SUPPLEMENTARY APPENDIX

# The negative impact of being underweight and weight loss on survival of children with acute lymphoblastic leukemia

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# Methods

## Study Population

This study included newly diagnosed pediatric ALL patients, aged 2-18 years, treated according to the Dutch Childhood Oncology Group (DCOG) – ALL9 protocol, between January 1997 and November 2004 (1). Patients were treated in 7 pediatric oncology centers in the Netherlands and were stratified at time of diagnosis into a non–high-risk treatment group (NHR) and a high-risk group (HR) as previously described (1). Briefly, HR criteria were: white-blood-cell (WBC) count higher than $50 \times 10^9$/L, T-cell immunophenotype, mediastinal mass, central nerve system (CNS) involvement, testicular involvement, and genetic aberrations (translocation t(9;22), BCR-ABL1, or any 11q23/*MLL* gene rearrangements). All other patients were classified as NHR. The two-year treatment schedules included dexamethasone during an induction period of 6 weeks, and repetitive pulses of dexamethasone for 2 weeks every 7 weeks during maintenance therapy (total cumulative dose: HR, 1,244 mg/m2; NHR, 1,370 mg/m2). None of the patient received CNS irradiation (1).

For the current study, patients with syndromes or pre-existent diseases affecting the locomotorsystem were excluded (figure 1) as these influence body composition and/or leukemia outcome (2). Patients were prospectively evaluated from the day of diagnosis to November 2012 at the central DCOG office. The Medical Ethical Committee approved the study in 1996. A written informed consent according to the Helsinki agreement was obtained from all caregivers and patients age ≥12 years (trial number NTR460/SNWLK-ALL-9).

## Body Composition

Clinical information on height and weight was measured at diagnosis (T0), after 32 weeks of treatment and start of ALL maintenance therapy (T1), at the end of the treatment protocol (T2, 109 weeks) and 1 year after end of treatment (T3) (figure 1).

Height (m) was measured using a Harpenden stadiometer and weight (kg) with a standard clinical scale. Absolute values of Body Mass Index BMI) were calculated as weight over squared height (3). A Z-score for age and gender was calculated for weight, height and BMI with reference values of healthy Dutch peers, and was expressed as standard deviation score ($_{SDS}$: Z-score) (4-6). Reference values for these healthy peers were available from age 2 up to 20 years (4).

$BMI_{SDS}$ at diagnosis was analyzed into categories: obese and overweight (>1.1 $_{SDS}$) normal weight (–1.8 to 1.1 $_{SDS}$) and underweight (<–1.8 $_{SDS}$) (4, 5). These cut-off values are conform the international task force on obesity of the World Health Organization (ITFO) and the European Childhood Obesity Group (ECOG) (7). The differences between the used definition and the definition for obese and overweight, normal weight, and underweight by the Centers for Disease Control and Prevention (CDC) are listed in supplemental table 4.

Change of BMI was measured between diagnosis and 32 weeks of treatment (T0 to T1), and from diagnosis and cessation of antileukemic treatment (T0 to T2). Patients were included into the analysis if BMI was measured at 32 (range: 30-40) weeks or at 109 (range: 104-114) weeks. BMI change was analyzed in categories: BMI loss (T1-T0 and T2-T0 are <0 SDS) and BMI gain (T1-T0 and T2-T0 are >0 SDS). We defined BMI loss <0 SDS as clinically relevant, as we expect that children will normally gain in BMI during therapy due to i.e. corticosteroid use and change of diet (8, 9); a BMI loss will therefore be unexpected.

To assess the relative contribution of body composition components to the change of BMI over time, the percentage (%) fat and total lean body mass (kilogram of organs and muscle tissue) were measured by Dual X-ray Absorptiometry (DXA: only on the GE Lunar Prodigy, LUNAR Corporation, Madison, WI, USA). DXA were made with patients older than 4 years of age, as reference values of healthy peers were only available from the age of ≥4 years (10, 11). DXA measurements were only performed in patients from one treatment center on time points T0, T1, T2 and T3.

### Treatment outcome

Primary outcome of interest were complete remission rate, event free survival (EFS), cumulative incidence of relapse (CIR) and overall survival (OS).

