	6 months	38	4.1 (2.5)	42	4.4 (2.7)		
	9 months	35	4.3 (2.5)	36	4.0 (2.2)		
	12 months	34	4.1 (2.5)	36	4.3 (2.5)	0.41	<0.001
Total body water (%)							
	Baseline	56	55.3 (6.7)	59	55.2 (7.1)		
	3 weeks	48	57.4 (7.2)	55	58.5 (7.9)		
	6 weeks	38	56.9 (7.1)	42	59.0 (8.3)		
	3 months	39	60.3 (7.5)	48	60.3 (7.5)		
	6 months	38	60.2 (7.1)	42	59.7 (6.9)		
	9 months	35	59.6 (7.3)	36	60.9 (6.1)		
	12 months	34	60.2 (7.4)	36	60.0 (6.8)	0.98	<0.001

*P-values from mixed model analysis.

Table S3. Hazard ratios (HRs) and 95% confidence intervals (CIs) for treatment group, body weight, fat-free mass index and body fat mass index, and risk of death post-discharge.

Variables	N	Cases	Univariable		Multivariable*	
			HR (95% CI)	P-value	HR (95% CI)	P-value
Discharge as time-varying variable added in the model						
Weight (kg)	117	35	1.03 (1.00-1.05)	0.02	1.04 (1.01-1.06)	0.01
FFMI (kg/m ²)	115	35	1.04 (0.93-1.17)	0.45	1.06 (0.90-1.25)	0.50
BFMI (kg/m2)	115	35	1.16 (1.04-1.30)	0.01	1.22 (1.08-1.38)	0.01

FFMI Fat-free mass index, *BFMI* body fat mass index, *NRM* non-relapse mortality, ^{*} Adjusted for treatment group, gender and age.

Table S4a. Hazard ratios (HRs) and 95% confidence intervals (CIs) for body weight, fat-free mass index and body fat mass index at baseline ^{*} and risk of death, relapse and non-relapse mortality

	Univariable				Multivariable**	
	N	Cases	HR (95% CI)	P-value	HR (95% CI)	P-value
Death						
Weight (kg)	117	35	1.01 (0.99-1.03)	0.23	1.02 (0.99-1.04)	0.21
FFMI (kg/m ²)	115	35	1.02 (0.90-1.14)	0.78	1.00 (0.84-1.20)	0.97
BFMI (kg/m2)	115	35	1.11 (0.99-1.24)	0.07	1.13 (1.00-1.27)	0.04
Relapse						
Weight (kg)	117	20	1.01 (0.98-1.03)	0.62	1.00 (0.96-1.03)	0.77
FFMI (kg/m ²)	115	20	1.07 (0.91-1.24)	0.42	0.96 (0.78-1.19)	0.74
BFMI (kg/m ²)	115	20	0.94 (0.80-1.11)	0.48	0.98 (0.83-1.16)	0.83
NRM						
Weight (kg)	117	20	1.02 (0.99-1.04)	0.21	1.03 (0.99-1.06)	0.14
FFMI (kg/m ²)	115	20	1.01 (0.87-1.18)	0.88	1.02 (0.79-1.31)	0.90
BFMI (kg/m ²)	115	20	1.16 (1.01-1.35)	0.04	1.19 (1.02-1.39)	0.03

FFMI Fat-free mass index, *BFMI* body fat mass index, *NRM* non-relapse mortality, ^{*} Modeled as continuous variables, ^{**} Adjusted for treatment group, gender and age.

Table S4b. Hazard ratios (HRs) and 95% confidence intervals (CIs) for weight change before baseline and risk of death, relapse and non-relapse mortality

	Univariable				Multivariable [*]	
	N	Cases	HR (95% CI)	P-value	HR (95% CI)	P-value
Death						
No weight loss ^{**}	47	13	Ref.		Ref.	
>0%-<5% loss	27	10	1.43 (0.63-3.25)	0.40	1.40 (0.61-3.24)	0.43
5%-<10% loss	21	4	0.68 (0.22-2.08)	0.50	0.66 (0.21-2.06)	0.48
≥10% loss	22	8	1.40 (0.58-3.38)	0.45	1.43 (0.58-3.50)	0.43
Relapse						
No weight loss ^{**}	47	8	Ref.		Ref.	
>0%-<5% loss	27	3	0.68 (0.18-2.55)	0.56	0.66 (0.17-2.50)	0.54
5%-<10% loss	21	4	1.12 (0.34-3.71)	0.86	1.26 (0.37-4.21)	0.71
≥10% loss	22	5	1.47 (0.48-4.50)	0.50	1.25 (0.40-3.88)	0.70
NRM						
No weight loss ^{**}	47	8	Ref.		Ref.	
>0%-<5% loss	27	7	1.57 (0.57-4.34)	0.38	1.61 (0.57-4.54)	0.37
5%-<10% loss	21	2	0.56 (0.12-2.64)	0.46	0.53 (0.11-2.58)	0.44
≥10% loss	22	3	0.84 (0.22-3.16)	0.79	0.94 (0.25-3.64)	0.93

NRM non-relapse mortality, ^{*} Adjusted for treatment group, gender and age, ^{**} The first category includes the patients that gained weight.

