Institut für Biologische Chemie und Ernährungswissenschaft
Universität Hohenheim

Nutritional status and its impact on outcome in patients undergoing allogeneic haematopoietic cell transplantation and an experimental trial to improve the supply of a specific micronutrient

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You never change things by fighting the existing reality. To change something, build a new model that makes the existing model obsolete.

Buckminster Fuller (1895 - 1983)

Original research articles addressing this thesis

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Urbain P, Ihorst G, Biesalski HK, Bertz H (2012) Course of serum 25-hydroxyvitamin D3 status and its influencing factors in adults undergoing allogeneic hematopoietic cell transplantation. Ann Hematol. 91:759–766. www.springerlink.com/content/pg0243530j725631/ DOI: 10.1007/s00277-011-1365-2 [2010 impact factor 2.69]

Urbain P, Singler F, Ihorst G, Biesalski HK, Bertz H (2011) Bioavailability of vitamin D_2 from UV-B-irradiated button mushrooms in healthy adults deficient in serum 25-hydroxyvitamin D: a randomized controlled trial. Eur J Clin Nutr 65 (8):965-971. www.nature.com/ejcn/journal/v65/n8/full/ejcn201153a.html DOI: 10.1038/ejcn.2011.53 [2010 impact factor 2.56]

Ready for submission

Urbain P, Birlinger J, Ihorst G, Biesalski HK, Bertz H. Body mass index and bioelectrical impedance phase angle as potentially modifiable nutritional markers are independent risk factors for outcome in allogeneic haematopoietic cell transplantation. Estimated submission: December 2011

Urbain P, Birlinger J, Bertz H, Lambert C, Biesalski HK. Longitudinal follow-up of nutritional status and its influencing factors in patients undergoing allogeneic hematopoietic cell transplantation. Estimated submission: November 2011

Further publications and presentations

2010

Oral presentations at the 29th "Deutscher Krebskongress", February 24-27, Berlin, Germany.

> Urbain P, Renger S (2010) oncoMAT (Malnutrition Assessment and Therapy) PC-gestütztes Programm zur Erfassung und Beurteilung des Ernährungsstatus onkologischer Patienten. Abstract FV229. Onkologie; 33(suppl 2): P190
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> Urbain P (2010) Die Rolle der Versorgung mit Antioxidantien für das orale Mukositirisiko nach Hochdosis-Chemotherapie und allogener peripherer Blutstammzelltransplantation. Abstract PO216. Onkologie; 33(suppl 2): P200
>
> Urbain P (2010) Parameter des Ernährungsstatus (SGA, Phasenwinkel, Albumin, CRP, BMI) als prognostische Faktoren vor Hochdosis-Chemotherapie und allogener peripherer Blutstammzelltransplantation. Abstract PO221. Onkologie; 33(suppl 2): P201

Oral and poster presentations at the "Jahreskongress Ernährung 2010 Mitten in der Medizin", June 17-19, Leipzig, Germany.

> Urbain P (2010) Die Rolle der Versorgung der Mundschleimhaut mit Antioxidantien für das Mukositisrisiko bei der Chemotherapie. Vortrag über Ergebnissen des DGEM Förderpreis 2008
>
> Urbain P, Singler F, Biesalski H-K, Bertz H (2010) Erste Humanstudie zur Verbesserung des Serum 25-Hydroxyvitamin D-Status durch UVB-behandelte Pilze in gesunden Erwachsenen. P 2.8. Aktuel Ernahrungsmed; 35: P144

Oral presentation at the 32nd congress of Clinical Nutrition and Metabolism (ESPEN), September 5-8, Nice, France.

> Urbain P, Singler F, Ihorst G, Biesalski HK, Bertz H (2011) Bioavailability of vitamin D_2 from UV-B-irradiated button mushrooms in healthy adults deficient in serum 25-hydroxyvitamin: a randomized controlled trial. Abstract OP035. Clinical Nutrition Supplements, Vol. 5, Issue 2, P15

2011

Poster presentation at the 48th scientific congress DGE ("Deutsche Gesellschaft für Ernährung"), March 16-18, Potsdam/Berlin, Germany.

> Urbain P, Raynor A, Bertz H, Lambert C, Biesalski H-K (2011) Role of antioxidants in plasma and buccal mucosa cells in the occurence of oral mucositits after allogeneic haematopoetic cell transplantation. Abstract P11-8. Germ. Nutr. Soc., Vol. 15

Poster presentation at the 37th Annual Meeting of the European Group for Blood and Marrow Transplantation, April 3-6, Paris, France.

Bertz H, Urbain P, Birlinger J, Zuercher G, Finke J (2011) Nutritional status deteriorates during allogeneic haematopoetic cell transplantation. Abstract P682. EBMT Final Programme Book: P107

Poster presentations at the Multinational Association of Supportive Care in Cancer (MASCC) Congress, June 23-25, Athens, Greece.

Urbain P, Raynor A, Bertz H, Lambert C, Biesalski H-K (2011) Antioxidants in plasma and buccal mucosa cells in the incidence and severity of oral mucositis after allogeneic haematopoietic cell transplantation. Abstract 343. Support Care Cancer 19 (Suppl 2): P184

Poster presentations at the 33rd congress of Clinical Nutrition and Metabolism (ESPEN), September 3-6, Goteborg, Sweden.

Urbain P, Birlinger J, Biesalski H-K, Bertz H (2011) Course of serum 25-hydroxyvitamin D3 status and its influencing factors in adults undergoing allogeneic haematopoietic cell transplantation. Abstract PP079-MON. Clinical Nutrition Supplements, Vol. 6, Issue 1, P144-145

Birlinger J, Urbain P, Biesalski H-K, Bertz H (2011) Nutritional status and overall survival in patients undergoing allogeneic haematopoietic cell transplantation. Abstract PP076-SUN. Clinical Nutrition Supplements, Vol. 6, Issue 1, P52

Poster presentations at the DGHO (Deutsche Gesellschaft für Hämatologie und Onkologie) congress, September 30-October 4, Basel, Switzerland.

Urbain P, Raynor A, Lambert C, Bertz H, Biesalski H-K (2011) Role of antioxidants in plasma and buccal mucosa cells in the incidence and severity of oral mucositis after allogeneic haematopoietic cell transplantation. Abstract 897. Onkologie; 34(suppl 6): P270

Urbain P, Birlinger J, Biesalski H-K, Bertz H (2011) Nutritional status and overall survival in patients undergoing allogeneic haematopoietic cell transplantation. Abstract 899. Onkologie; 34(suppl 6): P271

Urbain P, Biesalski H-K, Bertz H (2011) Course of serum 25-hydroxyvitamin D3 status and its influencing factors in adults undergoing allogeneic haematopoietic cell transplantation. Abstract 900. Onkologie; 34(suppl 6): P272

Scientific awards and grants

2006

PhD grant (4 years) from the National Research Fund of Luxembourg to carry out this research.

2008

Young Scientist Award (15000 €) for financial support for materials and analytics issued by the German Society for Clinical Nutrition (DGEM e.V.) to conduct the clinical trial presented under 2.3.

2010

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2011

Young Scientist Oecotrophica prize (500 €) awarded by the "Verband der Oecotrophologen e.V." to Fabian Singler for his diploma thesis on the topic addressed in the trial presented under 2.5.

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Abbreviations

aGVHD	acute graft-versus-host diesease
ALL	acute lymphocytic leukemia
alloHCT	allogeneic haematopoietic cell transplantation
alloHZT	allogene hämatopetische Zelltransplantation
AML	acute myeloblastic leukemia
ANOVA	analysis of variance
AOX	antioxidants
BCNU	carmustine or bis-chloronitrosourea
BIA	bioelectrical impedance analysis
BMC	buccal mucosa cells
BMI	body mass index
CI	confidence interval
CLL	chronic lymphocytic leukemia
CML	chronic myeloid leukemia
CMV	cytomegalovirus
CP	chronic phase
CR	complete remission
CsA	cyclosporine A
EDTA	ethylenediaminetetraacetic acid
e.g.	exempli gratia - for example
FBM	fludarabine, BCNU, melphalan
FBTT	fludarabine, BCNU, thiotepa
FTT	fludarabine, thiotepa
GVHD	graft-versus-host disease

HCT	haematopoietic cell transplantation
HCT-CI	haematopoietic cell transplantation comorbidity index
HLA	human leukocyte antigen
HSV	herpes simplex virus
iPTH	intact parathyroid hormone
KPS	Karnofsky performance status
MCL	mantle cell lymphoma
MDS	myelodysplastic syndrome
MM	multiple myeloma
MPS	myeloproliferative syndrome
MTX	methotrexate
NHL	Non-Hodgkin’s lymphoma
NRM	non-relapse mortality
ns	not significant
25(OH)D	25-hydroxyvitamin D
$25(OH)D_2$	25-hydroxyergocalciferol
$25(OH)D_3$	25-hydroxycholecalciferol
OM	oral mucositis
OS	overall survival
PA	phase angle
PFS	progression-free survival
RA	refractory anemia
RARS	refractory anemia with ringsideroblasts.
RIA	radioimmunoassay
RM	relapse mortality

SD	standard deviation
SGA	Subjective Global Assessment
SGA-A	well nourished
SGA-B	moderately malnourished
SGA-C	severely malnourished
SPA	standardised phase angle
TRM	transplant-related mortality
UV	ultra violet
WBCs	white blood cells
WHO	World Health Organisation

1 Introduction

1.1 Background

Approximately 400 patients with oncological or haematological neoplasias admitted for chemotherapy at the University Medical Centre Freiburg were recruited in different but overlapping prospective observation surveys. The surveys all incorporated a detailed assessment of nutritional status including anthropometric measurements, diet history, life style, medical records, serum 25-hydroxyvitamin D_3, and antioxidative status in serum and buccal mucosa cells. The three consecutive assessment events took place at the start of each new chemotherapy cycle, or at main diagnostic time points in patients undergoing allogeneic haematopoietic cell transplantation (alloHCT).

Complete data collection resulted in part from supervising five diploma theses and one medical PhD. Some of the data were utilised to develop and validate oncoMAT® software, which is programmed for the evaluation and prediction of cancer patients' nutritional status.

On behalf of the present cumulative doctoral thesis, a homogeneous collective of 105 patients admitted for high-dose chemotherapy followed by alloHCT was selected, resulting in four original research articles. These publications focused mainly on the potential impacts of nutritional and antioxidative status on clinically-relevant, post-transplant outcomes.

In addition, an experimental and randomised controlled clinical trial with 27 healthy young volunteers was carried out, leading to a further original research article. That trial investigated a new approach with which to improve a specific aspect of nutritional status, namely vitamin D, which is known to be insufficient in the general population, most particularly in patients undergoing alloHCT.

1.2 Topic of this thesis

Allogeneic haematopoietic cell transplantation (alloHCT) is a risky but potentially curative treatment in patients with diseases of the blood and bone marrow or certain types of cancer [1]. However, this procedure can cause many complications ranging

from mucositis, infections and graft-versus-host disease (GVHD) to treatment- or progression-related mortality [2-4].

Nutritional status in alloHCT

AlloHCT-associated complications and their medical treatments often cause tremendous changes in body composition, especially through disturbed muscle metabolism [5,6]. There is a paucity of studies assessing nutritional status before alloHCT, most of which only recorded body mass index (BMI) as a potential risk factor for overall survival [7-10]. Except for one report by Kyle et al. [11] on the development of lean body mass, there was until now no longitudinal data on nutritional status and body composition in the early post-transplant period.

The main objective of this prospective study (2.2 on page 18) was to assess the nutritional status in patients before alloHCT and its course in the early post-transplant period until day +100 via weight changes prior to alloHCT, body mass index (BMI) normalized for gender and age [12], the Subjective Global Assessment (SGA) questionnaire [13], and phase angle normalized for gender, age and BMI [14]. This is the first and in-depth evaluation of the risk factors associated with nutritional status and body composition in patients undergoing alloHCT.

Nutritional status - impact on outcome

There are great research efforts now being made to identify risk factors for outcome in patients under consideration for alloHCT so as to improve the selection for this intensive treatment [15,16] and, in the case of potentially modifiable factors, to develop new strategies for improving individual outcomes. Beyond the well-known risk factors such as recipient age, remission status at alloHCT, donor status, conditioning regimen, HLA has been evaluated and identified as being of prognostic value in these patients [7-10].

We have available a large number of parameters assessing nutritional status and validated as risk factors in cancer patients for a multitude of clinical and economic outcomes ranging from quality of life, length of hospital stay and infections to overall survival [17-20]. However, the relevance of common nutritional parameters other than BMI as risk factors for outcome in patients undergoing alloHCT remains unknown.

The aim of this prospective analysis (2.1 on page 5) was to investigate the validity of the major nutritional parameters: BMI, SGA questionnaire and phase angle as inde-

pendent risk factors for outcome until two years after alloHCT, using a multivariate model analysis including a variety of accepted risk factors.

Antioxidative status - impact on the incidence oral mucositis

Oral mucositis (OM), a leading adverse effect of radio- and chemotherapy conditioning preceding alloHCT, remains a significant complication during the early post-transplant period [21]. Consequences of OM range from soreness and painful ulcerations to malnutrition and life-threatening infections, and it is associated with significantly worse clinical and economic outcomes [22,23]. Furthermore, there is no preventive or treatment regime for OM, and little is known of risk factors for its occurrence [24,25].

A major stimulus in the pathogenesis of OM is the induction of reactive oxygen species [24] which leads to tissue damage triggered by the tissue damage caused by chemotherapy and/or radiation [26,27]. In contrast, the endogenous antioxidative system and exogenous antioxidants (AOX) such as α-tocopherol (vitamin E), ascorbic acid (vitamin C), and ß-carotene scavenge free radicals and may thus reduce the incidence and severity of OM [28,29]. AOX are usually measured in plasma, but this does not necessarily provide evidence of the supply in the target tissue. Therefore, the determination of AOX status at the main site of action, namely the buccal mucosal cells, could be more reliable [28,30].

The study under 2.3 on page 29 is the first to have investigated the association between the incidence and severity of OM and AOX status in buccal mucosa cells and plasma before conditioning chemotherapy followed by alloHCT.

Vitamin D status and its influencing factors

In addition to the potential impact of nutritional and antioxidative status on clinically relevant outcomes in patients undergoing alloHCT, recent studies suggest that hypovitaminosis D (<30 ng/ml) at diagnosis may be associated with poorer prognosis in a wide range of solid malignancies [31-34]. Moreover, a recent study by Shanafelt el al. [35] provided the first actual evidence that hypovitaminosis D could be associated with worse outcome in patients with a haematological malignancy. A further health implication of vitamin D is the potential reduction in adverse effects of graft-versus-host disease (GVHD) based on vitamin D's immunomodulatory properties [36].

Hypovitaminosis D is highly prevalent in adult [37,38] and paediatric patients [39] undergoing alloHCT, but potential influencing factors of serum vitamin D status (25-hydroxyvitamin D) have only been reported in paediatric patients [39].

To deepen understanding and develop future preventive and interventional approaches, the main objective of the research article 2.4 on page 39 was to determine those factors exerting significant influence on vitamin D status before conditioning chemotherapy followed by alloHCT in adult patients. A further objective was to describe the impact of influencing factors on the course of serum 25(OH)D_3 during the early post-transplant period.

New approach for improving vitamin D supply

As already mentioned, hypovitaminosis D is highly prevalent in patients undergoing alloHCT [39,37,38] and a public health issue prevalent worldwide [40-43], particularly in regions with a large seasonal shift in solar altitude, as the major source of vitamin D for humans is sunlight-induced cutaneous synthesis [44,45]. Moreover, few foods contain vitamin D in noteworthy concentrations; those that do are fish-liver oils, fatty fish, and egg yolk. Mushrooms contain very little if any vitamin D_2, but they are abundant in ergosterol [46,47], which can be converted into vitamin D_2 via ultraviolet (UV) irradiation [48,49].

The randomised controlled trial under 2.5 on page 52 the first report on the bioavailability of vitamin D_2 from vitamin D_2-enhanced mushrooms by UV-B in humans. Hence the primary objective of this randomised controlled trial was to demonstrate the potential of improving the 25-hydroxyvitamin D status with this natural food source via a higher serum 25OHD concentration in young and healthy adults with low 25-hydroxyvitamin D status. A secondary objective was to compare the bioavailability of vitamin D_2 from UV-B treated mushrooms with a vitamin D_2 supplement.

2 Original research articles

2.1 Body mass index and bioelectrical impedance phase angle as potentially modifiable nutritional markers are independent risk factors for outcome in allogeneic haematopoietic cell transplantation

2.1.1 Introduction

Allogeneic haematopoietic cell transplantation (alloHCT) is a risky but potentially curative treatment that can cause many complications, ranging from mucositis, infections, and graft-versus-host disease (GVHD), to treatment- or progression-related mortality [2-4]. There are great research efforts being made to identify risk factors for outcome in patients being considered for alloHCT so as to improve the selection for this intensive treatment [15,16] and as regards potentially modifiable factors, to develop new strategies for improving individual outcomes. Besides the widely-accepted risk factors such as recipient age, remission at HCT, donor status, conditioning regimen, and HLA compatibility, only body mass index (BMI) - weight normalized for height and a broad marker of nutritional status - has been evaluated so far and identified as having prognostic value in these patients. Under- and overweight patients are at increased risk for complications, non-relapse mortality and overall survival after alloHCT [7-10].

A large number of parameters assessing nutritional status are currently available and validated as risk factors for cancer patients for a multitude of clinical and economic outcomes, ranging from quality of life, length of hospital stay, infections, to overall survival [17-20]. One example is the phase angle, which is measured by bioelectrical impedance analysis and reflects cell membrane function [50] and indirectly the body's muscle mass [51]. It is an established parameter for the diagnosis of malnutrition, and a useful prognostic outcome marker in many clinical conditions [52] including several types of cancer [53,54,18,55]. Another well-established prognostic parameter is the Subjective Global Assessment (SGA) questionnaire according to Detsky et al. [13], which has been proven to provide the best combination of sensitivity and specificity in identifying complications related to malnutrition [56] and sur-

vival [57], making this questionnaire a validated tool to evaluate nutritional status in cancer patients [20]. However, the relevance of common nutritional parameters apart from BMI as risk factors for outcome in patients undergoing alloHCT is still unknown.

This is the first prospective study to investigate the validity of several nutritional parameters [weight change before admission; adjusted BMI, normalised for gender and age; SGA questionnaire; and standardised phase angle, normalised for gender, age and BMI] as independent risk factors for outcome until two years after alloHCT, using a multivariate model analysis including a variety of widely-accepted risk factors.

2.1.2 Subjects and methods

Study population

Adult patients with haematological malignancies admitted for alloHCT to our institution at University Medical Centre Freiburg between 2008 and 2010 were eligible to enter this prospective survey. The study protocol was approved by our local Ethics Committee, and all patients provided informed consent.

