Hyperlipidemia is a risk factor for osteonecrosis in children and young adults with acute lymphoblastic leukemia

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doi:10.3324/haematol.2016.160507

Supplementary material: "Hyperlipidemia is a risk factor for osteonecrosis in children and young adults with acute lymphoblastic leukemia."

Supplementary methods:

Therapy of acute lymphoblastic leukemia (ALL)

Below is described in details the therapy for the Nordic Society of Paediatric Haematology and Oncology (NOPHO) ALL2008 protocol. Patients were stratified into standard-risk (SR), intermediate-risk (IR) and high-risk therapy based on immunophenotype, white blood cell count at diagnosis, cytogenetics, central nervous system involvement and treatment response (i.e. minimal residual disease). Induction therapy consisted of doxorubicin, vincristine, intrathecal methotrexate and glucocorticosteroids. The glucocorticosteroid during induction was prednisolone for non-high-risk patients and dexamethasone for high-risk patients. Consolidation I (Cons-I) for SR and IR patients consisted of 6-mercaptopurine (6MP), vincristine, high-dose-methotrexate and five doses of pegylated asparaginase (PEG-ASP) injections with two weeks intervals. Delayed intensification I (DI-I) and Consolidation II (Cons-II) consisted of vincristine, intrathecal methotrexate, daunorubicine (only for IR patients), 6-thioguanine (6-TG), cyclofosfamide (only for IR patients), cytarabine, two seven-day-blocks of dexamethasone and PEG-ASP. PEG-ASP during DI-I and Cons-II included a randomization for SR/IR patients starting on Day 99 continuing into Maintenance I (MT-I). PEG-ASP was given as ten doses every two weeks (standard arm) or three doses every six weeks (randomization arm) (clinicaltrials.gov no: NCT00819351). MT-I consisted of high-dose methotrexate, vincristine and continuous oral 6MP/methotrexate. Dexamethasone therapy for SR patients, during MT-I consisted of five, five-day blocks and for IR patients of four, five-day blocks of vincristine-dexamethasone reinductions. In addition, IR patients received a

delayed intensification II (DI-II) including vincristine, cyclofosfamide, 6-TG, cytarabine and two seven-day-blocks of dexamethasone in week 60 and 62.

For high-risk patients, post-induction therapy consisted of three chemotherapy-blocks (Blocks A, B and C) with seven blocks for patients with minimal residual disease after block A1 below 0.1% (A1-B1-C1-A2-B2-A3-B3) and nine blocks for poorer responders (A1-B1-C1-A2-B2-C2-A3-B3-C3). In addition to a range of anti-leukemic agents all blocks included one PEG-ASP injection and block B included five-days of dexamethasone. After block-therapy, patients received MT-I, without PEG-ASP and dexamethasone. After MT-I high-risk patients received delayed intensification including vincristine, daunorubicine, 6-TG, cyclofosfamide, cytarabine two seven-day-blocks of dexamethasone and two injections of PEG-ASP with two weeks interval. Maintenance II continued without PEG-ASP and dexamethasone.

Lipid analyses

Lipid analyses were performed on Cobas 8000, c702 modul, enzymatic/absorption-fotometric methods. Highest measurable value of triglyceride (TG) was 62.7 mmol/L. One center contributing with measurements used for clinical purposes used Cobas c 501 (Roche Diagnostics) with enzymatic colorimetric methods. Low-density lipoprotein (LDL) was calculated by Friedewald Formula.

Sample validation

We performed a validation to test if analyses were comparable between i) two centers who contributed with clinical measurements and ii) clinical lipid analyses and analyses from thawed plasma samples. We analyzed TG, total cholesterol (CH), high-density lipoprotein (HDL) and LDL on blood samples with normal lipid levels (TG below 2 mmol/L) and blood samples with severe

hyperlipidemia (TG above 15 mmol/L). We found no deviation in lipid measurements during validation for either normal or hyperlipidemic plasma.

Statistics:

The Running mean with standard error (SE) is calculated, during the whole period for asparaginase treatment, i.e. Day 30–250 after ALL diagnosis, for the ON patients and the non-ON patients using the loess function in R. All measurements were included, if several measurements were taken on the same day. In the Cox regression analyses Day 0–250 we used only one measurement per day if several measurements were taken on the same day; we selected the highest value for TG, CH, and LDL and the lowest value for HDL. For these analyses, 1787 TG samples from 112 patients and 1555 CH samples, 855 LDL samples and 853 HDL samples from 110 patients were available. The median number of plasma samples Day 0–250 available per patient was 11 (range=1–104) and two non-ON patients had only TG measured. The receiver operating characteristic (ROC) curve does not necessarily provide us with the optimal cut-off because we do not have complete follow-up of all patients (some patients might be diagnosed with ON after study end). The follow-up time distribution was estimated by the reverse Kaplan-Meier method.