Table S5. Number of patients (%) with no weight gain/weight loss versus weight gain and glucocorticoid usage in the early phase

	Baseline to three weeks			Three to six weeks			Baseline to six weeks		
	Glucocorticoid			Glucocorticoid			Glucocorticoid		
	Yes	No	P-value	Yes	No	P-value	Yes	No	P-value
No weight gain/weight loss	11 (68.7)	63 (66.3)		28 (80.0)	64 (87.7)		26 (74.3)	69 (94.5)	
Weight gain	5 (31.3)	32 (33.7)	0.85	7 (20.0)	9 (12.3)	0.29	9 (25.7)	4 (5.5)	<0.004

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Paper III

Microbial diversity and mortality after allogeneic hematopoietic stem cell transplantation: secondary analysis of a randomized nutritional intervention trial

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Abstract

Microbial diversity is associated with survival and acute graft-versus-host disease (aGVHD) in allogeneic hematopoietic stem cell transplantation (allo-HSCT). Diet is a critical determinant of microbial diversity, but whether nutritional support affects microbiota and outcome after allo-HSCT, is unknown. We used data from a randomized controlled trial to investigate if gut microbial composition, short-chain fatty acids (SCFAs, markers of gut-brain signaling) and markers of gut barrier function were associated with survival and aGVHD, and if optimized energy and protein intake influenced these parameters. Fecal specimens available from 47/117 recipients were collected and subjected to 16S rRNA gene sequencing. We observed major depletion of microbiota, SCFAs, and altered markers of gut barrier function from baseline to 3 weeks post-transplant. One-year mortality was significantly higher in patients with lower diversity at 3 weeks post-HSCT, but not related to diversity at baseline. The relative abundance of *Blautia* genus at 3 weeks was higher in survivors. We observed minor associations between fecal SCFAs, markers of gut barrier function, and clinical outcomes. Importantly, we found no significant differences between the intervention group and controls in investigated parameters, suggesting that other strategies are needed to prevent any negative effects of the gut microbiome on clinical outcomes.

Introduction

Microbiota has shown to be a predictor of mortality in allogeneic hematopoietic stem cell transplantation (allo-HSCT) [1]. High diversity of the gut microbiota both before allo-HSCT and until engraftment is associated with improved overall survival and reduced acute graft-versus-host-disease (aGVHD) [1, 2]. Moreover, patients with preserved stable intestinal microbiota during the course of allo-HSCT had improved outcomes [3]. Specific bacteria have been associated with reduced mortality from GVHD and improved overall survival [4, 5]. Furthermore, short-chain fatty acids (SCFAs) are end-products of microbial metabolism important for the gut mucosal barrier and may have a role in mediating signaling between the gut and brain [6]. Depletion of SCFAs has been associated with aGVHD in children [7] and in animal models [8, 9]. Moreover, mucosal barrier injury and bacterial translocation have been associated with aGVHD and chemotherapy-induced gastrointestinal toxicity [10-12].

Diet is a major determinant of gut microbiota composition [13], and tube feeding may exert a beneficial effect on the intestinal flora. In one retrospective study, oral intake protected against aGVHD [14]. Moreover, tube feeding was associated with reduced aGVHD in a prospective, non-randomized study [15]. In a randomized controlled trial we found no effect of optimized energy and protein intake preferably via the enteral route on global quality of life, oral mucositis, and aGVHD three months post-HSCT [16]. Moreover, the nutrition intervention had no impact on one-year survival [17]. The aim of the present study was to use data from this trial to investigate if gut microbiota composition, microbial function (as measured by SCFAs) and markers of gut barrier function are (i) associated with overall survival and/or aGVHD, and/or (ii) altered by the nutritional intervention.

Materials and methods

Patients

Our randomized controlled trial (RCT) has been detailed [16]. Briefly, we included 117 adult patients undergoing allo-HSCT after myeloablative conditioning for a hematological malignancy. They were randomly assigned to receive optimized energy and protein intake preferably via the enteral route (intervention: n=57) or standard total parenteral nutrition (TPN) (control: n=60) [16]. Fecal samples were collected throughout the last five randomization blocks (block size of 10) resulting in fecal samples from 47 patients at baseline and 3 weeks post-HSCT (Supporting Information). The samples were immediately frozen at -80°C or immediately stored at 4°C and frozen within 24 hours at -80°C. Blood samples were collected from the 117 patients at baseline and 3 weeks post-HSCT, immediately frozen and stored at -80°C. Data were collected from August 2010 to February 2017 at Oslo University Hospital, Norway. The study was approved by The Regional Committee for Medical and Health Research Ethics South East Norway (#S-09136c 2009/2115) and the Data Protection Officer, Oslo University Hospital, and is registered with Clinical Trials (ID NCT01181076).