Complete remission is defined as a normal hematopoiesis with <5% leukemic blasts and an absence of detectable leukemic cells in the body on day 42 of ALL treatment (1). EFS was defined as time from date of diagnosis until time of first event. An event was defined as: no complete remission after induction phase, relapse, occurrence of a second malignancy or death (1). Patients who did not achieve complete remission because of resistant disease or death in induction phase, were included in the analyses and considered to have a treatment failure on day 0. Patients who were withdrawn from the ALL9 study (due to protocol deviations, patient/doctors choice, and hematopoietic stem-cell transplantation) were included in the analysis until date of last follow-up. CIR was defined as time from complete remission to date of relapse. Relapse was considered as an event together with resistant disease which was included as an event of day 0. Second malignancy and death in remission were considered as competing events within the CIR analyses. OS was defined as mortality in the period from date of diagnosis until date of death. Patients that were lost to follow-up,

and patients who remained alive and in remission, were censored at the last follow-up date. Causes of death were described and a distinction was made between death during treatment with protocol ALL9 and death during additional treatment due to detection of relapse or secondary malignancy.

### Statistical analysis

The Chi-squared test was used to compare frequencies in patient variables between the BMI (change) categories. The two sample t-test or the analysis of variance (ANOVA) were used to compare BMI categories between normally-distributed continuous patient variables. The Kruskall-Wallis of Mann-Whitney-U test was used to compare BMI categories between skewed continuous patient variables. To compare %fat and LBM across patient variables the two sample t-test statistic, ANOVA or simple linear regression was used. The paired t-test was used to compare change of weight, length, BMI, %fat and LBM over time.

The 10-year survival curves for EFS and OS were estimated by using the Kaplan-Meier methodology (12). Statistical differences between estimated survival corresponding to different BMI categories were assessed with the two-sided Mantel-Haenszel log-rank test (13). Univariate and multivariate cox-regression analyses were used to estimate the hazard ratio (HR) of BMI categories for EFS and OS (14).

To estimate the 10-year CIR curves, a competing risks model has been used with death and second malignancy as competing events (15). The Gray's test has been applied to assess the statistical difference between the CIR curves of different BMI categories (16). To model the effects of explanatory variables on the CIR Fine and Gray's model was employed ($HR_{F\&G}$) (17).

All multivariate regression analyses including BMI at baseline were adjusted for non-high risk (NHR) or high risk (HR)-group, since these risk group classifications represent characteristics that are associated with poor treatment outcome such as: white blood cell count (WBC), immunophenotype, and extramedullary involvement. All multivariate analyses including BMI change as prognostic factor, were adjusted for risk group, and BMI at diagnosis as a continuous variable. Landmark analysis at time points T1 and T2 were performed with BMI change as prognostic factor (18). Only patients still alive and on ALL9 treatment at T1 or T2 were included in the respective survival analyses according to BMI change. When using this approach the models are able to deal with time-varying effects of covariates or time-dependent covariates

Effect modification was tested to examine whether the effect of BMI categories on survival is different for age (≥10 years), gender or risk group. To consider these possible effects an interaction term (BMI * age≥10 years, BMI * risk group or BMI * gender) was incorporated to each separate multivariate Cox regression models.

Statistical analyses were performed with SPSS 20.0 (SPSS Inc., Chicago, IL, USA) and with R version 2.15.2 (The R Foundation for Statistical Computing). $P \leq 0.05$ (two sided) was considered statistically significant.

## References


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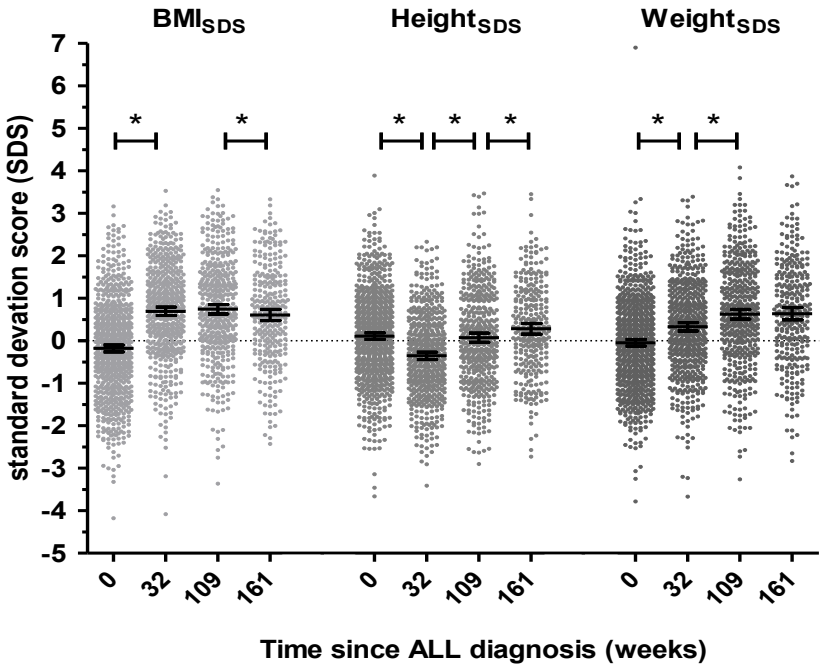
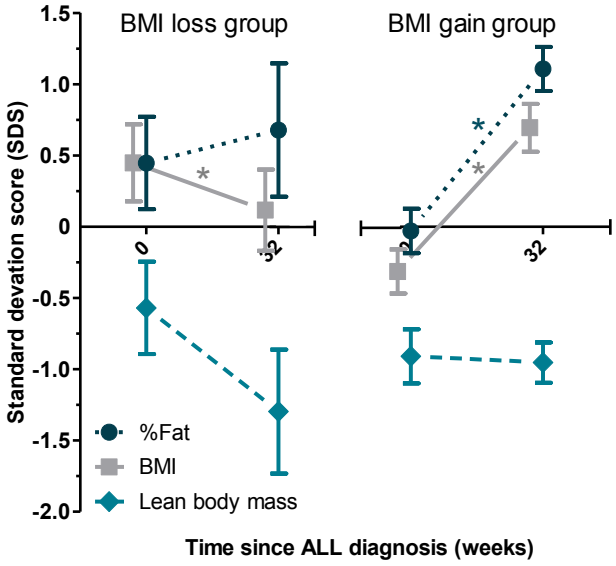
**Supplemental figure 1: Contribution of change in weight components during, and after ALL treatment.**