Study design and data collection

Nutritional status at admission was assessed in 105 patients by measuring weight, performing bioelectrical impedance analysis, and assessing nutritional status classification according to the Subjective Global Assessment (SGA) questionnaire. In addition, weight changes over the previous six months and body height were recorded. All other medical and outcome data were collected from hospital records.

Adjusted body mass index (BMI). Considering the BMI's age and gender dependencies, we used current and representative German BMI percentiles to categorise each value as low (<10th percentile), normal (10-90th percentile) or high (>90th percentile) according to age- and gender-specific BMI percentiles [12].

Subjective Global Assessment (SGA). The SGA questionnaire according to Detsky et al. [13] is a simple, validated bedside tool in cancer patients with which to subjectively evaluate nutritional status. It focuses on weight-loss history, relevant symptoms impairing nutrition intake, and a basic physical examination, and requires no equipment. Patients were classified as well nourished (SGA-A), moderately malnour-

ished (SGA-B), and severely malnourished (SGA-C). The SGA questionnaire score has been shown to be associated with quality of life [17], length of hospital stay [19], and survival in cancer patients [57].

Standardised phase angle*.* Bioelectrical impedance analysis is a validated, non-invasive and portable method to estimate body composition with specific algorithms based on the electrical properties of body tissues [58]. Bioelectrical impedance analysis can be useful in assessing and monitoring nutritional status [50,59]. Phase angle, one of the raw data obtained from bioelectrical impedance analysis measurements and thus independent from the equipment and internal algorithms used, has proved to be a good prognostic, nutritional, and membrane cell function marker in various diseases [53,60,61,55]. Sex-, age-, and BMI-specific reference values of phase angle from a large healthy German population [14] were used to calculated the standardised phase angle according to the formula standardised phase angle = (phase angle - phase angle$_{ref}$)/SD$_{ref}$, where phase angle$_{ref}$ and SD$_{ref}$ are the age-, sex- and BMI-specific mean and standard deviations in a healthy population, respectively. Positive values are expected for healthy individuals [62]. The transformation of phase angle values into standardised values (Z-score) allows us to quantify individual deviations from sex-, age-, and BMI-specific population averages and to compare subjects in heterogeneous groups. In addition, Norman et al. [51] demonstrated that this standardisation clearly enhances the predictive power of phase angle. Our analysis was performed using a multifrequency device (Body Scout instrument, Fresenius Medical Care, Germany) under standardised conditions [63]. The cut-off value was defined as the lower quartile (25th percentile = -2.26) of the standardised phase angle in our study sample [extremely low (≤-2.26) vs. low/normal (>2.26) values].

Statistical analysis

We used IBM SPSS 19 (IBM, NY, USA) and SAS 9.2 (SAS Institute Inc., Cary, NC, USA) software for statistical analysis. Mean and standard deviations (SD) were used in case of approximately symmetric distributions, and when useful min-max as well. Overall survival (OS) was calculated as time from transplantation until death from any cause, and progression-free survival (PFS) was calculated as time from transplantation to the first observation of disease progression or death from any cause. If the event of interest was not observed, observation times were censored at the time of last contact with the patient. We focused on the analysis of OS and PFS within the

2 years after transplantation, therefore observation times were censored after 2 years at the latest. Patients who died without evidence of relapse or progression were considered as cases of non-relapse mortality (NRM). NRM and relapse mortality (RM) were considered as competing risks, and cumulative incidence rates were calculated to estimate the respective rates. Prognostic factors were analysed by means of Cox proportional hazards regression models. We conducted univariate analyses first. A multivariate model was then applied including those factors with univariate P <0.2 as an initial variable set and performing a variable selection procedure (backward elimination, retaining factors if P<0.1). For RM and NRM, Cox models were applied with censoring for the competing event, thus analysing the impact of prognostic factors on event-specific hazard rates. In addition to the four nutritional parameters, we included as potential risk and confounding factors the following variables in the Cox regression: recipient age and gender, donor age, gender and status, remission status at HCT, diagnosis, HLA compatibility (C; A, B, DRB), conditioning type, HCT-Comorbidity Index score, Karnofsky Performance Status (KPS), and cytomegalovirus (CMV) serology. The Kaplan-Meier method was used to estimate OS and the log rank test to evaluate the difference between the curves.

2.1.3 Results

Baseline and transplant characteristics of the 105 patients are summarised in Tab. 1. Mean time between baseline and alloHCT was 11.6 ± 6.7 days. The 62.9% male and 37.1% female patients had a mean age of 56.1 ± 10.9 (22-76) years. Diagnoses were acute leukaemias or other myeloid malignancies (76.2%; AML, MDS, ALL, CML, MPS) and lymphoid malignancies excluding ALL (23.8%; MM, NHL, CLL). Patients underwent reduced-intensity regimens [64], and peripheral blood stem cells were the graft in all transplantations with 76.2% having a volunteer unrelated donor.

Mean BMI was 25.9 ± 4.1 kg/m^2 , and according to age- and gender-specific percentiles, 22.9% and 8.6% of the patients had low (<10th percentiles) and high (>90th percentiles) BMI values, respectively. Most patients (77.1%) were classified as well nourished and few (9.5%) as severely malnourished according to the SGA questionnaire at admission. A low overall phase angle was detected in this cohort with mean standardised phase angle of -1.31 ± 1.25. The probability of having positive standardised phase angle values, above age-, gender- and BMI-specific reference values, was unexpectedly low (12.6%).

Tab. 1: Patient and transplant characteristics (n = 105)

Sex n (%)	
male	66 (62.9)
female	39 (37.1)
Age (years)	
mean ± SD	56.1 ± 10.9
min-max	22 – 76
Diagnosis n (%)	
AML, MDS, ALL, CML, MPS	80 (76.2)
MM, NHL, CLL, MCL	25 (23.8)
Conditioning regimens n (%)	
Fludarabine, BCNU, Melphalan (FBM)	66 (62.9)
Fludarabine, Thiotepa (FTT)	17 (16.2)
Fludarabine, BCNU, Thiotepa (FBTT)	8 (7.6)
Busulfan, Cyclophosphamide (BC)	8 (7,6)
other[1]	6 (5.7)
myeloablative	9 (8.6)
reduced intensity	96 (91.4)
Remission at transplant n (%)	
early disease (CR1, CP1, RA, RARS, NHL/MM with tandem-HCT, CLL ≤2 therapy regimes pre-HCT)	36 (34.3)
advanced disease (all other stages)	69 (65.7)
Donor status n (%)	
related	25 (23.8)
unrelated	80 (76.2)
Graft source n (%)	
peripheral blood stem cells	105 (100)
GVHD prophylaxis	
containing cyclosporine A (CsA)[2]	95 (90.5)
containing Certican®[3]	10 (9.5)
BMI (kg/m^2)	
mean ± SD	25.9 ± 4.1
min-max	16.5-35.0
Adjusted BMI n (%)	

<10th percentile	24 (22.9)
10th - 90th percentile	72 (68.5)
>90th percentile	9 (8.6)
SGA questionnaire n (%)	
A - well nourished	81 (77.1)
B - moderately malnourished	14 (18.4)
C - severely malnourished	10 (9.5)
Standardised phase angle n (%)	
mean ± SD	-1.31 ± 1.25
≥0	13 (12.6)
<0	92 (87.4)

Abbreviations: ALL = acute lymphocytic leukaemia, AML = acute myeloid leukaemia, BMI = body mass index, CLL = chronic lymphocytic leukaemia, CML = chronic myeloid leukaemia, CR1 = complete remission, CP1 = chronic phase, MCL = mantle cell lymphoma, MDS = myelodysplastic syndrome, MM = multiple myeloma, MPS = myeloproliferative syndrome, NHL = Non-Hodgkin's lymphoma, RA = refractory anaemia, RARS = refractory anaemia with ringsideroblasts, SD = standard deviation, SGA = Subjective Global Assessment. [1] Modified regimens: melphalan (n = 1), fludarabine + melphalan (n = 2), fludarabine + melphalan + thiotepa (n = 1), fludarabine + thiotepa + treosulfan (n = 1), and total body irradiation + etoposid + cyclophosphamide (n = 1). [2] CsA/Campath (n = 83); CsA mono (n = 1), CsA/MMF (Mycophenolate mofetil)/ATG (Anti thymocyte globulin) (n = 2); CsA/MMF (Mycophenolate mofetil)/Campath (n = 1); CsA/MTX (Methotrexate)/ATG (n = 4). [3] Myfortic/Certican (n = 9), Myfortic/Certican/Campath (n = 2).

Univariate and multivariate analysis of risk factors

Overall Survival (OS). The 2-year NRM, PFS and OS rates for all patients were 64%, 41% and 49%, respectively. Tab. 2 summarises the results of the univariate analysis for risk factors for 2-year OS. Nine of 17 investigated factors showed P <0.2 in the univariate analysis and formed the initial variable set for the multivariate Cox regression model (Tab. 2). These included adjusted BMI (low), standardised phase angle (≤-2.26), recipient age (>60 years), remission status (advanced disease), KPS (≤80), donor status (unrelated), CMV serology (recipient positive), HLA-C and -A, -B, -DRB statuses (incompatible). Multivariate analysis after backward elimination revealed that only an extremely low standardised phase angle (≤-2.26) (HR: 1.97; *P* =

0.043), unrelated donor (HR: 2.64; *P* = 0.039) and HLA-C incompatibility (HR: 2.13; *P* = 0.024) were significant independent risk factors for 2-year OS. Less significant associations with OS were found for low BMI (HR: 1.82; *P* = 0.09), older age (≥60 years) (HR: 1.78; *P* = 0.062) and advanced disease (HR: 1.97; *P* = 0.056). Patients classified as malnourished by the SGA questionnaire did not reveal reduced survival. Kaplan-Meier curves of survival in the entire follow-up period for standardised phase angle and adjusted BMI categories are shown in Fig. 1 and Fig. 2.

Non-relapse Mortality (NRM). The significant multivariate analysis results for all nutritional markers as independent risk factors for the analysed outcomes are summarised in Tab. 3 and all further significant factors are listed in the table's legend. Besides low BMI (HR: 2.90; P = 0.018) as the sole nutritional marker, only unrelated donor (HR: 5.36; P = 0.028) were significant and high BMI (HR: 3.02; P = 0.062), HLA-C (HR: 2.14; P = 0.069) and HLA-A, -B, -DRB (HR: 2.37; P = 0.074) disparities were modestly independent risk factors for 2-year NRM.

Relapse Mortality (RM). Multivariate analysis revealed that an extremely low standardised phase angle and advanced disease were associated with elevated HRs of 3.18 (*P* = 0.017) and 4.19 (*P* = 0.025) for increased 2-year RM, respectively.

Progression-free survival (PFS). Multivariate analysis revealed extremely low standardised phase angle (HR: 1.91; *P* = 0.039), advanced disease (HR: 2.46; *P* = 0.025) and HLA-C disparity (HR: 2.1; *P* = 0.019) to be significant risks factors for PFS. A trend toward significance was found for unrelated donor (HR: 2.15 *P* = 0.053).

Tab. 2: Pretransplant risk factors for 2-year OS after alloHCT (Cox regression analysis, n = 105)

Variable	Value	HR	95% CI	*P* value
Univariate Cox regression analysis				
Weight change over previous 6 months	loss	0.97	0.55-1.72	0.93
Adjusted BMI	low	1.75	0.93-3.33	0.08
	high	1.25	0.48-3.24	0.64
SGA questionnaire	malnourished (B, C)	1.38	0.73-2.61	0.32
Stand. phase angle	≤-2.26	1.74	0.93-3.26	0.08
Recipient age	≥60 years	2.04	1.15-3.61	0.01
Recipient gender	male	0.95	0.53-1.71	0.87
Donor age	>40 years	0.96	0.54-1.71	0.90
Recipient:donor	male:female	0.86	0.45-1.66	0.66
Diagnosis	lymphatic	0.78	0.39-1.58	0.50
Remission status	advanced disease	1.86	0.97-3.59	0.06
Donor status	unrelated	2.89	1.23-6.82	0.02
Karnofsky Performance Status	≤80	1.66	0.92-2.98	0.09
HLA-C	incompatible	2.07	1.11-3.87	0.02
HLA-A, -B, -DRB	incompatible	1.88	0.84-4.21	0.13
Conditioning[1]	reduced	not estimable	-	-
HCT-CI	≥3 points	0.99	0.56-1.76	0.98
CMV serology	recipient positive	0.65	0.36-1.14	0.13
Multivariate Cox regression analysis after backward elimination[2]				
Adjusted BMI	high	2.22	0.81-6.02	0.12
	low	1.82	0.91-3.65	0.09
Stand. phase angle	≤-2.26	1.97	1.02-3.81	0.043
Recipient age	≥60 years	1.78	0.97-3.28	0.062
Remission status	advanced disease	1.97	0.98-3.94	0.056
Donor status	unrelated	2.64	1.05-6.63	0.039

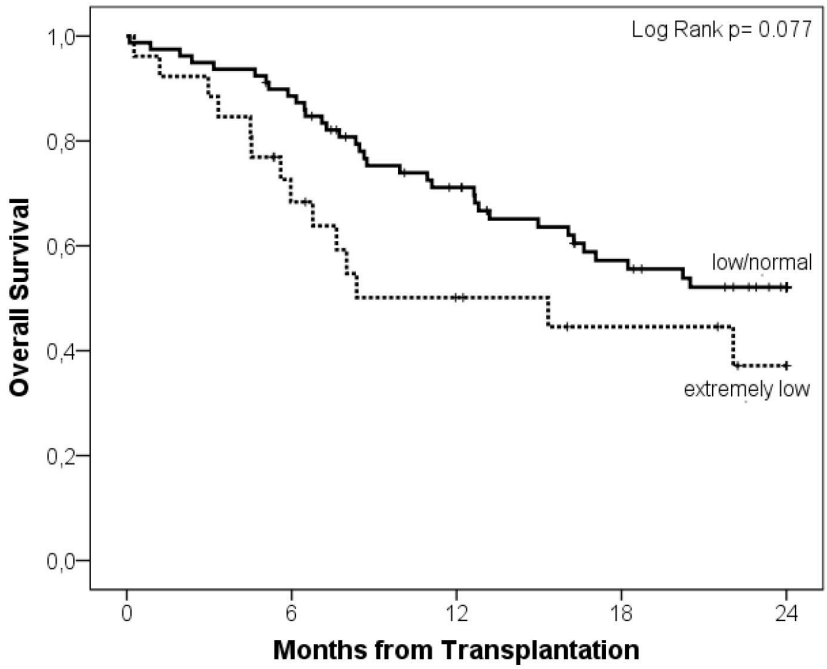
HLA-C	incompatible	2.13	1.11-4.10	0.024

Abbreviations: BMI = body mass index, CI = confidence interval, CMV = cytomegalovirus, HCT-CI = Haematopoietic Cell Transplantation Comorbidity Index, HLA = human leukocyte antigen, HR = hazard ratio, KPS = Karnofsky Performance Status. [1] No events occurred in the myeloablative group. [2] Multivariate model was applied, including those factors with univariate *P* <0.2 as an initial variable set and performing backward elimination, retaining factors if *P* <0.1.

Tab. 3: **Relevant results of multivariate Cox regression analysis of nutritional markers as risk factors for 2-year outcome (OS, NRM, RM, PFS) among patients undergoing alloHCT [n=105, HR (95% CI), *P* value]**

Variable	**OS[1]**	**NRM[2]**
adjusted BMI		
low	1.82 (0.91-3.66), 0.09[5]	2.90 (1.20-7.03), 0.018
high	-	3.02 (0.95-9.64), 0.06
SGA questionnaire		
malnourished (B, C)	-	-
Stand. phase angle		
extremely low (≤-2.26)	1.97 (1.02-3.81), 0.043	-
Variable	**RM[3]**	**PFS[4]**
adjusted BMI		
low	-	-
high	-	-
SGA questionnaire		
malnourished (B, C)	-	-
Stand. phase angle		
extremely low (≤-2.26)	3.18 (1.23-8.27), 0.017	1.91 (1.00-3.50), 0.039

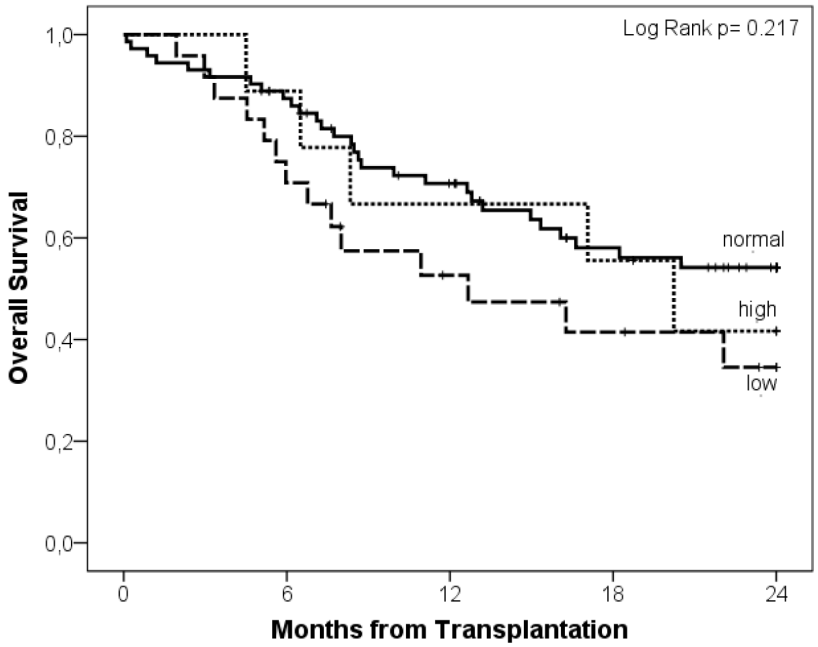
Abbreviations: CI = confidence interval, HR = hazard ratio, NRM = non-relapse mortality, OS = overall survival, PFS = progression-free survival, RM = relapse mortality, SGA = Subjective Global Assessment. [1] Detailed results are shown in Tab. 2. [2] Unrelated donor: 5.36 (1.20-24.04), 0.028; trend toward significance: HLA-C disparity: 2.14 (0.94-4.88), 0.069; HLA-A, -B, -DRB disparity: 2.37 (0.92-6.09), 0.074. [3] Advanced disease: 4.19 (1.19-14.71), 0.025. [4] Advanced disease: 2.46 (1.3-4.7), 0.006; HLA-C disparity: 2.1 (1.1-4.1), 0.019; trend toward significance: unrelated donor: 2.15 (1.13-4.09), 0.053.



Patients at Risk (n=)

low/normal	79	69	50	35	23
extremely low	26	16	10	7	3

Fig. 1: Survival distribution according to the standardised phase angle.