Treatment procedures

The procedures have been detailed [16]. The nutritional intervention aimed a minimum daily energy intake of 126 kJ/kg body weight and a protein intake of 1.5-2.0 g protein/kg body weight, preferably via the enteral route [16]. Patients received routine hospital food and were encouraged to consume energy-enriched and lactose-reduced snacks and oral supplements on a daily basis. A nasogastric tube was inserted within day +5 post-HSCT and a non-fiber, hydrolyzed medium triglyceride fat formula administered. We supplemented with parenteral nutrition (PN) if intolerance for enteral nutrition. The control group received routine practice

for nutritional support, energy and protein requirements were not calculated, dietary intake not recorded, and tube feeding not administered. TPN was given at the discretion of the physician. Trimethoprim-sulfamethoxazole was given prophylactic. Gentamicin and penicillin were preferred upon neutropenic fever [18] and ceftazidime or piperacillin-tazobactam and meropenem in combination with antifungal treatment as second and third line treatments. Acute GVHD prophylaxis consisted of cyclosporine and methotrexate.

Definition of study outcomes

In the current study, the outcomes were gut microbial diversity, fecal SCFAs, circulating gut leakage markers (a proxy for gut mucosal barrier function) at baseline and 3 weeks post-HSCT, one-year survival, and aGVHD. Gut microbiota composition was classified according to measures of alpha diversity (observed operational taxonomic units [OTUs] and Shannon diversity index), inter-individual (beta) diversity, as well as relative abundances of microbes at the genus level. Death was defined as death from any cause one year post-HSCT. We reported the highest grade of aGVHD [19]. Antibiotic usage was given in number of days of treatment.

Analyses of gut microbiota, short-chain fatty acids and markers of gut barrier function

Microbial DNA was extracted from fecal samples using PSP Spin Stool DNA Kit (Strattec Molecular GmbH) and the V3-V4 region of the 16S rRNA gene was amplified followed by sequencing on the Illumina MiSeq platform (REF PMID 24558975). Quality control and closed reference OTU mapping to the Silva database [20] (version 128, reference OTUs pre-clustered at 97% sequence similarity) was performed using Quantitative Insights Into Microbial Ecology version 1.9.1 [21] (Supportive Information). The samples were rarefied (sub-sampled) to an OTU count of 15,004 per sample, and all further analyses were performed

on this rarefied dataset.

Fecal concentrations of SCFAs were analyzed using vacuum distillation followed by gas chromatography [22-24]. Levels of the following SCFAs were assessed: acetic acid, propionic acid, iso-butyric acid, butyric acid, iso-valeric acid, valeric acid and total SCFAs. The plasma levels of (the gut-leakage markers) intestinal fatty acid binding protein (I-FABP), lipopolysaccharide binding protein (LBP) and soluble cluster of differentiation (sCD14) were measured by ELISAs (Hycult Biotech, Uden, The Netherlands and R&D Systems Europe, Oxfordshire, UK, respectively). The inter-assay coefficients of variation were 5·8%, 9·8% and 7·4%, respectively.

Statistical analyses

Comparison of continuous variables was performed with Mann-Whitney U test. We used Wilcoxon signed rank test for comparisons between baseline and 3 weeks samples. For analysis of bacterial abundances only genera present in at least 50% of the individuals in at least one of the groups analyzed were included. Correlation analysis was performed using Spearman's test. Acute GVHD was categorized as "yes" or "no". Due to few events we did not analyze associations between SCFAs at 3 weeks and death and aGVHD. Multivariate analysis of beta diversity between the intervention and control groups at baseline and 3 weeks and changes from baseline to 3 weeks was performed with a permutational multivariate analysis of variance (PERMANOVA). Due to multiple comparisons, false discovery rates were also calculated for the comparisons of bacterial taxa, according to the Benjamini-Hochberg method and denoted Q_{FDR} . A $p < 0.05$ was considered statistically significant. Analyses were performed using IBM-SPSS 26 (IBM Corp., Armonk, NY) and R package, version 3.6.1 (R Core Team, Vienna, Austria).

Results

No effects of the nutritional intervention on gut microbiota

Clinical characteristics of the 47 patients (intervention: n=23, control: n=24) are shown in Table 1. A total of 82 fecal samples were included in the final analysis; 44 collected at baseline (intervention: n=22, control: n=22) and 38 after 3 weeks (intervention: n=19 and control: n=19).

Allo-HSCT caused a large reduction in alpha diversity from baseline to 3 weeks ($p<0.001$ for both the intervention and the control group; Fig. 1a, b, Supplementary Tables S1-2). However, there were no differences between the intervention and control group at baseline or at 3 weeks for observed OTUs ($p=0.639$ and $p=0.293$, respectively; Fig. 1a-b) or Shannon diversity index ($p=0.606$, $p=0.737$, Supplementary Tables S1-2). We found no differences in beta diversity between the intervention and control groups at baseline or at 3 weeks (PERMANOVA pseudo-F=0.78, $p=0.661$ and pseudo-F=1.16, $p=0.292$, respectively). Beta diversity dropped in both the intervention and control group from baseline to 3 weeks (PERMANOVA pseudo-F=3.64, $p=0.002$ and pseudo-F= 4.38, $p=0.001$).