We used R for statistical analyses (Version 3.2.5, 2016, R Foundation for Statistical Computing, R Core Team: A language and environment for statistical computing).

Supplementary Table S1

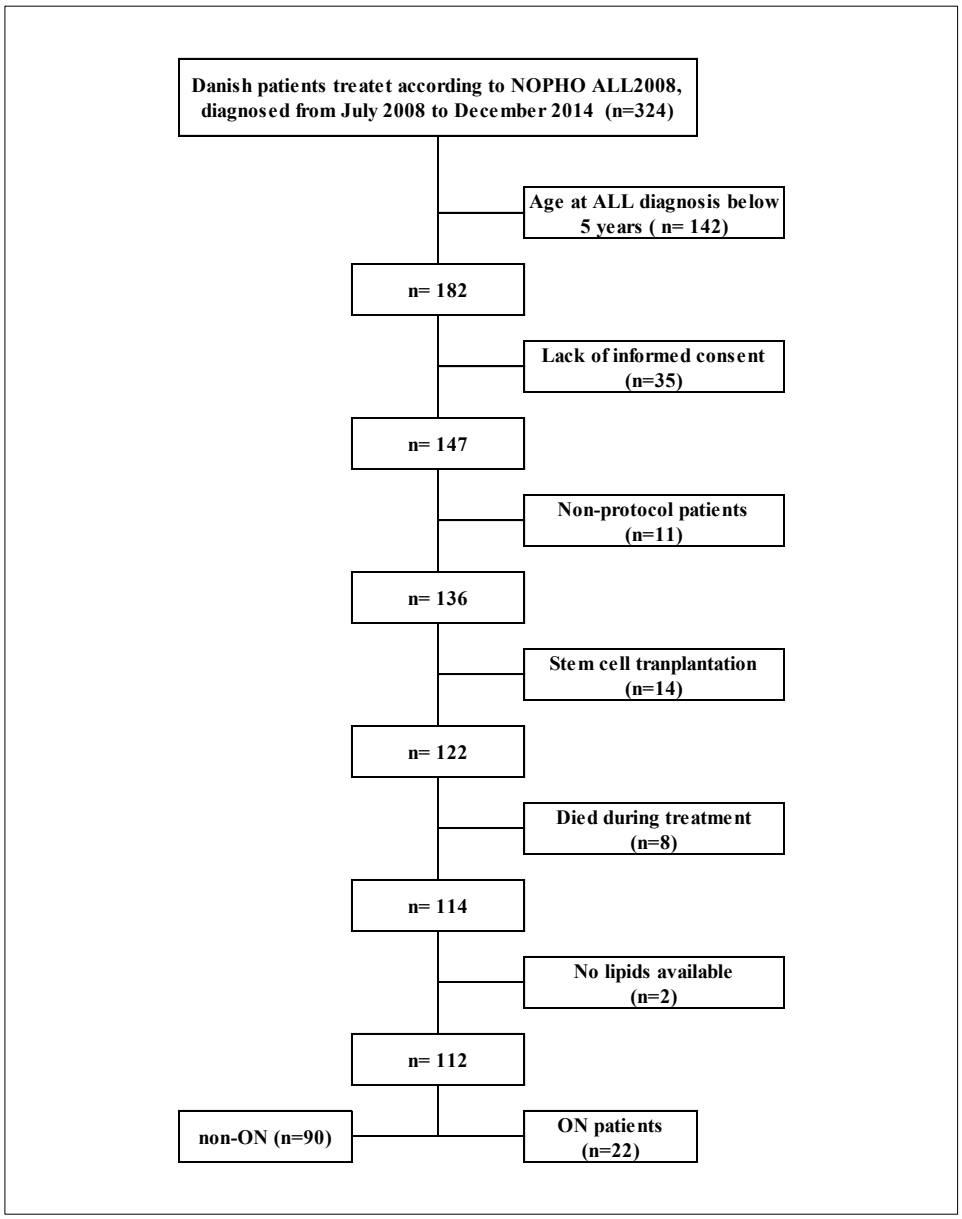
Table S1: Characteristics of 112 ALL patients

Characteristic	No. of patients	ON (n=22)	non-ON (n=90)
Age at ALL diagnosis (years)			
Median (IQR)	112	12.7 (8.35 –15.8)	10.9 (6.14–17.7)
Sex			
Males	65	12 (55%)	53 (59%)
Females	47	10 (45%)	37 (41%)
Risk Group			
Standard Risk	34	5 (23%)	29 (32%)
Intermediate Risk	61	15 (68%)	46 (51%)
High Risk	17	2 (9%)	15 (17%)
Highest WBC before ALL therapy ($x10^9$/l)			
Median (IQR)	112	11.1 (4.33–54.4)	11.2 (4.33–47.5)
Steroid during induction*			
Prednisolone	77	13 (59%)	64 (71%)
Dexamethasone	34	9 (41%)	25 (28%)
BMI at ALL diagnosis (kg/m^2)			
Median (IQR)	112	16.9 (15.9–19.5)	17.6 (15.1–21.0)