Patients at Risk (n=)

normal	72	60	44	30	21
high	9	7	6	5	3
low	24	17	10	7	4

Fig. 2: Survival distribution according to the adjusted BMI categories.

2.1.4 Discussion

The primary purpose of this study was to investigate nutritional markers as independent risk factors for major long-term outcomes after alloHCT. To the best of our knowledge, this is the first study to evaluate phase angle as a risk factor in these patients.

Patients with extremely low pretransplant standardised phase angles had a higher risk of death in the first 2 years after alloHCT. A reduced standardised phase angle was one of the few independent risk factors for 2-year OS besides unrelated donor and HLA-C incompatibility. Death was caused by relapse, as the patients with an extremely low phase angle had a risk of RM three times higher than those with higher phase angles. Furthermore, this parameter correlated significantly with PFS. The two most influential pretransplant risk factors identified in this study and associated with similarly increased HRs for three of four outcomes (OS, RM and PFS) were an extremely low standardised phase angle and advanced disease, respectively. The phase angle is one of the raw data from bioelectrical impedance analysis, a quick, non-invasive and safe method for evaluating a patients' body composition. It is known to be a valid independent prognostic factor for overall survival in patients receiving chemotherapy [53,54,18,51,60]. Reduced phase angle can be a result or the combination of either body cell mass loss (especially muscle mass via prolonged physical inactivity), cell membrane weakness, and increased body water displacement into extracellular space [65,51], which are the main characteristic changes triggered by malnutrition. Although the exact biological relevance of the phase angle remains unclear, previous studies report its association with physical functions such as handgrip strength, peak expiratory flow, and physical activity [65,66,51]. Considering that the most effective countermeasure against skeletal muscle atrophy is physical training combined with protein-rich nutrition [67-69], it is worth considering whether physical training together with the appropriate nutritional support before admission would improve both phase angles and outcomes after alloHCT in patients with reduced phase angles.

Of the other nutritional parameters considered, only underweight and overweight, according to age- and gender-specific BMI percentiles (≤10th and ≥90th percentile), demonstrated associations with increased risk of NRM that were not directly linked to the other risk factors considered here. Low BMI also correlated weakly with overall survival. Consistent with our results having observed increased NRM in patients un-

dergoing alloHCT with low BMI, Le Blanc et al. [8] demonstrated increased NRM in patients with BMI <20 kg/m^2. Similar outcomes also have been observed after autologous HCT in underweight adults [70,71]. The literature, however, is controversial about the relationship between BMI and outcome after alloHCT. Low BMI was found to be associated with decreased OS [72,8,73], decreased PFS [8], and later engraftment [74] after alloHCT. On the other hand, Fleming et al. [7] showed that high BMI was related to decreased OS (RM and NRM causes were equally responsible) in alloHCT. Contradictory to these results, Nikolousis et al. [10] reported that high BMI did not adversely affect either OS or PFS, showing however that high BMI had a significant impact on infection rates and duration of hospitalisation. One possible explanation for the worse outcomes observed in obese patients after conditioning chemotherapy followed by alloHCT is altered pharmacokinetics of chemotherapeutic agents compared to non-obese patients [70]. A principal pharmacokinetics parameter is volume of distribution in terms of the loading dose, which changes in the larger absolute lean body and fat masses in obese patients [75,76]. Furthermore, obesity may implicate impaired renal function [77,78] and hepatic drug metabolism due to liver damage induced by fatty infiltrations [79]. These factors may lead to the overdosage of chemotherapeutics in obese patients and thus a higher risk of NRM. On the other hand, undernutrition (low BMI) is known to be associated with many adverse outcomes [80], including depression of the immune system, which increases the likelihood of developing infections [81]. Le Blanc et al. [8] confirmed that alloHCT patients with low pretransplant BMI were affected by bacterial septicaemia significantly more often and required more granulocyte transfusions. However, Nikolousis et al. [10] reported results showing higher infection rates in overweight patients after alloHCT. Although the data from the literature are inconsistent and the precise pathophysiology of adverse outcomes in alloHCT patients with low or high body weight remains largely unknown, patients not falling within ideal weight ranges may be either at a higher risk of relapse mortality via insufficient dosage, or of NRM via drug toxicity.

Finally and surprisingly, we identified no association with any of the outcomes we investigated for the two main and most widely-used parameters in the screening and assessment of nutritional status (and valid predictors of worse outcome in cancer patients9, namely weight changes over the previous six months [82] and the SGA questionnaire [17,19,20]. This reveals that the significance of the phase angle and

BMI as risk factors is independent of weight dynamics before admission to transplantation.

A limitation of our study concerns the cut-off value defined as the lower quartile (25th percentile) of a standardised phase angle in our study sample, reducing the external validity of our results [62,60]. Therefore, Paiva et al. [83] propose using a cut-off value of the 5th percentile of a healthy reference population in studies. Our patients tended to present extremely low phase angle values at admission, making their proposed cut-off unusable for our analysis and obliging us to compare patients with extremely low values with those with low to normal values. A further limitation to consider has to do with our study's comparability with other studies - that we are the first to have categorised each BMI value in terms of age- and gender-specific BMI percentiles. We are convinced that this standardisation is the most suitable means of making heterogeneous cohorts comparable and of overriding the weakness of the fixed WHO classification, which fails to consider dynamic changes and gender-specific differences in body weight during the entire lifespan [12]. However, as all of the publications we have considered used different BMI risk categories, they are difficult to compare.

In summary, our results demonstrate for the first time that the pretransplant phase angle, here presented as a standardised value, was an independent predictor for 2-year OS, NRM and PFS in patients undergoing alloHCT. In line with other studies, BMI adjusted for age and gender emerged as an independent risk factor for OS and NRM. Both nutritional markers performed better than malnutrition according to the SGA questionnaire, weight history and numerous generally-accepted risk factors; they were the only significant prognostic values for outcome in our cohort beyond HLA-C compatibility, donor, and remission status.

Most of the many risk factors before alloHCT cannot be modified. On the other hand, BMI and phase angle are theoretically modifiable during the often lengthy treatment period before transplantation. Further investigation is necessary to demonstrate whether or not the phase angle can be increased by strategies that enhance muscle mass via physical training together with nutritional support, and whether this approach can lead to beneficial effects on outcome after alloHCT.

2.2 Longitudinal follow-up of nutritional status and its influencing factors in adults undergoing allogeneic haematopoietic cell transplantation

2.2.1 Introduction

AlloHCT is a potentially curative treatment in patients with diseases of the blood and bone marrow or certain types of cancer [1]. However alloHCT is a risky procedure that can cause many complications ranging from mucositis, infections, and graft-versus-host disease (GVHD) to treatment or progression-related mortality [2-4]. AlloHCT-associated complications and their medical treatments often cause tremendous changes in body composition, especially trough-disturbed muscle metabolism [5,6]. Furthermore, under- and overweight patients are at an increased risk increased risk for complications, non-relapse mortality, and overall survival after alloHCT [7-10].

To date, few studies have assessed nutritional status before alloHCT, and most of them only recorded body mass index (BMI) as a potential risk factor for survival [7-10]. Except for one report by Kyle et al. [11] on the development of lean body mass, there was until now no longitudinal data on nutritional status and body composition in the early post-transplant period.

There are now many nutritional parameters available and validated for cancer patients. The phase angle is one of them. It is measured by bioelectrical impedance analysis and reflects cell membrane function [50] and indirectly, the body's muscle mass [51]. It is an established parameter for diagnosing malnutrition, and a useful prognostic marker in many clinical conditions [52] including several types of cancer [53,54,18,55]. The Subjective Global Assessment (SGA) questionnaire according to Detsky et al. [13] is another means of demonstrating the best combination of sensitivity and specificity in identifying complications related to malnutrition [56], making the SGA questionnaire a validated tool in cancer patients with which to evaluate nutritional status [20].

The main objective of this prospective study was to assess the nutritional status in patients before alloHCT and its course during the early post-transplant period until day +100 via weight change before admission, body mass index (BMI) normalized

for gender and age, the SGA questionnaire, and the phase angle normalized for gender, age, and BMI. To deepen understanding and develop future preventive and interventional approaches, we also investigated the impact of influencing factors on the body weight course during the early post-transplant period.

2.2.2 Subjects and methods

Study population

Adult patients with haematological malignancies admitted for alloHCT to our institution at University Medical Centre Freiburg between 2008 and 2010 were eligible to enter this prospective survey. The study protocol was approved by our local Ethics Committee, and all patients provided informed consent.

Study design and data collection

Nutritional status at admission and on days +30 and +100 after alloHCT was assessed in 105 patients by measuring body weight, performing bioelectrical impedance analysis, and assessing nutritional status classification according to the SGA questionnaire. At admission we recorded weight changes in the previous six months, body height, HCT-Comorbidity Index (HCT-CI) [84] and Karnofsky Performance Status (KPS). Moreover, we assessed at each time point the actual portion size of food intake (100%, 75%, 50%, ≤25% of usual size) and relevant discomfort (anorexia, diarrhoea, taste disturbance, fatigue) graded as: no, mild, moderate or severe. Oral mucositis classified by the WHO oral toxicity scale [85] and infections as early toxicities and duration of parenteral nutrition were assessed until day +30. Maximum grade of acute graft-versus-host disease (aGVHD) (°0-°IV) and all further medical data were collected from hospital records. Nutritional interventions followed standard institutional clinical practice.

Adjusted body mass index (BMI): Considering the age and gender dependencies of BMI, we used current and representative German BMI percentiles to categorise each value as low (<10th percentile), normal (10-90th percentile) or high (>90th percentile) according to age- and gender-specific BMI percentiles [12].

Subjective Global Assessment (SGA): The SGA questionnaire according to Detsky et al. [13] is a simple, validated bedside tool for use in cancer patients with which to subjectively evaluate nutritional status, mainly according to weight-loss his-

tory, relevant symptoms impairing nutrition intake, and a basic physical examination requiring no equipment. Patients were classified as well nourished (SGA-A), moderately malnourished (SGA-B), and severely malnourished (SGA-C)., the SGA questionnaire classification has been shown to be associated to quality of life [17], length of hospital stay [19] and survival [57].

Standardised phase angle: Bioelectrical impedance analysis is a validated, non-invasive, portable method to estimate body composition with specific algorithms based on the electrical properties of body tissues [58]. Bioelectrical impedance analysis is useful in assessing and monitoring nutritional status [50,59]. The phase angle, one of the raw data obtained from bioelectrical impedance analysis measurements and therefore independent from the equipment and internal algorithms used, has proved to be a good prognostic, nutritional, and membrane cell function marker in various diseases [53,60,61,55]. Sex-, age-, and BMI-specific reference values of the phase angle from a large healthy German population [14] were used to calculated the standardised phase angle according to the formula standardised phase angle = (phase angle - phase angle$_{ref}$)/SD$_{ref}$, where phase angle$_{ref}$ and SD$_{ref}$ are the age-, sex- and BMI-specific mean and standard deviation of a healthy population, respectively. Positive values are expected for healthy individuals [62]. The transformation of phase angle values into standardised values (Z-score) allows us to quantify individual deviations from sex-, age-, and BMI-specific population averages and to compare subjects from a heterogeneous group. Furthermore, Norman et al. [51] showed that this standardisation clearly enhances the phase angle's predictive power. Our analysis employed a multifrequency device (Body Scout instrument, Fresenius Medical Care, Germany) under standardized conditions [63].

Statistical analysis

We used IBM SPSS 19 (IBM, NY, USA) and SAS 9.2 (SAS Institute Inc., Cary, NC, USA) software for statistical analysis. Mean and standard deviations (SD) were used in case of approximately symmetric distributions, and when useful min-max as well. Paired *t*-tests were used to analyze differences over time in continuous variables. To examine the impact of potential influencing factors on the body weight course from days +30 until day +100, we used linear regression models with change in BMI as the dependent variable. First, univariate models were employed for a preliminary assessment of single factors. Then, a multivariate model was constructed with those variables showing a univariate P <0.1. Results of the linear regression model are

presented as parameter estimates for the regression coefficients with accompanying 2-sided 95% confidence intervals (CI). For continuous independent variables (e.g. BMI at day +30), the regression coefficient represents the increase in serum 25(OH)D_3 concentration for a one unit increase in the respective continuous variable. For binary independent variables (all other variables), the regression coefficient describes the difference in BMI course between two groups.

2.2.3 Results

Patient and transplant characteristics

Baseline and transplant characteristics of the 105 patients are summarised in Tab. 1 on page 9. The 62.9% male and 37.1% female patients had a mean age of 56.1 ± 10.9 (22-76) years. The diagnoses were acute leukaemias or other myeloid malignancies (76.2%; AML, MDS, ALL, CML, MPS) and lymphoid malignancies excluding ALL (23.8%; MM, NHL, CLL). Patients were treated with reduced-intensity regimens [64] and peripheral blood stem cells were the graft in all transplantations with a volunteer unrelated donor in 76.2%. Advanced disease was diagnosed in 65.7% of the patients at transplantation. Mean time between baseline and alloHCT was 11.6 ± 6.7 days, day +30 was 28.6 ± 2.9 days, and day +100 was 99.4 ± 6.5 days after alloHCT.

Baseline nutritional status

Different aspects of baseline nutritional status are shown in Fig. 3. At admission, most (77.1%) of the patients were classified as well nourished and few (9.5%) as severely malnourished according to the SGA questionnaire. However, 23.8% of the patients reported significant weight loss during the last six months of 5-10% (10.5%) and >10% (13.3%), respectively. Mean BMI was 25.9 ± 4.1 (16.5-35.0) kg/m^2 and according to the WHO [86] 1.9% and 54.3% were classified as underweight (≤18.5 kg/m^2) and overweight (≥25 kg/m^2), respectively. In contrast, abnormal age- and sex-adjusted BMI values (<10^{th}, >90^{th} percentiles) were found in 31.5%, with 22.9% having low and 8.6% high values. An overall low phase angle was detected in this study population with a mean standardised phase angle of -1.31 ± 1.25. The probability of having positive standardised phase angle values, and

phase angle values above age-, gender- and BMI-specific reference values, was unexpectedly low (13.3%) in this cohort.

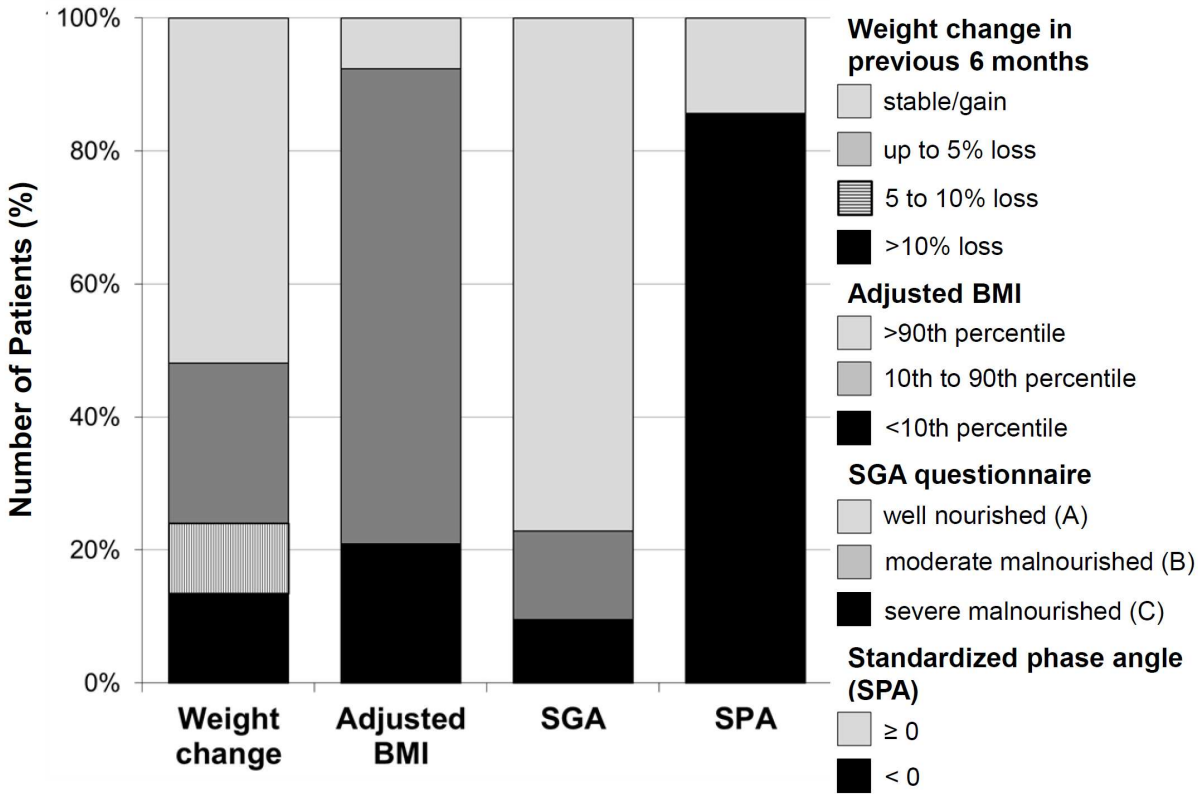


Fig. 3: Baseline nutritional status characteristics in 105 patients undergoing alloHCT just before conditioning chemotherapy started. Weight change over previous six months; age- and sex-adjusted BMI; Subjective Global Assessment (SGA) questionnaire and standardized phase angle (SPA) were classified.

Course of nutritional status

Longitudinal data (N = 78 patients) of weight, represented as BMI, and of standardised phase angle are shown in Fig. 4, revealing that BMI decreased significantly (P <0.0001) in both measured periods from 26.1 ± 3.8 kg/m^2 at admission to 23.2 ± 3.0 kg/m^2 at day +100 by 11% in total. In other words, this represents a total weight loss of 8.6 ± 5.7 kg during the study period. Simultaneously, the patients experienced an extraordinarily high 74.3% drop in the standardised phase angle during the first 40 days after admission (P <0.0001). We observed a trend towards fur-

ther standardised phase angle deterioration during the rehabilitation period after ward discharge till day +100 (P = 0.06).