Gut microbiota composition and clinical outcomes

The baseline clinical characteristics of the current subset (Table 1) were similar to those of the original trial cohort [16], and given the lack of effect of nutritional intervention, the intervention and control groups were pooled for further analysis of clinical outcomes. The alpha diversity at 3 weeks, but not at baseline, was significantly associated with one-year survival (Fig. 2a, b). Both survivors and non-survivors had a significant drop in alpha diversity from baseline to 3 weeks, both for observed OTUs and Shannon diversity index. The relative loss of observed OTUs from baseline to 3 weeks was larger in non-survivors (median 84%) than in survivors (median 63%), ($p=0.035$, Fig. 2c).

At baseline, the relative abundance of the *Subdoligranulom* genus was lower in patients dying during the first year than in the survivors ($p=0.023$). At 3 weeks, but not at baseline, the *Blautia* genus was less abundant in patients who died during follow-up than in those surviving ($p=0.049$, Fig. 3a). A reduction of *Blautia* abundance from baseline to 3 weeks was observed in those who died during follow-up ($p=0.047$, Fig. 3b), while a numerical reduction of *Blautia* abundance in those alive at one year was not significant ($p=0.127$, Fig. 3b). Regarding aGVHD during follow-up, we found no significant associations between aGVHD and microbial alpha diversity or relative abundance of the *Blautia* genus at baseline or 3 weeks (Supplementary Fig. 1a-c, 2a-e). Q_{FDR} was >0.75 for all analyses of bacterial taxa.

Regarding treatment-related factors, there was a negative correlation between the number of days with i.v. nutrition and alpha diversity measures at 3 weeks ($r=-0.35$, $p=0.031$ for Shannon diversity index and $r=-0.31$, $p=0.054$ for observed OTUs). There was no significant association between i.v. nutrition and *Blautia* abundance at 3 weeks ($p=0.254$). All patients received i.v. antibiotics in the early post-transplant period (Table 1), but there were no correlations between number of days with i.v. antibiotics and *Blautia* abundance or measures of alpha diversity at 3 weeks (data not shown).

Fecal short-chain fatty acids and clinical outcomes

Fecal SCFAs were analyzed in a total of 55 samples (n=39 at baseline and n=16 at 3 weeks). A large reduction in total SCFA concentration was observed from baseline to 3 weeks (from median 62.5 to 25.1 mmol/kg, $p=0.001$), in parallel with reductions in both the major SCFAs acetic acid, propionic acid, and butyric acid, as well as less abundant SCFAs like isobutyric acid, isovaleric acid, and valeric acid. No significant differences were observed related to SCFAs between the intervention and control group at baseline or 3 weeks (Supplementary Tables S3-4).

Considering alpha diversity measures, *Blautia* abundance and SCFAs, only minor correlations were found at baseline. At 3 weeks, positive correlations were found for OTUs, Shannon diversity index, and total SCFA ($r=0.61$, $p=0.016$ and $r=0.66$, $p=0.007$, respectively). In addition, there were specific correlations between Shannon diversity index and *Blautia* abundance and butyric acid ($r=0.63$, $p=0.011$ and $r=0.57$, $p=0.027$, respectively) (Supplementary Tables S5-6).

Markers of gut barrier function and clinical outcomes

Damage of epithelial surfaces could represent another link between gut microbiota and clinical outcomes. Therefore, all available blood samples from the original study cohort were analyzed for I-FABP, LBP and sCD14 (Supplementary Table S7). From baseline to 3 weeks, a decrease was observed in I-FABP (from median 432 to 214 pg/ml, $p<0.001$), an increase in LBP (from median 13.695 to 18.805 ng/ml, $p<0.001$), but no difference in sCD14 (from median 1.745 to 1.803 ng/ml, $p=0.444$). No significant associations were observed between baseline values, 3-week values or change from baseline to 3 weeks for I-FABP, LBP or sCD14 and 1-year survival or aGVHD (data not shown). No differences between the intervention and control group were found (Supplementary Tables S8-10).

In the subset with fecal microbiota composition available in addition to circulating markers, a correlation between Shannon diversity index and I-FABP was observed both at baseline (n=44) ($r=0.31$, $p=0.043$) and 3 weeks (n=38) ($r=0.45$, $p=0.005$). For LBP and sCD14, a correlation with Shannon diversity index was observed only at 3 weeks ($r=-0.35$, $p=0.032$ and $r=-0.35$, $p=0.034$, respectively). At 3 weeks, *Blautia* genus was correlated with I-FABP ($r=0.39$, $p=0.017$). We found no correlations between observed OTUs and SCFAs or markers of gut function (Supplementary Tables S5-6).

Discussion

In the present study we observed a major reduction in bacterial diversity and levels of fecal SCFAs from baseline to 3 weeks post-HSCT. Markers of gut barrier function were also altered. Moreover, low diversity and low abundance of *Blautia* genus at 3 weeks and high relative loss of diversity at 3 weeks were associated with death the first year, but not with aGVHD. Fecal total SCFAs and markers of gut barrier function correlated with diversity and *Blautia* genus at 3 weeks, but were less strongly associated with clinical outcomes. We observed no impact of the nutritional intervention on microbiota, SCFAs, or markers of gut barrier function.