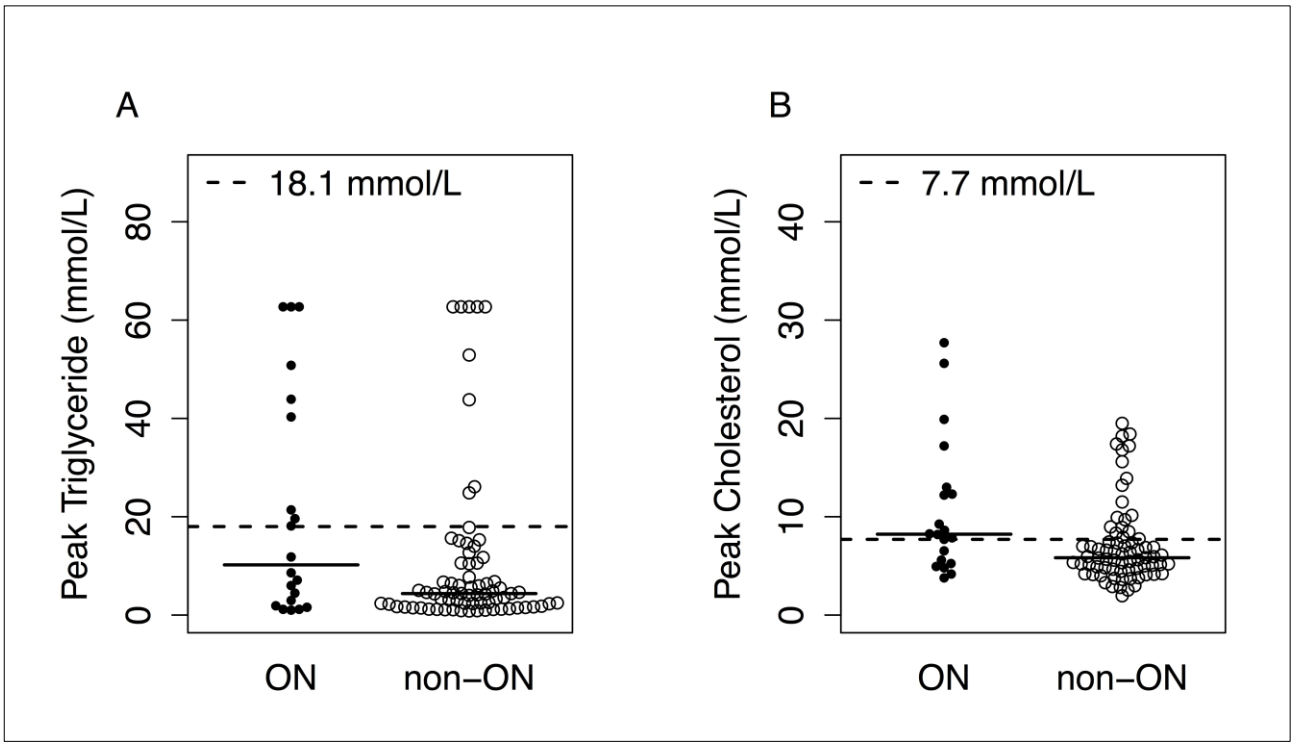
ALL: acute lymphoblastic leukemia, ON: osteonecrosis, IQR: interquartile range
WBC: white blood cell count, BMI: body mass index

*One non-ON patient had 'other' steroid during induction; thus, only 111 patients are included here.

Supplementary figures



Supplementary Figure S1: Flowchart of 324 patients in Denmark diagnosed with acute lymphoblastic leukemia (ALL) from July 2008 to December 2014 and treated according to the NOPHO ALL2008 protocol. Patients were excluded due to age below 5 years at ALL diagnoses (n=142), lack of consent (n=35), non-protocol treatment (n=11), stem cell transplantation (n=14) and death during therapy (n=8). Two patients did not have any lipid measurements available. Of the 112 patients included 22 were diagnosed with symptomatic osteonecrosis (ON) before end of follow-up August 27, 2015.



Supplementary Figure S2. Peak lipid levels and cut-off values. Illustration of peak levels and cut-off value for triglycerides (A) and cholesterol (B) during $DI_{ASP+DEX}$ (day 90–130 after diagnosis of acute lymphoblastic leukemia) for standard risk and intermediate risk patients. ● = ON patients and ○= non-ON patients. - - shows cut-off level at 18.1 mmol/L for triglyceride and 7.7 mmol/L for cholesterol. ─ shows the median for each group.