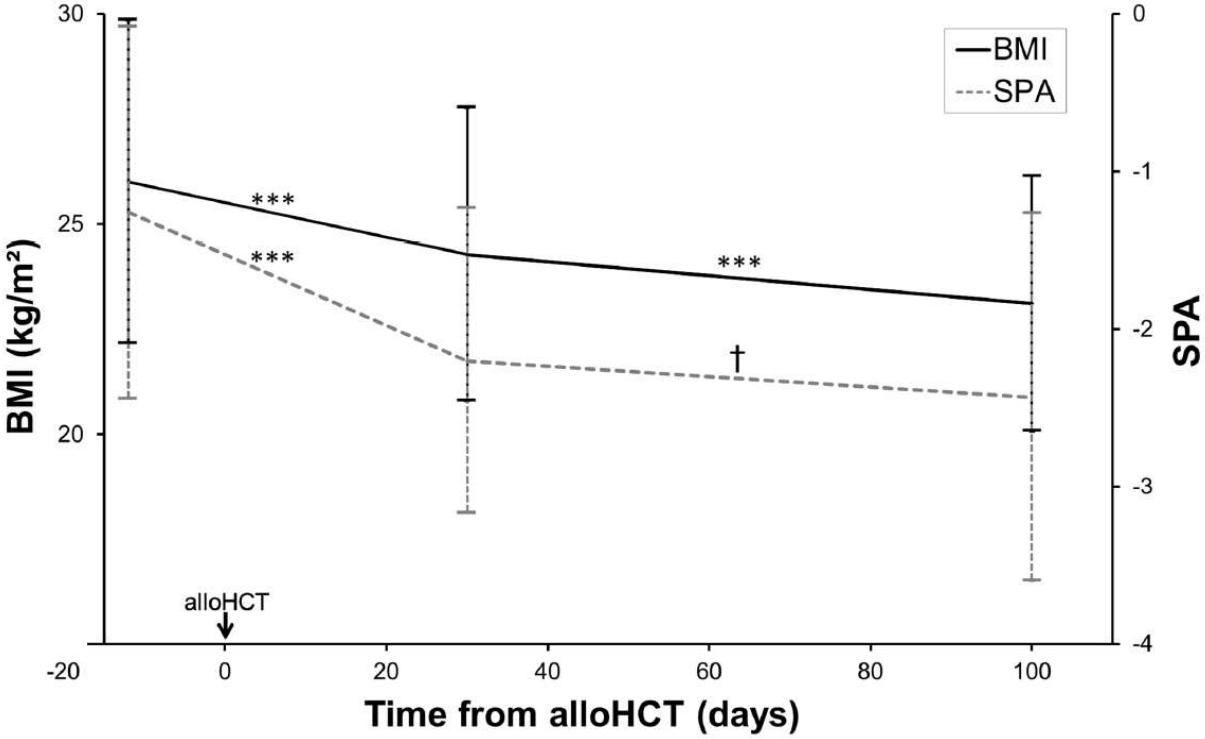


Fig. 4: Course of mean BMI (solid line) and standardised phase angle (SPA) (dashed line) during the study period (baseline, day +30 and day +100 after alloHCT). Figure represents data sets of 78 patients and error bars are +/- 1 SD. *** significant decrease P <0.0001. † nearly significant decrease P = 0.06 (paired t-test).

Impact of factors influencing the body weight course

As body weight loss remains significant during the rehabilitation period after ward discharge, we investigated the impact of a variety of potential influencing factors of body weight. We used change in body mass index (BMI), weight normalized for height, as the dependent variable. All the influencing factors for univariate analysis we considered are listed in Tab. 4. In univariate analysis, clinically-relevant aGVHD (P = 0.02) and anorexia (P = 0.02) were significantly associated with increased loss of BMI. A multivariate regression model was constructed with those variables showing a relationship (P <0.1) to BMI course. Only HCT-CI and BMI on day +30 were added in this model and after multivariable adjustment, clinically-relevant aGVHD

(parameter estimate -1.43; 95% CI: -2.61, -0.26; *P* = 0.02) and increasing grades of anorexia (parameter estimate -0.52; 95% CI: -0.99, -0.06; *P* = 0.03) were identified as independent influencing factors of BMI loss in the investigated period.

Tab. 4: Results of regression analysis investigating potential influencing factors of BMI course from day +30 to day +100 in patients undergoing alloHCT (n = 75)

Influencing factor	Unit	Parameter estimate (95% CI)	P value
Univariate analysis			
Recipient age	≥60 vs <60	-0.42 (-1.57, 0.72)	0.47
Recipient gender	male vs female	0.01 (-1.17, 1.18)	0.99
Diagnosis	lymphoid vs myeloid malignancies	-0.27 (-1.70, 1.15)	0.70
Donor status	unrelated vs related	1.26 (0.02, 2.51)	0.047
HCT-CI	high vs low risk	0.95 (-0.18, 2.08)	0.098
Conditioning chemotherapy	ablative vs reduced intensity	-0.72 (-2.52, 1.08)	0.43
HLA A, B, DRB	incompatible vs compatible	-1.15 (-3.16, 0.85)	0.25
Remission status at admission	advanced vs early disease	-0.31 (0.87, -1.49)	0.60
Karnofsky Performance Status	≤80 vs >80	-1.15 (-3.50, 1.19)	0.33
BMI at day +30	kg/m^2	-0.13 (-0.29, 0.02)	0.09
Serum CRP at day +30	mg/l	-0.01 (-0.04, 0.01)	0.29
aGVHD maximal grade till day +100	II°-IV° vs °0-°I	1.40 (0.23, 2.58)	0.02
Actual portion size of food intake at day +30	≤75% vs >75%	-0.14 (-0.67, 0.40)	0.61
Anorexia at day +30	grades 2-3 vs 0-1[1]	1.04 (-0.09, 2.18	0.07

Diarrhea at day +30	grades 2-3 vs 0-1	0.67 (-0.87, 2.22)	0.39
Taste disturbance at day +30	grades 2-3 vs 0-1	0.84 (-0.29, 1.99)	0.14
Fatigue at day +30	grades 2-3 vs 0-1	0.85 (-0.31, 2.02)	0.15
Multivariate analysis			
Donor status	unrelated vs related	0.76 (-0.50, 2.03)	0.23
HCT-CI	high vs low risk	0.91 (-0.17, 1.99)	0.10
BMI at day +30	kg/m^2	-0.10 (-0.25, 0.05)	0.19
aGVHD	II°-IV° vs °0-°I	1.46 (0.26, 2.66)	0.02
Anorexia	grades 2-3 vs 0-1	1.07 (-0.04, 2.18)	0.058

Abbreviations: aGVHD = acute graft-versus-host disease, BMI = body mass index, CI = confidence interval, CRP = C-reactive protein, HCT-CI = Hematopoietic Cell Transplantation Comorbidity Index, HLA = human leukocyte antigen. [1] grades 2-3 vs 0-1 = grades (moderate and severe) vs (no and mild)

2.2.4 Discussion

We herein present a longitudinal follow-up of nutritional status in adults undergoing alloHCT, and for the first time a multivariate linear regression model investigating the impact of factors on the course of body weight during the early rehabilitation period.

When adjusted BMI (78% in normal range) and the SGA questionnaire (77% well nourished) before alloHCT are considered, our findings on nutritional status in patients with haematological malignancies are consistent with other reports showing that the majority of these patients is well nourished [19,87,11,88]. However, approximately half of the patients experienced unintentional weight loss over the previous six months before alloHCT, revealing that changes in body composition were a frequent condition in these patients - a fact often overlooked when using BMI as sole tool in the determination of nutritional status.

According to the adjusted BMI classification, ten times more patients were classified as underweight according to age- and gender-specific percentiles than when referring to the standard WHO classification. The fixe WHO classification of e.g. normal weight (18.5-25 kg/m^2) does not consider the dynamic changes and gender-specific differences in body weight over the entire lifespan [12], nor does it take into account

that when considering all-cause mortality, optimal BMI varies by age and sex [89,90]. We therefore recommend that, when the BMI is used in the context of nutritional assessment, that it be employed to compare BMI calculations with age- and gender-specific BMI percentiles of an appropriate reference population.

Further, on closer inspection with bioelectrical impedance analysis measurements, we detected positive standardised phase angle values (expected in well-nourished, healthy individuals) in only 13.3% of our patients despite their overall normal BMI. This reveals that the phase angle, which is a valid nutritional marker and independent indicator of survival for patients receiving chemotherapy [53,54,18,51,60], was extremely low in this cohort. A reduced phase angle can be the result of or the combination of either body cell mass loss (especially muscle mass via prolonged physical inactivity), cell membrane weakness, and increased body water displacement into extracellular space [65,51], which are the main characteristic changes triggered by malnutrition.

Explanations for this completely different picture provided by the phase angle compared to BMI and the SGA questionnaire are that BMI is a limited measure of nutritional status, giving us only information on low or high fat mass; the SGA questionnaire's main classification criterion is weight change over the previous weeks. A major limitation of these quick tools (BMI, SGA questionnaire) is that relevant changes in body composition (e.g. loss of fat-free mass, mainly due to muscle atrophy) can be disguised by an increase in fat mass or fluid retention leading to stable overall weight [91]. This factor is highlighted by a small study in alloHCT patients showing significant fluid shifts to extracellular space, along with significant body cell mass loss till day +30, whereby body weight and fat mass remained constant [92].

Regarding changes in nutritional status during the early post-transplant period, we demonstrate convincingly that the losses of body weight and phase angle are especially high in the period between conditioning therapy until day +30 (a 40-day interval), and that they do not cease during the two months of rehabilitation after hospital discharge, although we did notice that this deterioration was somewhat alleviated. Lenssen et al. [9] reported that the frequency of depleted muscle reserves rose independently of weight changes during the first post-transplant year in alloHCT recipients. Moreover, Kyle et al. [11] even showed that any loss of weight and lean body mass experienced during the immediate post-transplant period (reflecting muscle mass amongst other factors) is not regained during the first year.

Possible explanatory models include persistent discomfort affecting oral intake and intestinal distress during that period, especially true in patients with chronic GVHD involving the oral mucosa [9], decline in physical activities for up to one year after transplantation [9], a negative energy balance due to hypermetabolism, and the catabolic effects on skeletal muscle exerted by medication with corticosteroids [5,6] as well as immunosuppressive drugs [93,94].

Summarising these results, changes in body weight are not sensitive enough to detect relevant changes in body composition, as the loss of fat-free mass is often masked by an excess of fat mass and/or extracellular fluid. This brings into focus the proposal that body composition measurements such as bioelectrical impedance analysis, muscular strength, and/or mid-arm muscle circumference are indicated for the assessment of nutritional status in these patients. Weight loss during the early post-transplant period from day +30 until day +100 rose significantly due to the severity of anorexia and aGVHD ≥°II, both predominant alloHCT-associated complications [95-97]. Depending on the organs affected by aGVHD (≥°II), it can alter nutritional status directly via food intake or nutrient uptake or indirectly via corticosteroid therapy, both of which have negative effects on muscle metabolism [5].

A limitation of this study relates to the lack of an exact record of the duration and quantity of corticosteroid medication during the study period, which is known to promote muscle atrophy and should therefore be included in future regression models.

In conclusion, we found as have others patients in good overall nutritional condition before alloHCT using quick screening tools for malnutrition such as BMI and the SGA questionnaire. However, upon closer inspection of their nutritional status, we observed unintentional weight loss before alloHCT to be a frequent condition, detecting many more underweight patients by using age- and gender-specific BMI classification, and overall low phase angle values at admission. The deterioration in our cohort's nutritional status was significant during the early post-transplant period, in spite of our clinic's internal standards of regular diet counselling, parenteral nutrition when needed, and high energy supplements ad libitum, combined with fast mobilisation and light physical activities. Furthermore, we identified anorexic patients and those with clinically-relevant aGVHD to be at an increased risk of decline in nutritional status during early rehabilitation. Beyond body weight measurements, our results reveal the key role of more reliable methods with which to identify those pa-

tients who need individually-adapted nutritional interventions and for monitoring the response to such treatment. Further studies are necessary to investigate new nutritional intervention strategies combined with physical training capable of preventing such deterioration in nutritional status during the early post-transplant period.

2.3 Role of antioxidants in buccal mucosa cells and plasma on the incidence and severity of oral mucositis after allogeneic haematopoietic cell transplantation

2.3.1 Introduction

Oral mucositis (OM) is an adverse effect of chemotherapy preceding HCT [21]. Consequences of OM range from soreness and painful ulcerations to malnutrition and life-threatening infections, and it is associated with significantly worse clinical and economic outcomes [22,23]. To date there is no preventative or treatment therapy for OM, and little is known of risk factors for its occurrence [24,25].

A major stimulus for the initiation of OM is the induction of reactive oxygen species [24] which result in tissue damage triggered by chemotherapy and/or radiation [26,27]. In addition to the endogenous antioxidative system, exogenous antioxidants (AOX) such as α-tocopherol (vitamin E), ascorbic acid (vitamin C), and ß-carotene scavenge free radicals and may therefore reduce incidence and severity of OM [28,29]. A small clinical trial in patients suffering from chemotherapy-induced OM showed that vitamin E applied locally induced a significantly more rapid resolution of lesions compared to the placebo group [98].

AOX are usually measured in plasma, but this does not necessarily provide evidence of the supply in the target tissue, because the plasma concentrations of several micronutrients are under homeostatic control. Therefore, the determination of their concentrations at the main site of action, namely the buccal mucosal cells (BMC) as in this study, could be more reliable [28,30].

To the best of our knowledge, this is the first report investigating prospectively the association between AOX status and the risk of developing OM in patients undergoing alloHCT after chemotherapy-containing conditioning regimens. Our primary objective was to assess the impact of plasma vitamin E status before alloHCT on the occurrence of ulcerative OM. Additional objectives included the influence of vitamin E concentrations on BMC, vitamin C and ß-carotene concentrations in BMC and plasma on the incidence and severity of OM. Furthermore, we investigated the influence of different maximum OM grades on the duration of parenteral nutrition and neutropenia,

the use of opioid analgesia, incidence of acute graft-versus-host disease (aGVHD) and herpes simplex virus (HSV) infections, respectively.

2.3.2 Patients and methods

Study population

This observational study began at the end of 2008 and adult patients with haematological malignancies admitted for alloHCT to our institution at University Medical Center Freiburg and receiving fludarabine-based, reduced-intensity conditioning regimens were eligible. The exclusion criterion was the presence of OM at baseline. The study protocol was approved by our local Ethics Committee, and all patients provided informed consent.

Study design and data collection

Samples of blood and BMC were collected at admission for assessing AOX status (vitamin E, C, and ß-carotene), and baseline characteristics were recorded. OM was assessed every second day, starting five days before alloHCT, and until discharge of the patients or day +30 after transplantation. We assessed OM daily only during the period in which OM is most likely to be maximal [22] (between days +4 and +10). To classify OM we used the WHO oral toxicity scale [85], which summarizes OM-related symptoms, signs, and functional disturbances on a five-point global scale. No or mild, ulcerative and severe OM were defined as WHO scale grades 0-I, II-IV and III-IV, respectively. Consistent quality of OM assessment was assured, as the three investigators conducting the study were supervised and trained by the same physician. In addition, the patients themselves estimated the intensity of OM-specific symptoms (dysphagia, diarrhoea, xerostomia and pain in the oral cavity) using a 4-point scale ranging from 0 (no symptoms) to 4 (severe symptoms).

All further data, including medical data, infections in the oral cavity, duration of parenteral nutrition, and maximum grade of oral/enteral aGVHD were collected from the hospital records. OM management followed standard institutional clinical practice, including oral care, topical antimicrobials, ice and analgesic support. Prophylaxis therapy consisted of an oral mouthwash with Salviathymol™ used 3 to times a day.

Sampling BMC and blood

BMC were collected using a kit from Day-med-concept GmbH, Berlin, Germany. Subjects first had to rinse their mouths with water thoroughly to remove food particles and then brush the inner lining of their cheeks with a soft toothbrush twenty times, twice on each side. The toothbrush was washed in 25 ml NaCl solution (0.9%) gently after each brushing. The samples were then centrifuged at 1600 rpm for 3 minutes. The supernatant was discarded; the cells were completely resuspended in rinsing solution (phosphor float 0.15% w/v) and centrifuged again at 1800 rpm for 3 minutes. After removal of the supernatant fraction, the stabilizing solution (heat-sensitive reducing agent 0.09% w/v) was added; and the cells were resuspended and stored.

The blood samples were taken into EDTA-Monovettes and stored in the dark for 10 minutes; the tubes were then centrifuged at 3000 rpm for 10 minutes at 4 °C. 200 µl from the resulting plasma were transferred into a 1.7 ml reagent tube with buffer solution (reducing agent) for vitamin C analysis and 500 µl in an empty reagent tube for vitamin E and ß-carotene analysis. All samples were frozen at -80 °C and shipped every 3 to 4 months on dry ice to the analyzing company. The AOX in BMC and plasma (pmol/µg DNA) of the samples were measured by BioTeSys GmbH (Esslingen, Germany) using an accredited RP-HPLC method according to DIN EN ISO/IEC 17025. The reference values are based on a statistical distribution reflecting the 25th and 75th percentile of a data set covering analysed BMC samples over one year.

Sample size calculation and statistics

This study was designed to detect a difference of 20% (6,1 µmol/L) in plasma vitamin E concentrations between the no or mild and ulcerative OM groups with a power of 80% with a two-sided t-test at a significance level of 5%. The assumed standard deviation was 9,3 µmol/L in the study population, derived from data on the vitamin E status of German adults [99]. No or mild OM and ulcerative OM groups were not expected to be equally large, as the mean ulcerative OM risk is 54% according to previous investigations. The expected ratio of no or mild to ulcerative OM groups was 0.85 and the resulting sample size was 76 patients in total. The duration of the study was scheduled for 14 months.

For descriptive data analyses, all values are presented as means ± SDs and if useful (min - max) when distributed normally or when not as median (min - max). Two

sample t-tests or Wilcoxon rang-sum tests were used for group comparisons of continuous variables distributed normally or not, respectively. Chi-square tests were used to analyze categorical variables between two groups. Pearson's correlation was calculated to assess the relation between variables.

For investigating the interactions of any one AOX in the antioxidative network, we compared patients with all plasma AOX in or above the normal range (AOX-sufficiency group) to patients with at least one suboptimal plasma AOX (AOX-inadequacy group) with regard to our outcome parameters. For statistical analysis we used SPSS version 16.0 (SPSS Inc, Chicago, IL, USA).

2.3.3 Results

Patient and transplant characteristics

After the defined study period, we enrolled 74 instead of the planned 76 patients. Four patients were excluded, two because the HCT had to be rescheduled, one patient died before finishing the study, and one required intensive medical care during the study and was no longer available. Baseline and transplant characteristics of the 70 evaluable patients are summarised in Tab. 5. The 47 (67%) male and 23 (33%) female patients with a mean age of 58.2 ± 10.2 (26-76) y had a mean body mass index (BMI) of 26.0 ± 4.0 (19-35) kg/m^2. The diagnoses were acute leukaemias or other myeloid malignancies (81.5%; AML, MDS, ALL, CML, MPS) and lymphoid malignancies excluding ALL (18.5%; MM, NHL, CLL, MCL).