A reduction in gut microbial diversity was observed from baseline to 3 weeks, in line with several studies [4, 25, 26]. Lower microbial diversity and less abundant *Blautia* at 3 weeks were observed in those who died during one-year follow-up than in the survivors. This is also in line with studies showing that microbial diversity around the time of engraftment is associated with mortality following allo-HSCT [4, 25], and that *Blautia* abundance below median after allo-HSCT is associated with increased lethal GVHD and reduced overall survival [4]. We found a large reduction in the concentrations of SCFAs from baseline to 3 weeks, possibly caused by both reduced bacterial load and fiber intake. We further found a correlation between microbial diversity, *Blautia* genus, and butyric acid at 3 weeks. Interestingly, low levels of butyrate were found in children with aGVHD [7] and butyric acid may mitigate GVHD in mice [8].

The gut barrier is compromised after allo-HSCT [27]. In line with one study [11], we found LBP to be increased from baseline to 3 weeks, which we interpret as a measure of increased bacterial translocation and circulating LPS. The monocyte activation marker sCD14 remained stable, which argues against a systemic inflammatory response driven by microbial products at week 3. We observed a decrease in I-FABP, which has been reported as a

biomarker of enterocyte turnover, but with no predictive value in line with data from other studies [10, 12]. In contrast to our observations, higher LBP levels at day +7 and +14 post-HSCT have indicated higher probability of aGVHD [11]. Inverse correlation was found for Shannon diversity index and LBP at 3 weeks, but not at all for sCD14.

In the present study, only data at 3 weeks was associated with clinical outcomes, while the baseline values were not. In contrast, two recent studies analyzed microbiota at several time points and found an association between microbiome disruption and poor clinical outcomes both before conditioning and after allo-HSCT [1, 3].

Whether nutritional intervention can modify microbial diversity, improve survival, and reduce the risk of aGVHD in recipients of allo-HSCT, is controversial. In our RCT, the intervention had no significant effect on aGVHD prevalence or one-year survival [16, 17]. In the present study we found that our intervention did not affect gut microbial diversity, SCFA concentrations, or markers of gut barrier function, except for a weak, inverse correlation between number of days with PN and alpha diversity. In contrast, one study reported prompt post-HSCT recovery of the gut microbiome and SCFAs in children receiving tube feeding compared to children with PN [28]. Furthermore, tube feeding and several days with oral intake have been associated with reduced aGVHD in two observational studies, both in comparison to PN [14, 15]. Energy and protein intakes were not reported in these studies. Microbiota produce SCFAs throughout fermentation of fiber, but the intervention group in the present study received a fiber-free enteral formula as used in previously studies [29, 30]. Similarly, a recent study used a fiber-free formula to optimize EN tolerance and found no difference in microbial diversity between patients receiving predominantly EN versus PN [31]. In contrast, a retrospective case-control analysis suggested that fiber supplementation in allo-HSCT was associated with fewer days of diarrhea and reduced early mortality, but the study design does not allow firm conclusions [32]. Overall, there is limited evidence to

support nutritional measures focusing on EN as a treatment to modify the gut microbiota to reduce mortality and aGVHD.

Our study had some limitations. We have used an explorative approach also reporting results not withstanding correction for multiple comparisons, but focusing the discussion of the results primarily on pre-existing hypotheses. Importantly, the relatively small, but well-characterized subset with fecal samples available did not differ from the cohort of the original RCT in important characteristics. All patients had a malignant blood disease and received myeloablative conditioning, in contrast to other studies including patients treated with different conditioning regimens known to have different impact on altered microbiota.

In conclusion, low bacterial diversity at 3 weeks and relative loss of diversity until 3 weeks as well as low abundance of *Blautia*, was associated with death the first year, but not with aGVHD. A major reduction of SCFAs and changes in circulating markers of gut barrier function appeared at 3 weeks, but were only weakly associated with clinical outcomes. The nutritional intervention had no effect on the microbiota, SCFAs and markers of gut barrier function, suggesting that other means of intervention are needed to prevent any negative effects of an altered gut microbiota after allo-HSCT.

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Conflict of interests

The authors declare no competing interests. J.R.H. has served on Advisory boards for Orkla Health and Novartis, and received research support from Biogen, all unrelated to the present study.

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Figure Legends

Fig. 1 Gut microbial diversity and the intervention and control group at baseline and 3 weeks.

a Observed operational taxonomic units (OTUs) and groups, **b** Shannon diversity index and groups. Data are observed operational taxonomic units (OTUs) and Shannon diversity index given as box plots (median and interquartile range) with minimum and maximum values.

Fig. 2 Gut microbiota diversity and one-year survival. **a** Observed operational taxonomic units (OTUs) at baseline and 3 weeks and one-year survival, **b** Shannon diversity index at baseline and 3 weeks and one-year survival, **c** Loss of observed OTUs from baseline to 3 weeks and one-year survival. Data are observed OTUs and Shannon diversity index, and given as box plots (median and interquartile range) with minimum and maximum values.

Fig. 3 *Blautia* abundance and one-year survival. **a** *Blautia* abundance at 3 weeks and one-year survival, **b** Drop in *Blautia* abundance from baseline to 3 weeks and one-year survival. Data are *Blautia* abundance given as box plots (median and interquartile range) with minimum and maximum values. Lines connect individual data points.