This figure shows the contribution of weight and height to the change of BMI over time. The mean and 95% Confidence Interval are represented in the whiskers. T=0 (*N*=738), start therapy; T=1 (*N*=509), 32 weeks after diagnosis; T=2 (*N*=435), 109 weeks, end of therapy; T=3 (*N*=265), one year after end therapy. **P*<0.01: the change is different from the previous measurement. – online only

**Supplemental figure 2. Change of body composition during anti-leukemic treatment.**




A single center subset of patients with changes of BMI and BC (*N*=70). Subgroup A (*N*=8) represents patients with a BMI loss, subgroup B (*N*=34) represent patients with a BMI gain. Lines indicate the mean of the BMI standard deviation score (SDS); bars indicate the standard error of the mean. Abbreviations: T=0 =start therapy; T=1 =32 weeks after diagnosis; LBM=lean body mass; %fat=percentage fat; BMI=Body Mass Index. *$P$<0.05. – online only

**Supplemental table 1: Patient characteristics of included patients.**

| | Study cohort (*N*=762) |
|---|---|
| Age (years) | |
| Median (range) | 5.0 (1.5-17.3) |
| Median Follow-up time (range) | 7.0 (0.1-14) |
| Sex, N (%) | |
| Female | 299 (39%) |
| Male | 463 (61%) |
| Risk group, N (%) | |
| NHR | 536 (70%) |
| HR | 226 (30%) |
| Immunophenotype, N (%) | |
| BCP-ALL | 583 (80%) |
| T-ALL | 144 (20%) |
| Not determined | 25 |
| Cytogenetics, N (%) | |
| ETV6-RUNX1 t(12;21) | 159 (21%) |
| MLL rearranged t(4;11) | 10 (1%) |
| Philadelphia Chromosome t(9;22) | 13 (2%) |
| Extramedullary involvement, N (%) | |
| Mediastinal involvement | 54 (7%) |
| Testis involvement | 3 (0.4%) |
| CNS involvement | 18 (2%) |
| WBC Status, median, range | |
| WBC x 10*9/L | 9.6 (0.2-926.0) |
| Unknown (n) | 3 |

Abbreviations: BMI=body mass index; *N*=number; HR=high-risk treatment group; NHR=non-high-risk treatment group; ALL = acute lymphoblastic leukemia; BCP = B-cell precursor; CNS=central nerve system; WBC=white blood cell count.

## Supplemental table 2. BMI at diagnosis, BMI change and the incidence of death by cause.

### A) BMI at diagnosis

| | Underweight (*N*=59) | Other weight (*N*=679) |
|---|---|---|
| **Deaths before and during first remission** | **0 (0%)** + | **24 (4%)** + |
| Infection | 0 (0%) | 12 (2%) |
| ARDS | 0 (0%) | 0 (0%) |
| Toxicity | 0 (0%) | 6 (1%) |
| Other | 0 (0%) | 2 (0%) |
| **Deaths before first remission and in first relapse** | **0 (0%)** + | **40 (6%)** + |
| Deaths before remission | 0 (0%) | 8 (1%) |
| Relapse | 0 (0%) | 32 (5%) |
| **Deaths after relapse** | **10 (17%)** + | **48 (7%)** + |
| Relapse | 10 (17%) | 39 (6%) |
| Treatment related | 0 (0%) + | 11 (2%) + |
| Infection | 0 (0%) | 8 (1%) |
| ARDS | 0 (0%) | 1 (0%) |
| Toxicity | 0 (0%) | 0 (0%) |
| Other | 0 (0%) | 0 (0%) |