Peripheral blood stem cells were the graft in all transplantations with a volunteer unrelated donor in 70%. All patients were treated with fludarabine-based, reduced-intensity regimens, mainly fludarabine, carmustine (BCNU) and melphalan (FBM) in 71.4% [64]. Sixteen (33%) of the patients received additional preconditioning chemotherapy because of high blast counts in peripheral blood or bone marrow. Advanced or active disease was diagnosed in 58 (82.9%) patients, and 12 (17.1%) were transplanted for early disease. Three (4.3%) had undergone one previous allogeneic and 9 (12.9%) one or more previous autologous HCT.

Tab. 5: Patient and transplant characteristics (n = 70)

Sex n (%)	
male	47 (67.0)
female	23 (33.0)
Age (years)	
mean ± SD	58.2 ± 10.2
median (min-max)	58.5 (26 – 76)
BMI (kg/m^2)	
mean ± SD	26.0 ± 4.0
median (min-max)	25.5 (19 - 35)
Diagnosis n (%)	
AML, MDS, ALL, CML, MPS	57 (81.5)
MM, NHL, CLL, MCL	13 (18.5)
Conditioning regimens n (%)	
Fludarabine, BCNU, Melphalan (FBM)	50 (71.4)
Fludarabine, Thiotepa (FTT)	9 (12.9)
Fludarabine, BCNU, Thiotepa (FBTT)	6 (8.6)
other[1]	5 (7.1)
Remission at transplant n (%)	
early disease (CR1, CP1, RA, RARS)	58 (82.9)
advanced disease (all other stages)	12 (17.1)
Donor status n (%)	
related	21 (30.0)
unrelated	49 (70.0)
Graft source n (%)	
peripheral blood stem cells	70 (100)
GVHD prophylaxis	
containing cyclosporine A (CsA)[2]	58 (82.9)
containing certican[3]	12 (17.1)

Abbreviations: ALL = acute lymphocytic leukaemia, AML = acute myeloid (myeloblastic) leukaemia, CLL = chronic lymphocytic leukaemia, CML = chronic myeloid leukaemia,

MCL = mantle cell lymphoma, MDS = myelodysplastic syndrome, MM = multiple myeloma, MPS = myeloproliferative syndrome, NHL = Non-Hodgkin's lymphoma. [1] Modified regimens: fludarabine and melphalan twice, fludarabine once, melphalan and thiotepa, fludarabine and thiotepa once and only melphalan once. [2] CsA/Campath 53 (75.7%); CsA mono 1 (1.4%), CsA/MMF (Mycophenolate mofetil)/ATG (Anti thymocyte globulin) 2 (2.9%);CsA/MTX (Methotrexate)/ATG 2 (2.9%). [3] Myfortic/Certican 9 (12.9%), Myfortic/Certican/Campath 2 (2.9%), Certican mono 1 (1.4%)

AOX status in plasma and BMC

Tab. 6: AOX concentrations in plasma and BMC at baseline and normal ranges

AOX concentrations	N	Mean ± SD	(min – max)	Normal range
Plasma (µmol/L)				
vitamin E	70	31.7 ± 14.9	(12.3 – 91.1)	15 – 40
vitamin C	70	42.3 ± 26.2	(3.7 – 101.3)	30 – 80
ß-carotene	70	0.5 ± 0.5	(0.0 – 2.9)	0.2 – 1.0
BMC (pmol/µg DNA)				
vitamin E	69	27.4 ± 12.5	(3.2 – 61.1)	9.5 – 20.3
vitamin C	47	4.5 ± 4.5	(0 – 16)	3.9 – 11.1
ß-carotene	63	0.4 ± 0.5	(0 – 2.5)	0.1 – 0.5

The mean BMC concentrations of vitamin E, vitamin C and ß-carotene per µg DNA were 27.4 ± 12.5 pmol, 4.5 ± 4.5 pmol, and 0.4 ± 0.5 pmol, respectively. Given the reference values (Tab. 6), 4/69 (5.8%), 28/47 (59.6%), 6/63 (9.5%) of the patients had inadequate BMC concentrations of vitamin E, vitamin C and ß-carotene, respectively.

Baseline AOX concentrations were measured in blood plasma in all patients and BMC in 69/70 of the patients due to insufficient cell numbers. Vitamin C and ß-carotene concentrations were not detectable in 23/69 and 7/69 of the BMC samples, respectively. The mean plasma concentrations at baseline of vitamin E, vitamin C and ß-carotene were 31.7 ± 14.9 µmol/L, 42.3 ± 26.2 µmol/L and 0.5 ± 0.5 µmol/L, re-

spectively. Plasma concentrations of vitamin E, vitamin C and ß-carotene were beneath normal range in 3 (4.3%), 26 (37.1%) and 22 (31.4%) of the patients, respectively (Tab. 6). We found significantly high positive correlations between the AOX concentrations in blood plasma and BMC for vitamin E (r^2 = 0.096, P = 0.009) and ß-carotene (r^2 = 0.823, P <0.001), visualized in Fig. 5, although not for vitamin C.

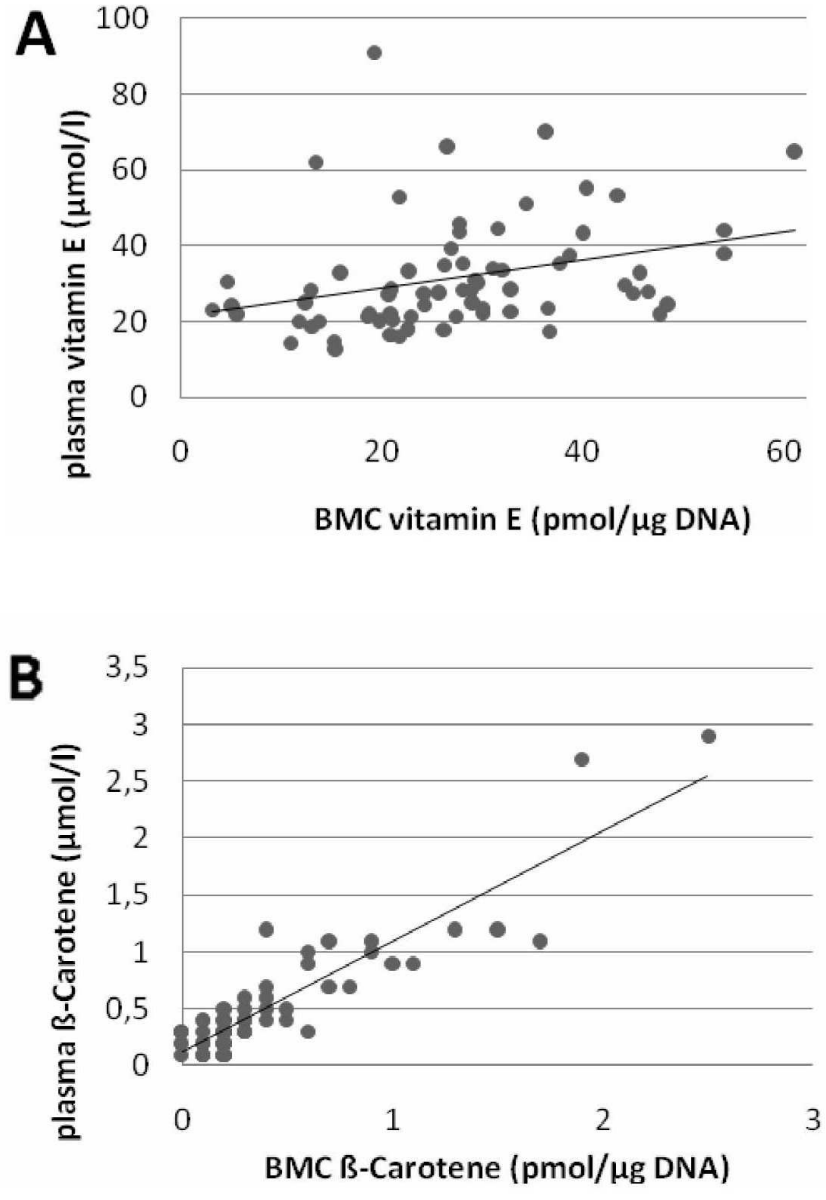


Fig. 5: Relation between the plasma and BMC concentrations of vitamin E and ß-carotene before conditioning chemotherapy. Simple linear regression was used to assess relations. **A**: n = 69, r^2 = 0.096, *P* = 0.009; **B**: n = 63, r^2 = 0.823, *P* <0.001

2.3.4 Discussion

Based on the hypothesis that oxidative tissue damage triggered by chemotherapy plays an eminent role in the pathogenesis of OM [26,27], we investigated the influence of the exogenous AOX status, mainly plasma vitamin E before conditioning chemotherapy on the incidence and severity of OM. Consistent with the observation in an earlier study [100], we observed no significant differences in plasma vitamin E status in this study population shortly before the conditioning therapy among patients suffering from different OM grades after alloHCT. We are the first to analyse the plasma and buccal mucosal tissue status of AOX in patients undergoing reduced-intensity conditioning regimens prior to alloHCT. However, we observed no single AOX concentration in plasma or BMC at the actual site of action to demonstrate a predictive value for the incidence or severity of OM. However, more than half of the patients were deficient in at least one plasma AOX (AOX-inadequacy group) and those tended to require longer parenteral nutrition, which is a relevant clinical and economic marker for OM treatment [24,23], than patients with all plasma AOX concentrations in or above the normal range - a finding that should be considered exploratory.

On average, our patients experienced their maximum OM grade on day 12 after the start of conditioning, consistent with the time point (on day 13) reported by Blijlevens et al. [22] In accordance with Horsely et al. [101], Blijlevens et al. [22] reported an incidence of ulcerative and severe OM in 64% and 44% of their patients receiving high-dose melphalan and BEAM conditioning chemotherapy followed by autologous HCT, respectively, whereas we report a markedly less frequent occurrence of ulcerative OM (20%). No or mild OM (°0-°I) was the most common diagnosis (54%) in our study population, which is perhaps due to the reduced-intensity conditioning regimens used [102]. Fludarabine-based, reduced-intensity conditioning regimens have been developed to provide a potentially curative treatment with reduced toxicity, specially for older and comorbid patients [64] or for those undergoing a second alloHCT [103,104]. Furthermore, Slavin et al. [105] showed that these conditioning regimens can reduce treatment-related toxicity including OM. Studies showing much higher incidences of ulcerative OM in patients undergoing HCT employed high-dose conditioning regimens sometimes containing total body irradiation [106,25,104]. No difference in maximum OM grades was detected for our different conditioning regimens (FBM, FTT and FBTT).

This study confirmed that patients with severe OM experienced a significantly higher incidence of a clinically-relevant aGVHD grade II-IV [107] and needed significantly longer parenteral nutrition [23] than patients with no or mild OM.

Consistent with our observation of overall adequate plasma vitamin E status (except in 4.3% of our patients), earlier studies reported similar circulating vitamin E concentrations prior to conditioning chemotherapy [108,100,109,110] and in a large group of patients with haematological malignancies [111], respectively. At admission, mean plasma vitamin C and ß-carotene concentrations were within normal ranges, in accordance with other studies [109,112,110]. However, around one third of our patients presented reduced plasma concentrations of vitamin C and ß-carotene, respectively.

Up to now, very few studies have investigated AOX status in BMC in the general population [113,114], let alone in patients undergoing alloHCT. Using the reference values provided by our analysing laboratory, few patients (6%) showed reduced vitamin E status in BMC. Inadequate concentrations of vitamin C and ß-carotene were found in 60% and 10% of the evaluable samples, respectively. The high incidence of low vitamin C concentrations in BMC may be due to a methodical error in the sample processing. In line with others [115,113,30], we found significantly high positive correlations between the AOX concentrations in blood plasma and BMC for vitamin E and ß-carotene.

A limitation of this study relates to the very low incidence of inadequate plasma vitamin E concentrations, while detecting a difference in patients with different OM grades was impeded. A further limitation is that the incidence of ulcerative OM was lower than expected. Reports on plasma vitamin E [116,109,117], ß-carotene [116,109] and overall AOX capacity [112,117] courses after high-dose chemotherapy followed by HCT showed significant decreases even when the patients received the recommended dietary allowances of vitamin E by total parenteral nutrition [109,117]. This implies that data on AOX status over the brief period after chemotherapy and before HCT may well be more reliable in providing further evidence of the role of a good AOX supply for OM prophylaxis.

A recent review by Block et al. [118] provided evidence that AOX supplementation during chemotherapy holds potential for reducing dose-limiting toxicities [119,120,98], however several studies reported that AOX might protect tumour cells and reduce treatment efficiency [121,122]. From this follows that well-designed ran-

domised trials investigating the cytoprotective properties of AOX in the cancer therapy-induced pathogenesis of OM should therefore administer AOX in a safe way to avoid impairing treatment efficacy. A safe and efficient way to improve the cellular supply in the buccal mucosa is the topical application of AOX via a rinsing solution [28,122,98].

In conclusion, no single AOX, either in plasma or BMC (vitamin E, vitamin C and ß-carotene), revealed predictive value for the incidence or severity of OM. Patients with an overall good plasma AOX status tended to require less parenteral nutrition, a common clinical marker for OM. We hypothesize that an intact antioxidative network may be more relevant than any one AOX to reduce the OM risk. Future studies should consider both the exogenous- and endogenous AOX systems.

2.4 Course of serum 25-hydroxyvitamin D_3 status and its influencing factors in adults undergoing allogeneic haematopoietic cell transplantation

2.4.1 Introduction

Vitamin D has biological effects far beyond its hormonal activity in maintaining calcium homeostasis [123], including immunomodulatory activity [124,125], regulating angiogenesis [126] and cellular differentiation [127], proliferation [128], and apoptosis [129] of both normal and malignant cells. Current studies suggest that hypovitaminosis D at diagnosis may be associated with poorer prognosis in a wide range of solid malignancies [31-34]. A study by Shanafelt el al. [35] recently provided the first direct evidence that low serum 25-hydroxyvitamin D_3 [25(OH)D_3] concentrations may be associated with poorer overall survival (OS) in haematological malignancies. Moreover, osteoporosis, a common, relevant complication in recipients of alloHCT, is one of the factors linked to immunosuppressive therapy and reduced serum 25(OH)D_3 concentrations [130,38,131]. Furthermore, vitamin D analogs alleviate graft-versus-host disease (GVHD) in cell cultures [36] and rats [132,133]. Thus, correcting vitamin D deficiency and maintaining adequate vitamin D status before and after alloHCT may improve short- and long-term outcome parameters.

Two studies with small numbers of patients have thus far assessed vitamin D status, revealing that vitamin D deficiency is highly prevalent in adult patients undergoing alloHCT [37,38], while one study showed 25(OH)D_3 concentrations within the lower normal range [134].

So far, only potential influencing factors on post-transplant vitamin D status at a mean time since HCT of 4.4 months and 4.2 years were investigated in paediatric patients [39] and paediatric and adult patients [135], respectively. To deepen understanding and develop future preventive and interventional approaches, the main objective of this study was to first determine those factors exerting significant influence on serum 25(OH)D_3 status before conditioning chemotherapy followed by alloHCT in adult patients. In addition, we are the first to describe the impact of influencing factors on the course of serum 25(OH)D_3 in the early post-transplant period until day +100.

2.4.2 Patients and methods

Study population

Adult patients with haematological malignancies admitted to our institution (the University Medical Centre Freiburg) between 2008 and 2010 for an alloHCT were eligible to enter this prospective survey. The study protocol was approved by our local Ethics Committee, and all patients provided informed consent.

Study design and data collection

Data were gathered at three time points: admission (baseline), day +30, and day +100 after alloHCT. At each time point, we assessed serum 25(OH)D_3 concentrations and if appropriate, factors potentially influencing serum 25(OH)D_3. All further (including medical) data were collected from hospital records. Nutritional interventions followed standard institutional clinical practice, including parenteral nutrition, dietary counselling, and prescription of a multivitamin supplement (vitamin D free) until day +100. Serum samples were collected routinely in the early morning at each time point. Our Department of Clinical Chemistry (certified according to ISO 9001) used an electrochemiluminescence immunoassay with specificity only to 25(OH)D_3. Hypovitaminosis D was defined as a serum 25(OH)D_3 concentration <30 ng/ml (75 nmol/L) and the lower limit of analytical determination was 4 ng/ml.

Factors potentially influencing serum 25(OH)D_3

Outdoor activities*:* As the major source of vitamin D in humans is sunlight-induced cutaneous vitamin D_3 synthesis, we assessed at baseline and day +100 the patients' sun exposure habits and time per day usually spent outdoors during the last month. Mean daily outdoor activity was graded as: outdoors <1 h, low, 1-3 h moderate, and >3 h high.

Season: Considering the seasonal fluctuation of UV-B irradiation, we divided the year in one period from April through September (the "increasing" season), when sun exposure at 48° north latitude suffices for cutaneous vitamin D synthesis [136] and a period from October through March (the "decreasing" season), when UV-B energy is low, leading to a continuous drop in serum 25(OH)D_3.

Dietary and supplementary vitamin D intake: We interviewed the patients at baseline and day +100 about their dietary habits in the last month using a short, semiquantitative food-frequency questionnaire including all food groups containing vitamin D_3 in noteworthy concentrations, i.e. fish, eggs and dairy products. In this dietary interview, patients were asked about their use of vitamin D_3 supplements and frequency of intake. We categorized the mean vitamin D_3 content of these foods, usual serving sizes, and frequency of consumption as low, medium, or high due to their potential impact on serum 25(OH)D_3: fish (≤1, 2-3, ≥4 times monthly), eggs (≤1, 2-3, ≥4 times weekly), dairy products (≤3, 4-6, ≥7 times weekly). Although mushrooms are a good source of vitamin D_2, we disregarded their consumption because our analytical method was not sensitive to serum 25(OH)D_2.

Parenteral nutrition: As parenteral nutrition (when supplemented by lipid-soluble vitamins) is an important source of vitamin D during the very early post-transplant period during hospitalization, we recorded the duration of parenteral nutrition and administration of lipid-soluble vitamins (200 IU of vitamin D_3/d).

Body fat mass*:* To investigate the influence of body fat on serum 25(OH)D_3, we used the fat mass in percent of body weight measured by bioelectrical impedance analysis (BIA). The BIA instrument used was a multifrequency device (Body Scout instrument, Nutritional Management Tool software version 2.0, Fresenius Medical Care, Germany) and conditions were standardized when measuring [63].

We also assessed age, gender, body mass index (BMI) and the Karnofsky performance status (KPS). We documented the maximum grade of acute graft-versus-host disease (aGVHD) (°0-°IV) as a factor potentially influencing the serum 25(OH)D_3 course according the criteria of Przepiorka et al. [137].