Table

Table 1. Clinical characteristics of the two study groups

	Intervention (n=23)	Control (n=24)
Age y - median (range)	45 (19-63)	35 (21-54)
Female	9 (39.1)	13 (54.2)
Underlying disease		
AML	15 (65)	15 (62)
ALL	1 (4)	3 (13)
CML	-	3 (13)
CMML	2 (9)	-
aCML	1 (4)	-
MDS	4 (18)	1 (4)
ABL	-	1 (4)
MCL	-	1 (4)
Donor		
HLA - identical sibling	4 (17)	5 (21)
HLA - identical unrelated	19 (83)	19 (79)
Stem-cell source		
Bone marrow	17 (74)	19 (79)
Peripheral-blood hematopoietic cells	6 (26)	5 (21)
Sex mismatch*	8 (35)	-
Conditioning		
Busulfan + Cyclophosphamide	23 (100)	22 (92)
Total body irradiation + Cyclophosphamide	-	2 (8)
HCTI - CI risk groups		
Low risk	15 (66)	18 (75)
Intermediate risk	4 (17)	5 (21)
High risk	4 (17)	1 (4)
EBMT score		
0-3	17 (74)	19 (79)
4	5 (22)	3 (13)
5-7	1 (4)	2 (8)
Performance status ECOG		
0	23 (100)	24 (100)

Table 1. Clinical characteristics of the two study groups

	Intervention (n=23)	Control (n=24)
BMI		
Underweight	-	4 (17)
Normal weight	15 (65)	11 (46)
Overweight	7 (31)	8 (33)
Moderately obese	-	1 (4)
Severely obese	1 (4)	-
Numbers of days with i.v. antibiotic (median, range)	15 (8-22)	16 (8-31)
aGVHD		
0-1	18 (78)	18 (75)
2-4	5 (22)	6 (25)
Non-survivors at 1-year	6 (26)	6 (25)

Values are numbers (%) unless otherwise stated. AML Acute myeloid leukemia, ALL Acute lymphocytic leukemia, CML Chronic myeloid leukemia, CMML Chronic myelomonocytic leukemia, aCML atypical Chronic myeloid leukemia, MDS Myelodysplastic syndrome, ABL Acute basophilic leukemia, MCL Mast cell leukemia, HCTI-CI Hematopoietic Cell Transplantation-specific comorbidity index, EBMT score European Group for Blood and Marrow Transplantation score, ECOG Eastern cooperative oncology group, BMI Body mass index, aGVHD acute graft-versus-host-disease.

* Sex mismatch was defined as female donor-to-male recipient.

Fig. 1

a

b

Fig. 2

a

b

c

Fig. 3

a

b

Supplementary Information for:

Microbial diversity and mortality after allogeneic hematopoietic stem cell transplantation: secondary analysis of a randomized nutritional intervention trial

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Methods

When our randomized controlled trial was designed and initially implemented the role of gut microbiota in allo-HSCT was scarcely reported, thus we did not collect fecal samples from the initially enrolled patients.

We used a standard Fecal Container (Thomas Scientific, Swedesboro, NJ) to store the fecal samples.

Reads containing Illumina Universal Adapters or PhiX were discarded using bbdut version 37.55 (BBTools, <https://jgi.doe.gov/data-and-tools/bbtools>), and the remaining reads were demultiplexed using je version 1.2 [1]. Indices, heterogeneity spacers and primers were trimmed with cutadapt version 1.14 [2] and the paired end reads were subsequently quality trimmed and merged using bbmerge version 37.55 [3]. The merged contigs were quality filtered using default values in Quantitative Insights Into Microbial Ecology (QIIME) version 1.9.1 [4] using SortMeRNA version 2.0 [5] through QIIME. OTUs with a number of sequences <0.005% of the total number of mapped sequences were discarded as recommended [6].

Results

Table S1. Microbial diversity between the two study groups at baseline and after 3 weeks

	Baseline		3 weeks	
	Intervention (n=22)	Control (n=22)	Intervention (n=19)	Control (n=19)
Observed OTUs	192 (104-435)	209 (89-371)	54 (13-167)	209 (89-371)
Shannon index	4.91 (3.26-6.41)	4.73 (2.97-6.24)	2.74 (0.72-5.11)	2.98 (0.91-5.44)
Blautia	0.01 (0.00-0.60)	0.01 (0.00-0.08)	0.00 (0.00-0.03)	0.00 (0.00-0.02)

Values are given as median (range), *p* value from Mann-Whitney U test.

Table S2. Microbial diversity at baseline and at 3 weeks for the pooled study cohort

	Baseline (n=44)	3 weeks (n=38)	<i>p</i> value
Observed OTUs	198 (89-435)	57 (13-229)	<0.001
Shannon index	4.84 (2.97-6.41)	2.80 (0.72-5.44)	<0.001
Blautia	0.01 (0.00-0.60)	0.00 (0.00-0.03)	0.015

Values are given as median (range), *p* value from Wilcoxon signed rank test.