### B) BMI change during 32 weeks of therapy

| | BMI loss (*N*=87) | BMI gain (*N*=421) |
|---|---|---|
| **Deaths before and during first remission** | **2 (2%)** + | **4 (1%)** + |
| Infection | 1 (1%) | 2 (0%) |
| ARDS | 0 (0%) | 0 (0%) |
| Toxicity | 0 (0%) | 2 (0%) |
| Other | 1 (1%) | 0 (0%) |
| **Deaths before first remission and in first relapse** | **7 (8%)** + | **8 (2%)** + |
| Deaths before remission | 0 (0%) | 0 (0%) |
| Relapse | 7 (8%) | 8 (2%) |
| Deaths after relapse | 7 (8%) + | 30 (7%) + |
| Relapse | 4 (5%) | 27 (6%) |
| Treatment related | 3 (3%) + | 3 (1%) + |
| Infection | 2 (2%) | 3 (1%) |
| ARDS | 1 (0%) | 0 (0%) |
| Toxicity | 0 (0%) | 0 (0%) |
| Other | 0 (0%) | 0 (0%) |

Abbreviations: *N*=number; ARDS=acute respiratory distress syndrome.

## Supplemental table 3. Characteristics of relapse in patients with BMI change during 32 weeks of treatment

| | Patients with BMI loss during 32 weeks of treatment | | |
|---|---|---|---|
| | **Alive (*N*=71)** | **Deceased (*N*=16)** | |
| | *N* (%) | *N* (%) | ***P*-value** |
| **Relapse** | | | P<0.001 [d] |
| Relapse | 3 (18%) | 14 (82%) | |
| No relapse | 68 (83%) | 2 (17%) | |

| | Patients without BMI gain during 32 weeks of treatment | | |
|---|---|---|---|
| | **Alive (*N*=379)** | **Deceased (*N*=42)** | |
| | *N* (%) | *N* (%) | ***P*-value** |
| **Relapse** | | | P<0.001 [d] |
| Relapse | 28 (42%) | 38 (58%) | |
| No relapse | 351 (99%) | 4 (1%) | |

| | BMI change groups during 32 weeks of treatment | | |
|---|---|---|---|
| | **BMI loss (*N*=17)** | **No BMI loss (*N*=66)** | ***P*-value** |
| | *N* (%) | *N* (%) | |
| **Patients with relapse** | | | |
| Time to first relapse (yrs.) Median (range) | 1.91 (0.81-3.83) | 1.63 (0.18-7.35) | P=0.090 [c] |
| Time from first relapse to death (yrs.), median (range) | 1.17 (0.40-2.64) | 0.75 (0.04-6.20) | P=0.495 [c] |
| **Location of 1ste relapse** | | | NA |
| Bone Marrow/Blood | 9 (53%) | 40 (61%) | |
| Isolated CNS | 3 (18%) | 15 (23%) | |
| Isolated Testis | 0 (0%) | 3 (5%) | |
| Other | 1 (6%) | 3 (5%) | |
| Bone Marrow + CNS + Other | 1 (6%) | 0 (0%) | |
| Bone Marrow + CNS | 2 (12%) | 2 (2%) | |
| Bone Marrow + Testis | 1 (6%) | 3 (5%) | |

*[a] chi-square test ; [b] linear-by-linear association ; [c] man whitney test ; [d] Fisher's exact test*

**Supplemental table 4: Summary of the Dutch and the CDC definitions of BMI categories used in children.**

| | Dutch cohort (SDS) | Dutch cohort (percentile) | Dutch cohort (*N*,(%)) | CDC 2000 (percentile) | CDC 2000 (SDS) | CDC 2000 (*N*,(%)) |
|---|---|---|---|---|---|---|
| Underweight | <-1.8 | <4th | 59 (8) | <5th | <-1.6 | 81 (11) |
| Normal weight | -1.8 - 1.1 | 4 - 86th | 584 (77) | 5 - 85th | -1.6 - 1.0 | 553 (73) |
| Overweight | 1.1 - 2.3 | 86 - 99th | 81 (11) | 85 - 95th | 1.0 – 1.6 | 90 (12) |
| Obese | >2.3 | >99th | 14 (2) | >95th | >1.6 | 14 (2) |

Abbreviations: BMI=body mass index; *N*=number; CDC = Centers for Disease Control and Prevention.