Statistical analysis

We used IBM SPSS 19 for statistical analysis (IBM, NY, USA). For descriptive data analyzes, all values are presented as means ± SDs and when useful (min - max). Paired *t*-tests were used to analyze differences over time in continuous variables. Two sample t-tests were employed for group comparisons. To examine the impact of potentially influencing factors for serum 25(OH)D_3 at transplantation, we used linear regression models with serum 25(OH)D_3 concentrations as the dependent variable. First, univariate models were employed for a preliminary assessment of single factors. Then, a multivariate model was constructed with those variables showing a uni-

variate P <0.1. Results of the linear regression models are presented as parameter estimates for the regression coefficients with accompanying 2sided 95% confidence intervals (CI). For continuous independent variables (e.g. age, BMI, fat mass), the regression coefficient represents the increase in serum 25(OH)D_3 concentration for a one-unit increase in the respective continuous variable. For binary independent variables (all other variables), the regression coefficient describes the difference in serum 25(OH)D_3 concentrations between two groups.

2.4.3 Results

Patient and transplant characteristics

Baseline and transplant characteristics of the 102 Caucasian adults are summarized in Tab. 7. The 37% female and 63% male patients with a mean age of 56 ± 11 years (range: 22-76) had a mean BMI of 25.9 ± 4.1 kg/m^2. Diagnoses were acute leukemias or other myeloid malignancies (76.5% AML, ALL, CML, MDS, MPS) and lymphoid malignancies excluding ALL (23.5%; NHL, MM, CLL, MCL). Peripheral blood stem cells were the graft in all transplantations. Seventy-three patients (71.6%) had advanced or active disease; 29 (28.4%) patients with early disease were transplanted.

By day +30, three patients had dropped out, as did another 11 patients by day +100 due to death or poor general condition. We had no data from day +30 from six patients, mainly because of early discharge. Mean time between baseline and alloHCT was 11.6 ± 6.7 days, whereas day +30 was 28.6 ± 2.9 days, and day +100 was 99.4 ± 6.5 days after alloHCT, respectively.

Tab. 7: Patient, transplant characteristics and baseline serum 25(OH)D_3 (n = 102)

Gender n (%)	
male	64 (63.0)
female	38 (37.0)
Age (years)	
mean ± SD	56 ± 11
min-max	22-76

BMI (kg/m^2)	
mean ± SD	25.9 ± 4.1
Diagnosis n (%)	
AML, MDS, ALL, CML, MPS	78 (76.5)
MM, NHL, CLL, MCL	24 (23.5)
Conditioning regimens n (%)	
reduced-intensity (fludarabine-based) [64]	93 (91.2)
myeloablative	9 (8.8)
Remission at transplant n (%)	
early disease (CR1, CP1, RA, RARS)	29 (28.4)
advanced disease (all other stages)	73 (71.6)
Donor status n (%)	
related	24 (23.5)
unrelated	78 (76.5)
Graft source n (%)	
peripheral blood stem cells	102 (100%)
GVHD prophylaxis	
containing cyclosporine A	89 (87.2)
containing certican	13 (12.8)
Serum 25(OH)D_3 (ng/ml) n (%)	
mean ± SD	16.4 ± 8.9
min-max	4-47
sufficient (≥30 ng/ml)	11 (10.8)
insufficient (10-30 ng/ml)	67 (65.7)
deficient (≤10 ng/ml)	24 (23.5)

Abbreviations: ALL = acute lymphocytic leukemia, AML = acute myeloid leukemia, BMI = body mass index, CLL = chronic lymphocytic leukemia, CML = chronic myeloid leukemia, CR1 = complete remission, CP1 = chronic phase, MCL = mantle cell lymphoma, MDS = myelodysplastic syndrome, MM = multiple myeloma, MPS = myeloproliferative syndrome, NHL = Non-Hodgkin's lymphoma, RA = refractory anemia, RARS = refractory anemia with ringsideroblasts, SD = standard deviation.

Serum 25(OH)D_3 concentrations

The mean serum 25(OH)D_3 concentration was 16.4 ± 8.9 ng/ml (range: 4-47) at admission, thus most our study cohort 90/102 (89.2%) had a status under the normal range (30-70 ng/ml), whereby 23.5% presented extremely low concentrations (<10 ng/ml) (Tab. 7). When evaluating the serum 25(OH)D_3 course during the study period, we only considered the 67 patients from whom we had complete data records of 25(OH)D_3. [N = 67 originates from: 14 drop-outs during the study, 6 examples of missing serum samples due to early hospital discharge, and 16 insufficient samples. One patient's sample was insufficient twice (on days +30 and +100). As a result, we had complete 25(OH)D_3 data sets of 67 patients available in total.] We found a slight and constant, but statistically not significant overall decrease in serum 25(OH)D_3 by 8.4% from baseline (16.2 ± 8.1 ng/ml) until day +100 post alloHCT (14.9 ± 7.5 ng/ml). Serum 25(OH)D_3 concentration at day +30 was 15.5 ± 8.7 ng/ml.

Exo- and endogenous vitamin D supply

The relevance of the two major vitamin D sources, namely sunlight-induced endogenous vitamin D_3 synthesis and exogenous oral vitamin D intake at baseline and day +100 is illustrated in Fig. 6 and Fig. 7, respectively. We had access to and evaluated the complete baseline and day +100 lifestyle data from 73 of the patients. Fig. 6 shows the percentage of patients engaging in a low, moderate or high degree of daily outdoor activities. At admission, 23/73 (32%) of the patients reported a high average time spent outdoors each day, while only 7% did so 100 days after alloHCT due to guidelines recommending the avoidance of direct sunlight exposure after alloHCT. Fig. 7 shows the percentage of patients consuming the three vitamin D_3-rich food categories at baseline and day +100 at a frequency with a potentially low, medium, or high impact on vitamin D supply. A high intake (≥7 times weekly) of dairy products remained constant in approximately 70% of the patients before and 100 days after alloHCT. High fish consumption (≥4 times monthly) was reported by 36% and 42% at baseline and day +100, respectively. High egg consumption (≥4 times weekly) was rare during the study. Eating habits in terms of vitamin D-rich foods 100 days after transplantation did not change noticeably from baseline habits. Eight patients took vitamin D_3 supplements daily at baseline [a median amount of 200 IU (range: 100-500)], whereas only one patient did so on day +100.

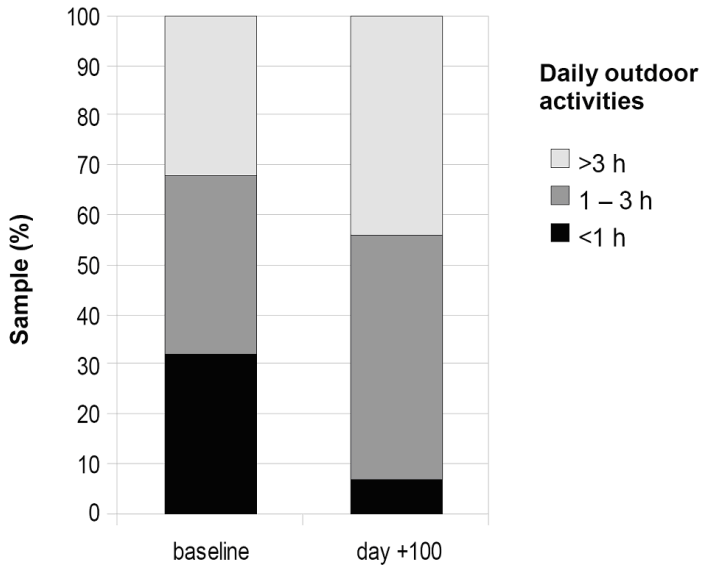


Fig. 6: Percentage of patients reporting low (<1 h), medium (1-3 h), or high (>3 h) daily outdoor activities in the month prior to baseline, and on day +100. N = 73, where outdoor activities data from baseline and day +100 were complete.

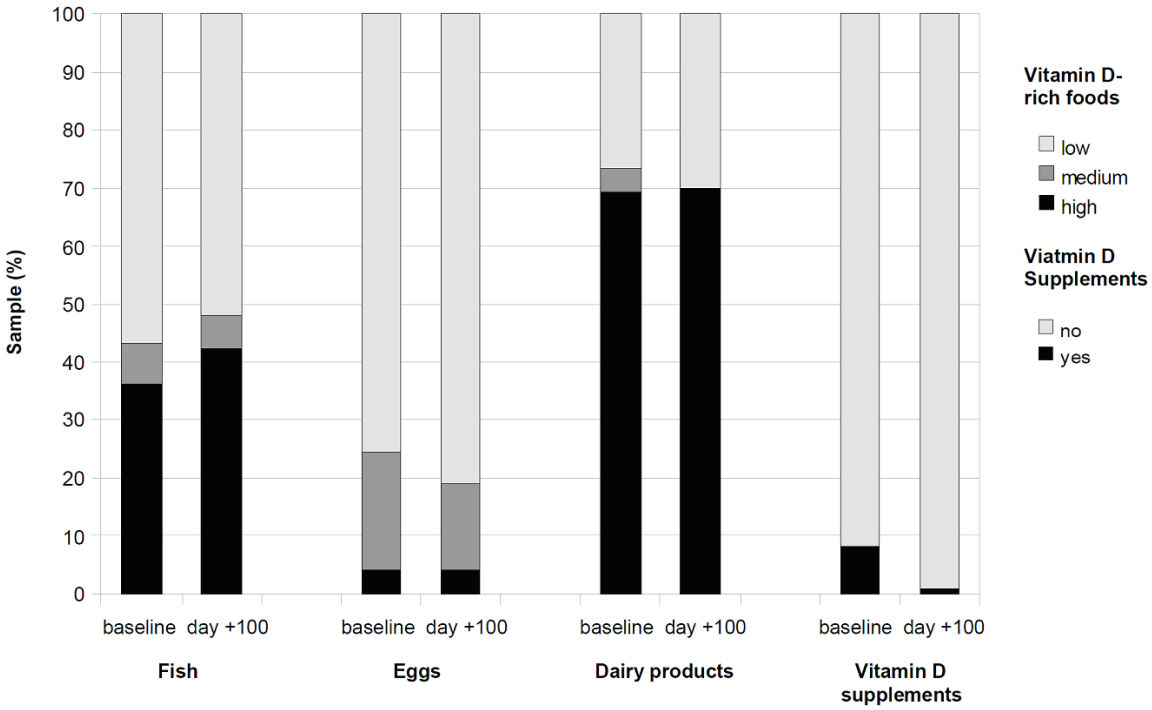


Fig. 7: Percentage of patients with a potentially low, medium, or high impact on serum 25(OH)D_3 due to their consumption of vitamin D-rich foods (fish, eggs, dairy products) and due to their daily intake of vitamin D supplements. Low, medium, or high impact

groups were defined as follows: fish (≤1, 2-3, ≥4 times monthly), eggs (≤1, 2-3, ≥4 times weekly) and dairy products (≤3, 4-6, ≥7 times weekly). N = 73, where nutrition data were complete from baseline and day +100.

Impact of influencing factors on baseline 25(OH)D_3 status

In univariate analysis, only female sex and higher body fat mass (%) had a significant impact on reduced 25(OH)D_3 concentrations (Tab. 8). In contrast, age, BMI, KPS, days between primary diagnosis and HCT, number of chemotherapy cycles before HCT, season, daily outdoor activities, use of vitamin D supplements, consumption of fish, eggs and dairy products were not associated with baseline vitamin D status. A multivariate regression model was constructed with those variables showing a relationship (*P* <0.1) to baseline vitamin D status. Our results are presented in Tab. 8. After multivariable adjustment, only fat mass (%) was identified as an independent influencing factor of baseline 25(OH)D_3 concentrations (parameter estimate -0.27; 95% CI: -0.47, -0.08; P = 0.007). After multivariate adjustment, the gender association disappeared. Female patients had a significantly higher percentage of body fat mass than men (38.2 ± 9.3% vs. 29.6 ± 9.3%, P <0.0001), suggesting that the gender difference in serum 25(OH)D_3 in the univariate analysis is based on the women's higher body fat mass.

Tab. 8: Results of regression analysis investigating potential influencing factors of baseline serum 25(OH)D_3 in patients undergoing alloHCT

Influencing factors of baseline serum 25(OH)D_3	**Unit**	**Parameter estimate (95% CI)** [1]	***P* value**
Univariate analysis (n = 102)			
Age	years	0.07 (-0.09, 0.23)	0.41
Gender	male vs female	-3.50 (-7.10, 0)	0.05
BMI	kg/m²	-0.10 (-0.53, 0.33)	0.65
Time between primary diagnosis and HCT	days	0.04 (-0.002, 0.003)	0.70
Chemotherapy cycles before HCT[2]	numbers	-0.10 (-0.90, 0.28)	0.30

Remission at HCT	early vs advanced disease	0.19 (-0.17, 7.46)	0.06
Body fat mass	% (n = 100) [3]	-0.29 (-0.47, -0.11)	0.001
KPS	≤80 vs >80	2.68 (-1.06, 6.42)	0.16
Season	decrease vs increase	-1.31 (-4.81, 2.19)	0.46
Outdoor activities	low vs high (n = 50)	2.76 (-2.89, 8.41)	0.33
Food consumption			
Fish	low vs high (n = 90)	0 (-3.91, 3.91)	1.00
Eggs	low vs high (n = 78)	3.59 (-5.48, 12.66)	0.43
Dairy products	low vs high (n = 94)	1.64 (-2.52, 5.80)	0.44
Vitamin D supplements	daily use vs no use	2.96 (-3.60, 9.52)	0.37
Multivariate analysis (n = 100)[3]			
Gender	male vs female	-0.03 (-4.37, 3.43)	0.81
Fat mass (%)	%	-0.28 (-0.46, -0.07)	0.009
Remission at HCT	early vs advanced	0.15 (-0.93, 6.63)	0.14

Abbreviations: BMI = body mass index, CI = confidence intervals, HCT = haematopoietic cell transplantation, KPS = Karnofsky performance status. [1] Parameter estimates represent the change in baseline serum 25(OH)D_3 concentrations for a one-unit increase of a continuous factor (e.g. age), or the difference in baseline serum 25(OH)D3 concentrations when comparing two levels of a binary factor (e.g. gender). [2] Excluding extra conditioning. [3] n = 100 instead of 102, because body fat mass via bioelectrical impedance analysis was only doable in 100 patients at baseline.

Factors potentially influencing the course of 25(OH)D_3

Due to the tremendous loss of body fat mass in 17% of the cohort (baseline: 26.2 ± 9.8 kg, day +100: 21.8 ± 8.9 kg; P <0.0001) during the early post-transplant period, we investigated the possible effect of vitamin D mobilization in body deposits, mainly in adipose tissue, by comparing patients showing a high to those presenting a low decrease or even increase in fat mass during the study period. In the end, we observed no differences in the amount of change in serum 25(OH)D_3 concentrations in either group.

We identified no significant difference in serum 25(OH)D_3 changes when comparing a subgroup of patients not receiving lipid-soluble vitamins between baseline and day +30 (n = 42) to those receiving parenteral nutrition (including 200 IU vitamin D_3) on ≥20% of the days [n = 23; 35% (range: 21-84)].

Corticosteroid-treated, clinically-relevant aGVHD (°II-°IV) developed in 35 (43%) of 82 patients with all relevant data. One hundred days after alloHCT~~,~~ a trend (P = 0.066) toward lower serum 25(OH)D_3 concentrations was found in patients developing clinically-relevant aGVHD (°II-°IV) with a decrease~~d by~~ of 23.1% (n = 24; serum 25(OH)D_3 baseline: 17.3 ± 8.3 ng/ml, day +100: 13.3 ± 7.8 ng/ml). In contrast, serum 25(OH)D_3 remained constant in patients with no or mild aGVHD (°0-°I) (n = 58; serum 25(OH)D_3 baseline: 15.6 ± 8.4 ng/ml, day +100: 15.3 ± 7.7 ng/ml).

2.4.4 Discussion

Very striking was our study cohort's overall low 25(OH)D_3 status before conditioning chemotherapy started, whereas a quarter showed extremely low serum concentrations (under 10 ng/ml), revealing a much higher prevalence of extreme low 25(OH)D_3 concentrations than the 16% reported in a representative sample of the German adult population [41]. Apart from one report in which median serum 25(OH)D_3 concentrations fell within the lower normal range [134], the prevalence of low 25(OH)D_3 status before alloHCT in this study is similar to results reported by Schulte et al. [38] and Kreutz et al. [37]. As those studies reported significant drops in serum 25(OHD)D_3 at the time point of engraftment [37] or on day +28 [38], we also noted a slight trend towards a continuous fall until day +100. Interestingly, Robien et al. [135] found in long-term HCT survivors 4.2 years after transplantation serum 25(OH)D concentrations above 30 ng/ml, whereby the majority of participants reported regular use of vitamin D supplements.

We are the first to prospectively investigate the impact of well-known influencing factors including age, gender, body fat mass, oral vitamin D intake, plus season and outdoor activities, as surrogates of UV-B irradiation and endogenous vitamin D synthesis on baseline serum vitamin D status in adults undergoing alloHCT. Surprisingly, we observed no seasonal variation in baseline serum 25(OH)D, which is the strongest influencing factor reported consistently in healthy subjects [41,138,139]. Likewise, a recent study in paediatric HCT demonstrated that the testing time of year was not a risk factor for vitamin D deficiency, most likely due to little outdoor activity

and the frequent use of sunscreen [39]. We assume that this lack in our study can be partly explained by our high number of patients with advanced disease at admission, which is often associated with prolonged disease history and hospitalization. The season after discharge till day +100 had no influence on serum 25(OH)D_3 changes during that period either, most probably due for the most part to the medical advice to avoid unprotected sun exposure. Univariate analysis revealed that no one food, nor the use of vitamin D supplements had any noticeable influence on vitamin D status in this cohort.

Obesity, thus increased body fat stores, have consistently been found to be associated with significantly lower serum 25(OH)D concentrations [140,141,135,142], probably because of augmented deposits in fat stores [142]. Baseline body fat mass was the only independent predictor of vitamin D status in our study cohort. To expand previous reports [140,142], we investigated the impact of increased fat mass breakdown with the potential release of stored vitamin D on vitamin D status, and failed to observe a positive effect of losses in body fat mass on serum 25(OH)D_3 during the study period. Changes in serum 25(OH)D_3 seemed to be independent of body composition changes during the first 100 days after alloHCT.

The trend toward a decrease in serum 25(OH)D_3 concentrations during the first 100 days after alloHCT in patients with clinically-relevant and corticosteroid-treated aGVHD (°II-°IV) observed in our study resembles the results reported by Kreutz et al. [37], who showed this effect in patients with severe aGVHD (°III-°IV). It is believed that this inverse association between aGVHD and serum vitamin D is due to the intestines' impaired capacity to absorb lipid-soluble vitamins [37]. Clinically-relevant aGVHD (≥°II) is treated with corticosteroids, potential confounders due to their known negative effects on vitamin D metabolism [143,144]. Future studies should assess GVHD-associated gastrointestinal disorders (e.g. diarrhea) to be able to discern effects of corticosteroids from those of malabsorption on vitamin D status.