Table S3. Fecal short chain fatty acids at baseline and at 3 weeks for the pooled study cohort

	Baseline (n=39)	3 weeks (n=16)	<i>p</i> value
Acetic acid	37.12 (6.41-77.20)	17.17 (4.46-54.53)	0.002
Propionic acid	4.74 (0.00-18.37)	2.60 (0.00-12.36)	0.008
Iso-butyric acid	0.92 (0.00-2.89)	0.27 (0.00-1.42)	0.008
Butyric acid	5.65 (0.00-34.44)	1.05 (0.00-7.53)	0.001
Iso-valeric acid	1.35 (0.13-3.66)	0.33 (0.00-2.17)	0.004
Valeric acid	0.15 (0.00-3.16)	0.00 (0.00-1.18)	0.002
Total short chain fatty acids	62.45 (28.24-101.20)	25.11 (5.39-68.04)	0.001

Values (mmol/kg) are given as median (range), *p* value from Wilcoxon signed rank test.

Table S4. Fecal short chain fatty acids in the two study groups at baseline and at 3 weeks

	Baseline		3 weeks		<i>p</i> value
	Intervention (n=18)	Control (n=21)	Intervention (n=11)	Control (n=5)	
Acetic acid	35.17 (12.82-58.89)	39.88 (6.41-77.2)	17.65 (5.39-43.18)	16.69 (4.46-54.53)	0.777
Propionic acid	4.13 (0.00-18.37)	4.74 (0.19-17.64)	2.90 (0.00-8.06)	2.29 (0.00-12.36)	0.955
Iso-butyric acid	1.19 (0.13-2.27)	0.91 (0.00-2.89)	0.24 (0.00-1.06)	0.39 (0.18-1.42)	0.234
Butyric acid	5.97 (0.13-16.34)	5.65 (0.00-34.44)	1.20 (0.00-4.12)	0.89 (0.30-7.53)	0.461
Iso-valeric acid	1.55 (0.22-3.66)	1.22 (0.13-3.47)	0.32 (0.00-1.23)	0.54 (0.21-2.17)	0.335
Valeric acid	0.14 (0.00-3.16)	0.18 (0.00-2.89)	0.00 (0.00-0.18)	0.00 (0.00-0.12)	0.491
Total short chain fatty acids	51.38 (14.39-101.20)	63.52 (7.19-135.17)	27.00 (5.39-46.63)	23.21 (6.27-68.04)	0.692

Values (mmol/kg) are given as median (range), *p* value from Mann-Whitney U test.

Table S5. Correlations among alpha diversity, short chain fatty acid and markers of gut barrier function at baseline

	OTUs		Shannon		<i>Blautia</i> abundance	
	r	p value	r	p value	r	p value
Short chain fatty acid*						
Acetic acid	0.14	0.395	0.17	0.308	-0.05	0.759
Propionic acid	0.22	0.186	-0.03	0.851	-0.39	0.016
Iso-butyric acid	0.17	0.136	0.16	0.319	-0.16	0.348
Butyric acid	0.30	0.076	0.24	1.160	0.02	0.895
Iso-valeric acid	0.24	0.152	0.27	0.101	-0.26	0.126
Valeric acid	0.41	0.012	0.17	0.305	-0.28	0.091
Total short chain fatty acid	0.30	0.070	0.21	0.224	-0.10	0.552
Markers of gut barrier functioning						
I-FABP (pg/ml)	0.21	0.167	0.31	0.043	0.03	0.870
LPB (ng/ml)	0.25	0.099	0.13	0.399	-0.28	0.062
sCD14 (ng/ml)	-0.06	0.693	-0.07	0.659	-0.26	0.090

*Values are (mmol/kg), r and p value are from Spearman's correlation coefficient.

Table S6. Correlations among alpha diversity, short chain fatty acid and markers of gut barrier function at 3 weeks

	OTUs		Shannon		<i>Blautia</i> abundance	
	r	p value	r	p value	r	p value
Short chain fatty acid*						
Acetic acid	0.49	0.061	0.59	0.020	0.32	0.249
Propionic acid	0.63	0.011	0.61	0.017	0.36	0.185
Iso-butyric acid	-0.02	0.950	0.34	0.221	0.20	0.467
Butyric acid	0.51	0.050	0.63	0.011	0.57	0.027
Iso-valeric acid	0.18	0.534	0.31	0.259	0.22	0.423
Valeric acid	0.27	0.329	0.32	0.239	0.12	0.683
Total short chain fatty acid	0.61	0.016	0.66	0.007	0.47	0.078
Markers of gut barrier functioning						
I-FABP (pg/ml)	0.27	0.098	0.45	0.005	0.39	0.017
LPB (ng/ml)	-0.24	0.140	-0.35	0.032	-0.20	0.239
sCD14 (ng/ml)	-0.22	0.184	-0.35	0.034	-0.074	0.659

*Values are (mmol/kg), r and p value are from Spearman's correlation coefficient.