Patients receiving 200 IU of vitamin D_3 via a daily infusion of lipid-soluble vitamins on at least 20% of the days spent on transplantation ward did not reveal a different course in serum 25(OH)D_3 than patients without parenteral nutrition, never mind an improvement in serum 25(OH)D_3. Strengthened by results from Schulte et al. [38] [who reported that serum 25(OH)D concentrations decreased during the first 4 weeks after alloHCT although all patients received vitamin D supplementation in their parenteral nutrition (200 IU/d)] and in accordance with Robien et al. [135], we be-

lieve that the current recommended daily allowance for adults in Germany is insufficient to meet these patients' specific micronutrient requirement in this extreme situation. But what the appropriate vitamin D supplementation for these patients could be, as their individual needs differ considerably due to divergent clinical conditions and medications, still remains unknown.

Hypovitaminosis D is a major risk factor for bone loss leading to osteoporosis via secondary hyperparathyroidism [145]. In addition to widely-used corticosteroids and cyclosporine A in post-alloHCT believed to be responsible for bone loss [146], hypovitaminosis D may play an important role in the dramatically increased bone resorption before alloHCT and in the poor recovery of bone mineral density one year after alloHCT [38]. A recent health implication of vitamin D is the potential reduction in adverse effects of GVHD while maintaining the graft vs tumour effect. Based on vitamin D's immunomodulatory properties, Rosenblatt et al. [36] published *in vitro* data showing that vitamin D exposure inhibits differentiation of dendritic cells (DCs) resulting in immature DCs exerting a tolerating influence on allo-reactive T-cell populations.

A study limitation is the semi-quantitative method we used instead of a validated dietary assessment, which would have enabled us to estimate individual vitamin D intakes. Further limitations for this analysis are associated with the great difficulty to accurately assess sunlight exposure. We thus documented the season and outdoor activities as approximate measures of sunlight. Furthermore, issues of statistical power should certainly be considered when inspecting the results of our analyses. When investigating the impact on $25(OH)D_3$ concentrations of factors with a strongly unbalanced distribution [Table 2, e.g. daily use of vitamin D supplements (n = 8) vs no use (n = 94)], results are based on small group sizes, leading to large confidence intervals.

In conclusion, hypovitaminosis D was a frequent condition in patients undergoing alloHCT in this monocentric study within our specific geographical region. Surprisingly, the most important influencing factors according to the current literature, such as season and vitamin D-rich foods, seemed to have an only minor impact on baseline vitamin D status. As expected, $25(OH)D_3$ status remained low in the early post-transplant period, showing a trend towards further deterioration, especially in patients with corticosteroid-treated aGVHD. In addition, reduced outdoor activity combined with the medical advice to avoid unprotected sun exposure, and an inadequate

supply via nutrition and vitamin D supplements, mean that without appropriate measures e.g. sufficient vitamin D supplementation, achieving higher serum 25(OH)D_3 concentrations is virtually impossible. Moreover, our results provide a clear rationale for the use of at least vitamin D containing multivitamin preparations in the case of standard multivitamin prescriptions and for the monitoring of vitamin D status at regular intervals from the date of diagnosis.

2.5 Bioavailability of vitamin D_2 from UV-B-irradiated button mushrooms in healthy adults deficient in serum 25-hydroxyvitamin D: a randomized controlled trial

2.5.1 Introduction

Low vitamin D status, defined as a serum 25-hydroxyvitamin D [25(OH)D] concentration <50 nmol/L [147,148], is a public health issue prevalent worldwide [40-43], particularly in regions with a big seasonal shift in solar altitude, as the major source of vitamin D for humans is sunlight-induced cutaneous synthesis [44,45]. Other criteria, like dark skin [149,150], old age [140], and immobility [151] further reduce the endogenous vitamin D synthesis. Moreover, few foods contain vitamin D in noteworthy concentrations; those that do are fish-liver oils, fatty fish, and egg yolk. Furthermore, there is a wide variety of foods fortified with vitamin D across the world [152], e.g. dairy products, bread, and recently, orange juice [153-155].

Naturally, the vitamin D_2 content of cultivated mushrooms is almost nil [<0.1 µg/100 g fresh weight], yet they are very rich in ergosterol [46,47]. Ergosterol is the principal sterol in fungi, and several studies have reported that mushrooms can be greatly enhanced with vitamin D_2 by ultraviolet (UV) irradiation, resembling the cutaneous synthesis of vitamin D_3 in humans [48,156]. The conversion rate of ergosterol to vitamin D_2 under UV irradiation depends on the UV spectrum (UV-B or -C), irradiation dose, moisture content, and the mushrooms' orientation toward the UV source [46,157,158].

Outila et al. (1999) were the first to demonstrate that vitamin D_2 was well absorbed from lyophilized and homogenized mushrooms in humans. Jasinghe et al. (2005, 2006) were the first to publish in vivo studies on the bioavailability of vitamin D_2 from UV-irradiated mushrooms, showing as others [159] that vitamin D_2 from vitamin D_2-enhanced mushrooms is well absorbed and metabolized in rodents, and that it improves bone mineralization.

Recently the case history of a patient with vitamin D deficiency and secondary hyperparathyroidism was published, who refused to take supplements, but self-treated his deficiency by consuming mushrooms daily, which he had exposed to UV-B irradiation [160].

To the best of our knowledge, this is the first report on the bioavailability of vitamin D_2 from UV-treated mushrooms in humans. Hence the primary objective of this randomized controlled trial was to demonstrate the possibility of improving the 25(OH)D status with this natural food source in terms of a higher serum 25(OH)D concentration in young adults with low 25(OH)D status 4 weeks after a weekly vitamin D_2 dose of 28 000 IU (700 µg) compared to placebo. A secondary objective was to compare the bioavailability of vitamin D_2 from UV-B treated mushrooms with a vitamin D_2 supplement.

2.5.2 Subjects and methods

Subjects

Subjects were recruited from employees of the University Medical Center Freiburg by advertising. The study protocol was approved by our Ethics Commission. Exclusion criteria included kidney stones, pregnancy, anticonvulsant or steroid therapy in any form, frequenting a tanning salon, or residence in the mountains or southern countries right before or during the study. The subjects were not allowed to take vitamin D supplements or fish liver oils, and were asked to eat fish no more than once a week.

Caucasian adults in good general health, younger than 45 years with a body mass index (BMI) between 18.5-26 kg/m², and not fulfilling any exclusion criterion were eligible to provide blood specimens for further testing after having signed a written consent form. Out of 49 female and male volunteers we randomized 27 subjects with low serum vitamin D (25(OH)D ≤50 nmol/L) and normal serum calcium concentrations (2.2-2.7 mmol/L) to enter the study.

Study design

This study was a 5-week, prospective, randomized, 3-arm, single-blind, placebo-controlled trial to investigate the bioavailability of vitamin D_2 from UV-B-irradiated button mushrooms and vitamin D_2 supplement, respectively.

The four first weekly visits (weeks 0, 1, 2, 3) constituted the interventional part of the study and the two last visits (weeks 4, 5) served as follow-up. The primary objective and further endpoints were analyzed till week 4, because that is when we expected the strongest interventional effect. At the initial, baseline visit (week 0),

weight and height were documented. At each subsequent weekly visit at the same time of day blood was drawn. The study was performed during the winter from late January till early March 2010, when a) a low vitamin D status in healthy subjects is most likely, and b) solar UV-B radiation is minimal to avoid the confounding effect of cutaneous vitamin D_3 synthesis on our intake-response evaluation.

Our 27 blinded subjects were randomly assigned into 3 equal groups [a) mushroom, b) supplement, c) placebo] using a computer-generated sequence to receive 4 times at weekly intervals either a) 28 000 IU vitamin D_2 via 365 g of the experimental soup containing the UV-B-irradiated mushrooms (vitamin D_2 content of 191.8 µg/100 g) and placebo, or b) 60 IU vitamin D_2 via a conventional mushroom soup and 28 000 IU vitamin D_2 via a supplement (equivalent of 70 drops), or c) 60 IU vitamin D_2 by a conventional mushroom soup and placebo, respectively. The liquid supplement used (Stérogyl, Desma Pharma, Paris, France) provided 400 IU per drop (verified as 393 IU per drop by SGS Institut Fresenius, Berlin, Germany), consisted of an ethanol formulation of vitamin D_2 and was dissolved in orange juice. The placebo consisted of pure orange juice. The supplement or placebo was served shortly before the soup. Soup intake was supervised and remains of the soup were absorbed by bread and eaten.

Blood sample analysis

The blood samples were stored for coagulation about 30-60 min in the dark at room temperature previous to centrifugation (2.000 rpm for 7 min). The serum samples were frozen at -78 °C until weekly analysis of all samples of one blood drawing by the laboratory MVZ Clotten (Freiburg, Germany). Serum $25(OH)D_2$ and serum $25(OH)D_3$ were measured combined as 25(OH)D by a radioimmunoassay (RIA) purchased from DiaSorin Inc. (Stillwater, MN, USA). The quality and accuracy of the serum 25(OH)D analysis were monitored by interlaboratory tests evaluated by INSTANT e.V. (Duesseldorf, Germany). The detection limit for the RIA assay was 10 nmol/L, inter- and intraassay coefficients of variation for 25(OH)D were 11.1% and 10.1%. The cross-reactivity of each compound, normalized to $25(OH)D_3$ and above 1% is specified by the manufacturer as 104%, 40% and 17% with $25(OH)D_2$, $1,25OH_2D_2$, and $1,25OH_2D_3$, respectively. Serum intact parathyroid hormone (iPTH) was measured by non-competitive immunoassay on the Roche Modular Analytics E170. Serum calcium was measured using a photometric color test with Olympus calcium Arsenazo III OSR60117. The reference ranges were 50-175 nmol/L,

1.2-4.5 pmol/L and 2.2-2.7 mmol/l, for 25(OH)D, iPTH and serum calcium, respectively. Serum 25(OH)D and calcium were measured weekly, serum iPTH was measured twice at week 0 and week 4.

Irradiation of mushrooms

We used fresh brown button mushrooms (Agaricus bisporus) provided by a local mushroom producer (Schlossbergpilze, Freiburg, Germany) with a moisture content of 91.4%, determined by the vacuum oven method. To produce vitamin D_2-enhanced mushrooms, they were placed completely separated from each other on a 2 cm meshed grid, and each side was irradiated simultaneously with UV-B (306 nm) at an irradiation dose of 1.5 J/cm^2 after 25 min at ambient temperature (22 °C). The custom-made UV unit was equipped with 8 UV-B lamps 176 cm in length (UV21, Waldmann, Villingen-Schwenningen, Germany). The total irradiation area was 0.72 m^2 with a homogeneous intensity of UV-B. The radiation dose was measured by a radiometer (UV34, PCE Group, Meschede, Germany).

Soup preparation and analytic method

Directly after irradiation, the mushrooms were diced and used in a puréed mushroom soup. All the experimental and conventional mushroom soups needed for the study were portioned out and stored in a freezer at -20 °C. Soup ingredients were water, button mushrooms, soy cream, flour, olive oil and spices [4.2% fat, 0.8% protein, 2.9% carbohydrates (weight/weight)]. Soup and mushroom samples were shipped on dry ice to SGS Institut Fresenius (Berlin, Germany) for vitamin D_2 analysis. The assay is based on semipreparative HPLC purification followed by analytical reversed-phase HPLC. Preliminary tests (data not shown) showed that the experimental soup's vitamin D_2 remained very stable during cooking, freezing, defrosting, and reboiling. The mushroom soups were well tolerated.

Sample size calculation and statistics

The study was designed to detect a difference of 20 nmol/L in 25(OH)D serum concentrations between the mushroom and placebo groups with a power of 80% with a one-sided *t*-test at a significance level of 5%. The assumed standard deviations (SD) were 13.0 in the placebo and 14.7 in the mushroom groups, derived from previous investigations. The resulting sample size was 7 per group. The study was analyzed

using SPSS version 16.0 (SPSS Inc, Chicago, IL, USA) and SAS version 9.2 (SAS Institute Inc, Cary, NC, USA).

For descriptive data analyses, values are presented as means ± SDs. Group comparisons were made using two sample *t*-tests and one-way ANOVA with post hoc Tukey tests. P-values will be provided for the comparisons of secondary objectives and should be regarded as exploratory. Results with $P < 0.05$ will be denoted as significant. The development of 25(OH)D concentrations during the study was investigated using a random effects model (SAS proc mixed) with 25(OH)D as dependent variable, different time slope parameters for each treatment group (i.e. time*treatment interaction) and subject specified as a random effect. Treatment differences during the study can thus be investigated by testing the differences in time slopes. Results will be given in terms of estimates for regression slope parameters which represent the 25(OH)D increase per week in each group and their accompanying 95% confidence intervals.

2.5.3 Results

Baseline characteristics of subjects

Of the 27 subjects recruited for the study, one from the mushroom group dropped out because of pregnancy before the first visit, while 26 completed the interventional phase and were used for the further evaluation. One subject had to miss the last follow-up blood withdrawal (week 5). Characteristics of the study population are summarized in Tab. 9. Mean age and BMI of the subjects were 30.8 ± 5.8 y and 22.1 ± 2.5 kg/m^2, respectively. There were no significant differences in these parameters among the 3 study groups at baseline. In addition, the 3 study groups were similar with regard to initial serum concentrations of 25(OH)D, iPTH, and calcium (Tab. 10). At 4.08 (1.89-7.70) pmol/L at baseline, median serum iPTH was in the upper normal range, and 8 subjects (30.8%) already presented secondary hyperparathyroidism as a result of vitamin D deficiency. Furthermore, we found a statistically significant negative linear correlation between iPTH and serum 25(OH)D ($r = -0.463$, $P = 0.017$).

Tab. 9: Baseline characteristics of subjects and dropouts (n = 26)[1]

Variables	Mushroom group (n = 8)	Supplement group (n = 9)	Placebo group (n = 9)
male:female (n)	3:5	4:5	2:7
Age (y)	28.6 ± 4.3[2]	31.1 ± 6.7	32.4 ± 6.0
BMI (kg/m^2)	22.0 ± 1.7	23.7 ± 2.1	20.6 ± 2.6
study dropouts	1 at week 0	1 at week 5	-

[1] There were no significant differences between the groups at baseline. [2] Means ± SDs (all such values).

Mushrooms' vitamin D_2 content

Our study's brown button mushrooms cultivated in the dark had very low concentrations of vitamin D_2 (0.18 µg/100 g fresh weight). The conversion of ergosterol to vitamin D_2 under UV-B irradiation in this study was very high, and we achieved concentrations of 491 µg/100g fresh weight (56.8 µg vitamin D_2/g dry solids).

Time course of the main serum parameters

The time course data of serum 25(OH)D concentrations for each study group over the 5-week period are presented in Tab. 10 and Fig. 8. The primary objective was to test the efficacy of the vitamin D_2-enhanced mushrooms to improve the 25(OH)D status. The data show that one week after the last consumption of such mushrooms at week 4, the mushroom group's serum 25(OH)D was significantly higher than that in the placebo group ($P < 0.0001$) (Tab. 10).

A secondary objective consisted of testing the bioavailability of vitamin D_2 from the UV-B treated mushrooms compared to a common vitamin D_2 supplement. When modelling the development of 25(OH)D concentrations as a linear function of time (mixed regression model), we found that the mushroom and supplement groups increased their 25(OH)D concentrations significantly over the study period by 3.9 nmol/L per week (95% CI: 2.9, 4.8; $P < 0.0001$) and by 4.7 nmol/L per week (95% CI: 3.8, 5.7; $P < 0.0001$) (Fig. 1). Regression slopes of the concentrations of the serum 25(OH)D in the mushroom and supplement groups did not significantly

differ from one another (P = 0.20). The mean increase in serum 25(OH)D in the first 4 weeks per 100 IU of vitamin D_2 was 0.5 nmol/L.

Tab. 10: Laboratory values in the three study arms[1]

Serum parameter and time (week)	**Mushroom group** (n = 8)	**Supplement group** (n = 9)	**Placebo group** (n = 9)	**P[2]**
25(OH)D (75-175 nmol/L)[3]				
0	34.0 ± 11.0[6]	28.7 ± 10.0[6]	38.7 ± 14.2[4]	0.16
1	45.2 ± 7.0	42.0 ± 9.2[4]	35.0 ± 13.0	0.12
2	47.2 ± 8.0[a]	46.2 ± 8.0[a, 4]	31.2 ± 9.7[b, 4]	0.001
3	51.0 ± 11.2[a]	50.7 ± 7.7[a]	27.5 ± 7.7[b, 4]	<0.0001
4	51.5 ± 7.7[a, 5]	48.2 ± 8.7[a, 4]	24.5 ± 7.2[b, 4]	<0.0001
5	56.7 ± 7.2[a]	58.0 ± 11.2[a]	28.7 ± 8.7[b]	<0.0001
iPTH (1.2-4.5 pmol/L)				
0	3.36 ± 1.01	4.67 ± 1.56	4.28 ± 1.95	0.24
4	3.20 ± 1.35	4.24 ± 1.21	3.78 ± 1.26	0.26
Calcium (2.2-2.7 mmol/L)				
0	2.53 ± 0.08	2.47 ± 0.08	2.48 ± 0.09[6]	0.33
4	2.43 ± 0.07	2.40 ± 0.13	2.37 ± 0.09	0.44

[1] Mean ± SD (all such values). iPTH, intact parathyroid hormone. **[2]** Values at each time of measurement with different superscript letters are significantly different from each other (one-factor ANOVA and post hoc analysis with Tukey's test). **[3]** Reference range. **[4-6]** Significantly different from the following mean in the same column (paired *t* test): **[4]**P <0.05, **[5]**P <0.01, **[6]**P <0.001.

Further analysis showed that already two weeks after the first consumption of the vitamin D_2-enhanced mushrooms, the two interventional groups' serum 25(OH)D concentrations were significantly higher than in the placebo group (P = 0.001) (Tab. 10). Furthermore, the data reveal significant within-subject changes in serum 25(OH)D in all three study groups already at week 1. During the first week, serum 25(OH)D rose significantly (P <0.001) by 33.1% and 46.1% in the mushroom and supplement groups, respectively. In addition, serum 25(OH)D decreased significantly

(P = 0.03) by - 6.5% in the placebo group. The development of hypercalcaemia (serum calcium >2.7 mmol/L) was the main safety criteria for the vitamin D_2 administration. Serum calcium remained within the reference range at all time points. No physical symptoms were reported during the study. Neither at baseline nor week 4 did the serum concentrations in iPTH and calcium differ significantly among the three study groups (Tab. 10). The correlation between the 4-week changes in both intervention groups from serum 25(OH)D and iPTH at baseline revealed a negative but non-significant association (r = -0.449, P = 0.071).

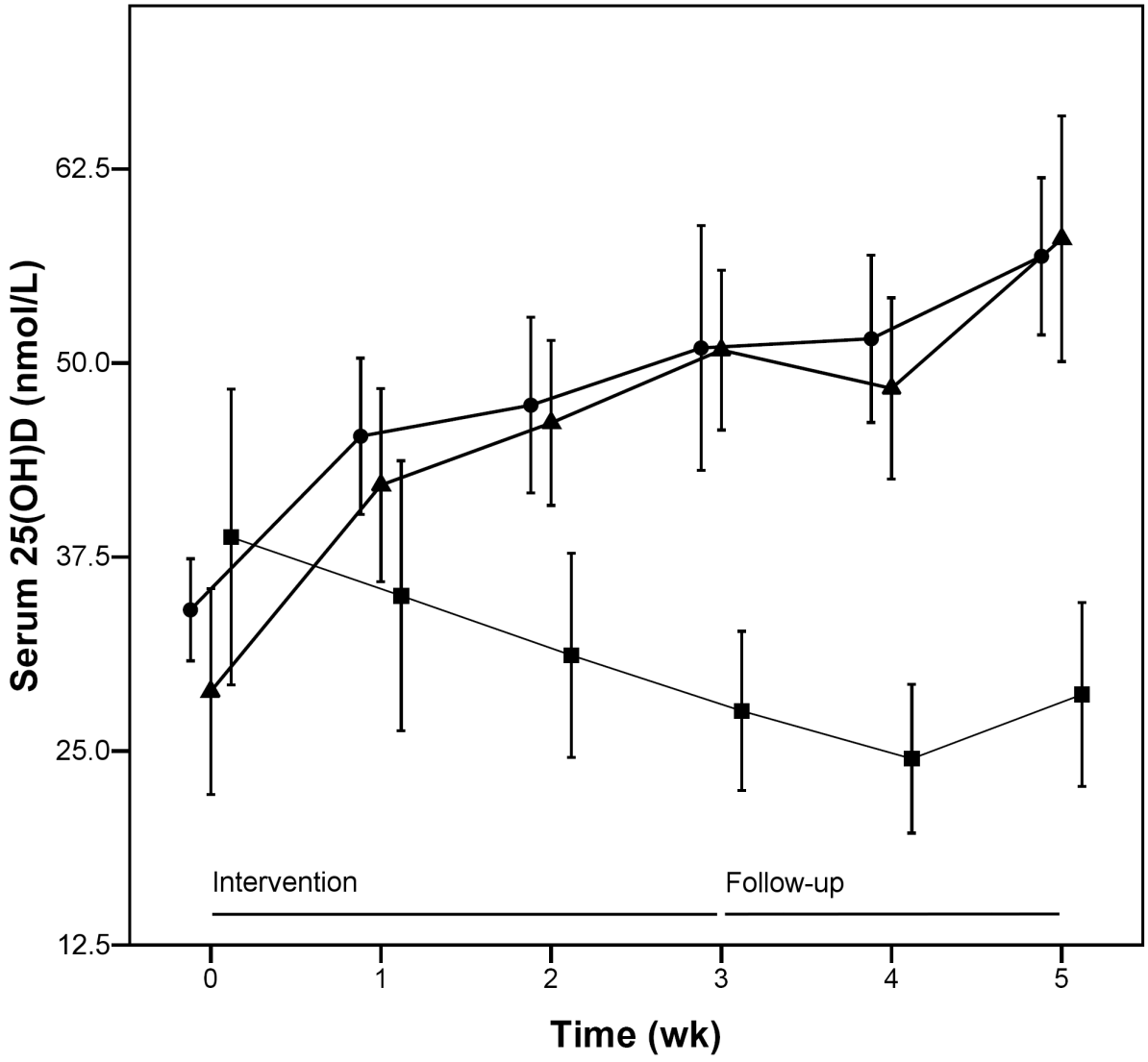


Fig. 8: Time course of the mean changes in serum 25(OH)D over the 5-week study period in subjects who consumed four times (weeks 0, 1, 2, 3) mushrooms enhanced with vitamin D2 via UV-B irradiation (mushroom group, n = 8, ●) or vitamin D2-containing supplements (supplement group, n = 9, Δ) or placebo (placebo group, n = 9, □) at the

end of the winter. Error bars are 2 SE. At week 5 one subject dropped out of the supplement group. Concentrations were significantly different (ANOVA, Tukey's test) between the mushroom and placebo groups, and between the supplement and placebo groups from week 2 onward (wk 2: P = 0.002; P = 0.004, respectively; weeks 3, 4, 5: P <0.0001). Time courses for serum 25(OH)D over the study period in the mushroom and supplement groups did not differ significantly.

2.5.4 Discussion

Here we describe for the first time in humans that UV irradiation of mushrooms creates an excellent source of vitamin D_2 which has equivalent bioavailability as a vitamin D_2 supplement.

The rapid serum 25(OH)D increase in our mushroom group is a clear demonstration that ingesting 28 000 IU vitamin D_2 once a week for 4 weeks via UV-B-irradiated and vitamin D_2-enhanced mushrooms is effective in improving vitamin D status in young, healthy adults. Already one week after their first ingestion of UV-B-irradiated mushrooms, serum 25(OH)D rose significantly. Furthermore, it was significantly higher than in placebo group one week after the second administration of enhanced mushrooms (week 2).

Most studies on the bioavailability of vitamin D in humans have been conducted using supplements, not natural food sources. Consistent with our observation of equivalent vitamin D_2 bioavailability from a soup prepared with UV-irradiated mushrooms and supplement, an earlier study [161] demonstrated the same efficiency with non-irradiated, but lyophilized and homogenized mushrooms in humans. Both findings disprove the hypothesis of Van-den-Berg (1997), who maintained that the bioavailability of vitamin D from natural food sources is probably lower than from supplements.

By our weekly vitamin D_2 supplementation of 28 000 IU (daily equivalent of 4000 IU), we found after one month a mean increase in serum 25(OH)D of 0.5 nmol/L for every 100 IU of vitamin D ingested. Evaluating our dose-response effect with the reported increase by approximately 1–2 nmol/L in 25(OH)D for each additional 100 IU of vitamin D3 [162] is not appropriate, because a plateau in serum 25(OH)D was not achievable over such a short period. Therefore, we examined sup-

plementation studies using comparable doses of vitamin D_2 and time periods. Mastaglia et al. reported a dose-response in the first month of 0.43 nmol/L for every 100 IU vitamin D_2 by supplementing weekly 35 000 IU of vitamin D_2, resembling our results although they used a 25% higher dose [163]. At their study's conclusion (3 months), dose-response rose to 0.71 nmol/L. Considering the differences in doses and time, our results also concur with dose-responses reported by others [164,148].

One of our study's limitations is that the accuracy of the RIA method used to determine 25(OH)D_2 was found to be lower than indicated by the manufacturer [165,166], so it is possible that total serum 25(OH)D was underestimated.

Other authors showed that a single dose of 50 000 IU of vitamin D_2 or vitamin D_3 produced similar increases in serum 25(OH)D over the first 3 days, but serum 25(OH)D began to fall immediately thereafter in the vitamin D_2 group until, by day 14, it reached baseline concentrations [167]. In contrast, serum 25(OH)D concentrations continued to rise until day 14 in the vitamin D_3 group, then falling slowly over the following 14 days.

We did not compare the potencies of these two types of vitamin D, but we could not confirm the reported short initial increase followed by a rapid fall in serum 25(OH)D after vitamin D_2 supplementation [167]. When adjusted for a concomitant increase in serum 25(OH)D in the placebo group in the last week (due to the confounding effect of cutaneous vitamin D_3 synthesis), the high serum 25(OH)D concentrations in both interventional groups achieved remained constant during the follow-up period.

Secondary hyperparathyroidism at baseline as a major clinical sign of vitamin D deficiency was present in almost a third of our subjects with low 25(OH)D status, especially in those with very low serum 25(OH)D concentrations (≤25 nmol/L). In addition, we observed a negative correlation ($r = -0.449$, $P = 0.071$) between serum 25(OH)D increase due to the weekly intake of 28 000 IU vitamin D_2 and the change in serum iPTH.

As anticipated, and in light of the current state of knowledge [168,169], the vitamin D_2 dose of an average of 4 000 IU/d used in this study was safe, and we observed no cases of hypercalcaemia or any adverse events.

Our results demonstrate for the first time that the bioavailability of vitamin D_2 from vitamin D_2-enhanced button mushrooms via UV-B irradiation was effective in improving vitamin D status in young, healthy adults. Furthermore, we did not observe any

differences in the absorption rate and metabolism of vitamin D_2 from UV-B-irradiated mushrooms and a vitamin D_2 supplement in raising circulating serum 25(OH)D concentrations.

In conclusion, as the vitamin D_2 enhancement of mushrooms boosts their nutraceutical value, it would provide a worthwhile means of improving the vitamin D maintenance in the general population. Further research and development are required to find solutions for making such vitamin D_2-enhanced mushrooms commercially available in a safe and affordable manner.

3 Summary

The first two studies investigated the course of the nutritional status in patients undergoing allogeneic haematopoietic cell transplantation (alloHCT) and the validity of nutritional markers as independent risk factors for outcome. In line with others, we detected an overall good nutritional status before alloHCT by employing quick screening tools such as BMI and the SGA questionnaire for identifying malnutrition. However, upon closer inspection, we observed unintentional weight loss previous to alloHCT to be a frequent condition, detecting many more underweight patients using age- and gender-specific BMI classification and overall low bioelectrical impedance phase angle values at admission. Deterioration in nutritional status during the early post-transplant period was significant in the cohort. Furthermore, we identified anorexic patients and those with clinically-relevant aGVHD (≥°II) to have an increased risk for a decline in nutritional status during early rehabilitation. In addition, it could be demonstrated for the first time that the pretransplant phase angle (≤25th percentile) used as a standardised value was an independent predictor for 2-year overall survival, non-relapse mortality and progression-free survival. BMI adjusted for age and gender emerged as an independent risk factor for OS and NRM. Both of these nutritional markers performed better than numerous generally-accepted risk factors, and they were the only significant prognostic values for outcome in this cohort besides HLA-C compatibility, donor and remission status. In addition, these two nutritional markers are theoretically modifiable during the often lengthy treatment period before transplantation. Further investigation is necessary to demonstrate whether or not the phase angle can be increased by strategies that enhance muscle mass via physical training together with nutritional support, and whether it can prevent deterioration in nutritional status and lead to beneficial effects on outcome after alloHCT.

The third study investigated the association between AOX status (α-tocopherol, ascorbic acid and ß-carotene) in buccal mucosa cells (BMC) or plasma and the risk of developing oral mucositis (OM) after conditioning chemotherapy. We observed no significant differences in baseline AOX concentrations in plasma or BMC among the different OM groups (no or mild, ulcerative and severe OM), revealing that no single AOX has predictive value for the incidence or severity of OM after conditioning chemotherapy. However, patients with an overall good plasma AOX status tended to require a shorter duration of parenteral nutrition, which is a relevant clinical and economic marker for OM treatment compared to patients with at least one plasma AOX

beneath the normal range. These findings may indicate that an intact antioxidative network is more relevant than any one AOX for lowering the OM risk. Future studies should consider both the exogenous- and endogenous AOX systems.

The fourth study revealed a generally low serum 25-hydroxyvitamin D [25(OH)D_3] status in these patients at admission. Furthermore, their 25(OH)D_3 status remained low during the early post-transplant period, showing a trend towards further deterioration, especially in patients with corticosteroid-treated aGVHD. In addition, we conducted a detailed investigation of the impact of well-known influencing factors on baseline serum 25(OH)D_3 status, revealing only higher body fat mass to be an independent risk factor for reduced baseline status. In fact the most important influencing factors, namely dietary factors and season, revealed no detectable impact, most probably due to inadequate oral intake, prolonged disease history, and hospitalisation. In conclusion, these results provide a clear rationale for the use at least of vitamin D-containing multivitamin preparations in standard multivitamin prescriptions, and for the monitoring of vitamin D status at regular intervals from the date of diagnosis.

The fifth study was a randomised controlled trial revealing for the first time in humans that the bioavailability of vitamin D_2 from vitamin D_2-enhanced button mushrooms via UV-B irradiation is effective in improving vitamin D status in young, healthy adults and not different than a vitamin D_2 supplement. The vitamin D_2 enhancement of mushrooms boosts their nutraceutical value by creating both an abundant and excellent source of vitamin D_2 that opens up new opportunities in fighting widespread vitamin D deficiency.

4 Zusammenfassung

Die ersten zwei Studien untersuchten den Verlauf des Ernährungsstatus bei Patienten, die eine allogene hämatopoetische Zelltransplantation (alloHZT) erhielten, sowie die Validität von Ernährungsparametern als unabhängige Risikofaktoren für das Outcome. In Übereinstimmung mit anderen Studien stellten wir vor Beginn der alloHZT mithilfe einfacher Screening-Verfahren für Mangelernährung wie BMI und SGA-Fragenbogen einen insgesamt guten Ernährungsstatus fest. Jedoch bei näherer Betrachtung zeigte sich häufig ein unbeabsichtigter Gewichtsverlust vor Beginn der alloHZT. Des Weiteren wurden bei Aufnahme deutlich mehr untergewichtige Patienten durch den Einsatz von alters- und geschlechtsspezifischer BMI-Klassifikation ermittelt und allgemein niedrige Phasenwinkel-Werte mittels bioelektrischer Impedanzanalyse gemessen. Signifikante Verschlechterungen des Ernährungsstatus in der frühen Phase nach Transplantation waren in dieser Kohorte häufig. Darüber hinaus identifizierten wir anorektische Patienten und solche mit einer klinisch relevanten aGVHD (≥°II) als solche mit erhöhtem Risiko für eine Verschlechterung des Ernährungsstatus während der frühen Rehabilitationsperiode. Diese Ergebnisse zeigen erstmals, dass der Phasenwinkel vor Transplantation (≤25te Perzentile) als standardisierter Wert, ein unabhängiger Prädiktor für das 2-Jahres Gesamtüberleben, therapiebedingte Mortalität (NRM) und progressionsfreies Überleben (PFS) ist. Des Weiteren stellte sich der alters- und geschlechtsadaptierte BMI als ein unabhängiger Risikofaktor für Gesamtüberleben und therapiebedingte Mortalität heraus. Beide Ernährungsparameter übertrafen zahlreiche allgemein anerkannte Risikofaktoren in ihrer Aussagekraft und waren neben HLA-C-Kompatibilität, Donor- und Remissionsstatus die einzigen signifikanten prognostischen Werte für das Outcome in der vorliegenden Kohorte. Zusätzlich lassen sich diese Ernährungsparameter während der häufig langen Behandlungsperiode vor Transplantation theoretisch beeinflussen. Weitere Untersuchungen sind nötig um zu zeigen, ob der Phasenwinkel mittels Strategien erhöht werden könnte, welche beispielsweise die Muskelmasse durch körperliches Training in Kombination mit Ernährungstherapie steigern, und ob auf diese Weise eine Verschlechterung des Ernährungsstatus verhindert würde und zu positiven Effekten für das Outcome nach alloHZT führen würde.

In der dritten Studie wurde der Zusammenhang untersucht zwischen dem AOX-Status (α-Tocopherol, Ascorbinsäure und ß-Carotin) in den bukkalen Mukosazellen (BMZ) sowie dem Plasma und dem Risiko eine orale Mukositis (OM) nach der Konditi-

onierungschemotherapie zu entwickeln. Es konnten jedoch keine signifikanten Unterschiede in den AOX-Ausgangswerten im Plasma oder in den BMZ zwischen den verschiedenen OM-Gruppen (keine oder leichte, ulzerierende und schwere OM) festgestellt werden. Dies zeigte, dass kein einzelner AOX-Wert einen prädikativen Wert für die Inzidenz oder die Schwere der OM nach Konditionierungschemotherapie hat. Hingegen benötigten Patienten mit einem insgesamt guten AOX-Status im Vergleich zu Patienten mit mindestens einem AOX-Wert unterhalb des Normbereiches tendenziell weniger Tage parenterale Ernährung, was ein relevanter klinischer und ökonomischer Parameter für die Behandlung von OM ist. Diese Ergebnisse können darauf hinweisen, dass für die Reduktion des OM-Risikos ein intaktes antioxidatives Netzwerk wichtiger ist, als ein einzelner AOX. Weiterführende Studien sollten sowohl die exogenen wie auch die endogenen AOX-Systeme berücksichtigen.

Die vierte Studie zeigte bei diesen Patienten einen insgesamt niedrigen 25-Hydroxyvitamin-D [25(OH)D_3] Status im Serum zum Zeitpunkt der Aufnahme. Darüber hinaus blieb der Vitamin D Status in der frühen Posttransplantationsphase erniedrigt und zeigte eine Tendenz zu einer weiteren Verschlechterung, besonders bei Patienten mit Kortikosteroid-behandelter aGVHD. Außerdem führten wir eine detaillierte Untersuchung bekannter Einflussfaktoren auf den 25(OH)D_3 Basiswert durch, bei der sich lediglich eine erhöhte Körperfettmasse bei Aufnahme als unabhängiger Risikofaktor für einen reduzierten Ausgangswert herausstellte. Die wichtigsten Einflussfaktoren, nämlich Ernährungsfaktoren sowie Jahreszeit, hatten hingegen keinen feststellbaren Einfluss, wahrscheinlich aufgrund unzureichender oraler Aufnahme, prolongiertem Krankheitsverlauf und häufigem stationären Aufenthalt. In der Zusammenschau liefern diese Ergebnisse ein klares Argument für den Einsatz von mindestens Vitamin D-haltigen Multivitaminpräparaten und für die Überwachung des Vitamin D Status in regelmäßigen Abständen ab dem Zeitpunkt der Diagnose.

Die fünfte Studie war eine randomisierte Kontrollstudie, die erstmals am Menschen zeigen konnte, dass Vitamin D_2 aus UVB-behandelten und dadurch Vitamin D_2-optimierten Champignons den Vitamin D Status junger, gesunder Erwachsener signifikant verbessern konnte. Weiterhin war die Bioverfügbarkeit vergleichbar mit einem herkömmlichen Vitamin D_2 Präparat. Die Vitamin D_2 Optimierung von Champignons ist eine reichhaltige und hervorragende Quelle für Vitamin D_2 und eröffnet neue Möglichkeiten, die weitverbreitete Vitamin D Unterversorgung zu bekämpfen.

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Declaration/Erklärung

I hereby certify that this thesis is my own work entirely. All materials and references required for this research have been indicated.

Hiermit erkläre ich, dass ich die vorliegende Dissertation selbständig und unter Verwendung der angegebenen Quellen und Hilfsmittel angefertigt habe.

Freiburg, den 11.11.2011