Table S7. Clinical characteristics at inclusion of the full cohort (n=117)

	Intervention (n=57)	Control (n=60)
Age years, median (range)	45 (19-65)	41 (18-62)
Female	20 (35)	25 (42)
Diagnosis		
AML	36 (63)	31 (51)
ALL	6 (10)	10 (17)
CML	2 (4)	7 (12)
CMML	3 (5)	3 (5)
aCML	1 (2)	-
MDS	6 (11)	5 (8)
APL	1 (2)	-
ABL	-	1 (2)
AUL	-	1 (2)
MCL	-	1 (2)
MF	2 (4)	-
MS	-	1 (2)
Donor		
HLA - identical sibling	17 (30)	13 (22)
HLA - identical unrelated	40 (70)	47 (78)
Stem-cell source		
Bone marrow	25 (44)	27 (45)
Peripheral - blood hematopoietic cells	32 (56)	33 (55)
Sex mismatch*	17 (30)	10 (17)
Conditioning		
Busulphan + Cyclophosphamide	56 (98)	56 (93)
TBI + Cyclophosphamide	1 (2)	4 (7)
HCTI - CI risk groups		
Low risk	42 (74)	45 (75)
Intermediate risk	8 (14)	10 (17)
High risk	7 (12)	5 (8)

Table S7. Clinical characteristics at inclusion of the full cohort (n=117)

	Intervention (n=57)	Control (n=60)
EBMT score		
0-3	33 (58)	36 (60)
4	14 (24)	14 (23)
5-7	10 (18)	10 (17)
Performance status ECOG		
0	55 (96)	54 (90)
1	2 (4)	6 (10)
BMI		
Underweight	2 (4)	4 (7)
Normal weight	31 (54)	27 (45)
Overweight	17 (30)	26 (43)
Moderately obese	4 (7)	3 (5)
Severely obese	3 (5)	0 (0)

Values are numbers (%) unless otherwise stated. AML Acute myeloid leukemia, ALL Acute lymphocytic leukemia, CML Chronic myeloid leukemia, CMML Chronic myelomonocytic leukemia, aCML atypical Chronic myeloid leukemia, MDS Myelodysplastic syndrome, APL Acute promyelocytic leukemia, ABL Acute basophilic leukemia, AUL Acute undifferentiated leukemia, MCL Mast cell leukemia, MF Myelofibrosis, MS Myeloid sarcoma, TBI Total body irradiation, HCTI-CI Hematopoietic Cell Transplantation-specific comorbidity index, EBMT score European Group for Blood and Marrow Transplantation score, ECOG Eastern Cooperative Oncology Group, * Sex mismatch was defined as female donor-to-male recipient.

Table S8. Markers of gut barrier function at baseline and at 3 weeks and difference from baseline to 3 weeks

	Baseline (n=114)	3 weeks (n=108)	Difference (n=105)	<i>p</i> value
I-FABP (pg/ml)	432 (60-2346)	214 (47-3820)	-199 (-2227-1003)	<0.001
LBP (ng/ml)	13695 (4410-31896)	18805 (4905-43176)	3788 (-9747-33473)	<0.001
sCD14 (ng/ml)	1745 (882-3214)	1803 (906-3011)	4 (-1454-1271)	0.444

Values are given as medians (range). *P* value from Wilcoxon signed rank test.

Table S9. Markers of gut barrier function between the two study groups at baseline and at 3 weeks

	Baseline			3 weeks		
	Intervention (n=55)	Control (n=59)		Intervention (n=53)	Control (n=55)	<i>p</i> value
I-FABP (pg/ml)	397 (122-1442)	473 (60-2346)		242 (47-3820)	204 (47-1373)	0.50
LBP (ng/ml)	13794 (4410-28500)	13389 (7098-31896)		18618 (4905-39149)	18618 (4905-39149)	0.82
sCD14 (ng/ml)	1651.9 (881.9-3213.8)	1820.6 (987.7-2842.6)		21750.5 (990.4-3010.5)	1829.7 (905.9-2853.3)	0.04

Values are given as medians (range), *p* value from Mann-Whitney U test.

Table S10. Markers of gut barrier function: Differences between baseline and 3 weeks between the groups

	Intervention (n=51)	Control (n=54)	<i>p</i> value
I-FABP (pg/ml)	-182 (-1395-841)	-207 (-2228-1003)	0.41
LBP (ng/ml)	2759 (-9747-25708)	4268 (-8818-33473)	0.92
sCD14 (ng/ml)	4.4 (-1453.9-1270.6)	9.9 (-964.7-1106.0)	0.77

Values are given as medians (range), *p* value from Mann-Whitney U test.

Fig. 1 The relationship between microbial diversity at baseline and aGVHD. **a** Observed OTUs and aGVHD, **b** Shannon diversity index and aGVHD, **c** *Blautia*, abundance and aGVHD. Data are observed operational taxonomic units (OTUs), Shannon diversity index and *Blautia* abundance given as box plots (median and interquartile range) with minimum and maximum values.

a

b

c

Fig. 2 The relationship between microbial diversity at 3 weeks and aGVHD. **a** Observed OTUs and aGVHD, **b** Reduction in observed OTUs and aGVHD, **c** Shannon diversity index and aGVHD, **d** *Blautia* abundance and aGVHD, **e** reduction in *Blautia* abundance and aGVHD. Data are observed operational taxonomic units (OTUs), Shannon diversity index and *Blautia* abundance given as box plots (median and interquartile range) with minimum and maximum values.

a

b

c

d

e

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