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Management of hyperleukocytosis in pediatric acute myeloid leukemia using immediate chemotherapy without leukapheresis: results from the NOPHO-DBH AML 2012 Protocol

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Authors' contribution

BZ, JA, HH and GK designed the research study. BZ, NAC, DC, BDM, JMFN, HH, KJ, KLJ-D, GK, ZK, OGJ, BL, MM-K, JP, RP, CJP, KS and JA provided patient consent and clinical information. BZ, UNN, AT and JA analyzed the results. BZ and JA wrote the paper. All authors commented and approved the manuscript.

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Requests for access to data used in this article for research purposes can be made to the senior author.

Abstract:

Hyperleukocytosis (HL) in pediatric acute myeloid leukemia (AML) is associated with severe complications and inferior outcome. We report results on HL patients included in the NOPHO-DBH AML 2012 study. We recommended immediate start of full dose chemotherapy (etoposide [ETO] monotherapy for 5 days as part of the first course), avoiding leukapheresis (LA) and prephase chemotherapy (PCT).

Of 714 included patients, 122 (17.1%) had HL, and 111 were treated according to the recommendations with ETO upfront without preceding LA or PCT. The first dose was applied the same day as the AML diagnosis or the day after in 94%. ETO was administered via peripheral veins in 37% of patients without major complications. After initiation of ETO the remaining WBC on days 2-5 was 69%, 36%, 17% and 8% of the pre-treatment level. On day 3, 81% had a WBC<100 $x10^9$/L. Five-year event-free/overall survival (EFS/OS) for all HL patients was 52.9% (CI 44.4-63.0)/74.1 (66.4-82.6), compared to 64.9% (60.9-69.1)/78.9 (75.4-82.4), for non-HL patients (EFS *P*<0.001, OS *P*=0.1). Six-week early mortality was 4.1% for all HL patients (2.7% for the 111 patients treated with ETO upfront).

We conclude that management of HL in pediatric AML with immediate ETO monotherapy without LA/PCT is feasible, safe and effective. WBC reduction during the first days is comparable to reported results of LA, and outcomes seem at least equivalent to therapies including LA. Based on our results, we advocate abandoning LA in HL in pediatric AML. Instead, starting induction chemotherapy as early as ever possible is crucial.

Introduction

Hyperleukocytosis (HL) is commonly, but arbitrarily, defined as WBC above $100x10^9$/L (1). In pediatric acute myeloid leukemia (AML), HL carries an increased risk of early morbidity and mortality, mainly due to the effects of leukostasis (infarction, pulmonary involvement) and bleeding (2, 3), whereas tumor lysis syndrome is less frequent than in acute lymphoid leukemia (ALL) (4).

Both in adult and pediatric reports there has been an ongoing debate regarding the benefit of cytoreductive measures such as prephase chemotherapy (PCT) and leukapheresis (LA) or exchange transfusion (ET) preceding initiation of full AML therapy (5-8). The technical feasibility and safety of LA has been confirmed in various studies, but several authors have questioned its clinical benefit. Still, many authors advocate LA in situations complicated by severe leukostasis (9-11). Others have discussed possible drawbacks of LA, particularly delaying full AML therapy, and have advocated immediate AML chemotherapy (12, 13).

After evaluation of the existing literature and analysis of the Nordic Society of Pediatric Hematology and Oncology (NOPHO) experiences (14), the NOPHO-DBH consortium issued recommendations on the management of HL as an appendix to the NOPHO-DBH 2012 protocol (15). Since we considered the benefit of LA/ET as uncertain, we instead recommended to immediately initiate the first induction course (which starts with five days monotherapy with etoposide, ETO), as soon as the AML diagnosis was established, avoiding both PCT and LA/ET. Our hypothesis was that immediate chemotherapy would be at least as effective and safe as initial therapy with LA or ET. The aim of the present paper is to report results of this rather uncommon approach.

Methods

Definitions

Hyperleukocytosis (HL) was defined as a highest WBC before therapy of ≥ $100x10^9$/L. *Early death* is defined differently in various studies, so we decided to report on early death both within two weeks and within six weeks from diagnosis. To categorize WBC response to ETO therapy, we arbitrarily used the term *slow responder* (WBC >$100x10^9$/L on day 3, before 3rd dose of ETO).

Patients and therapy

The study includes patients enrolled in the NOPHO-DBH AML 2012 protocol, diagnosed between March 1st, 2013 and September 30th, 2021 from the NOPHO-DBH-SHIP consortium. This consists of the Nordic countries (Sweden, Denmark, Finland, Norway, Iceland), the Baltic countries (Lithuania, Estonia, Latvia), Belgium, the Netherlands, Hong-Kong, Israel, Spain and (after conclusion of this study) Portugal. Ethical committees of each country approved the protocol and all parents/participants provided informed consent. Eligibility criteria were age under 18 years and de novo AML, excluding patients with Down syndrome, myelodysplastic syndrome, acute promyelocytic leukemia, secondary AML and (appropriate only for non-HL patients) isolated chloromas without recurring AML related fusion genes.

Patients with HL were identified in the study database, and relevant data extracted. In addition, questionnaires were sent to the treating hospitals, asking for more detailed information about HL patients. Questions included WBC on day 1-6 of therapy, use of peripheral lines for chemotherapy, date of AML diagnosis and initiation of hydration, time point of first ETO dose, modifications of ETO dose, use of LA/ET and PCT, as well as questions on ICU treatment, ventilator therapy, and complications of HL and/or therapy. We received completed questionnaires for all of the 122 included HL patients.

AML treatment, management of HL

All patients were treated according to the NOPHO-DBH AML 2012 protocol, registered at the European Medical Agency (EUDract 2012-002934-35) and at www.clinicaltrials.gov (NCT01828489), with two randomized questions in first and second induction courses (16). The first AML course was either MEC (standard arm, mitoxantrone-ETO-cytarabine), or DxEC (experimental arm, liposomal daunorubicin [DNX]-ETO-cytarabine). The MEC course stems from the Japanese AML-99 protocol (17). Both MEC and DxEC commenced with five days of ETO monotherapy (150 mg/m^2 as 2-hour infusion daily), continuing with anthracycline (mitoxantrone or DNX) and cytarabine from day six. From November 2017 all patients received MEC since DNX became unavailable.

Guidelines related to the protocol included recommendations on the management of HL. In addition to hydration, cautious use of red blood cell transfusions, but liberal use of platelet transfusions, and use of rasburicase was encouraged. The first ETO dose (as part of course 1) was

to be started immediately after the AML diagnosis was confirmed. ETO could be administered via peripheral venous lines if insertion of a central venous line was considered too risky. Use of PCT and LA/ET was discouraged regardless of WBC and complications.

Statistics

Statistical analysis was performed using IBM SPSS statistics version 28 or R version 4.0.3 (R Foundation, Vienna, Austria). The chi-square test was used to compare the frequency of events. Continuous data were compared using the Student's t-test or Mann Whitney U test, as appropriate. Probability of overall survival (pOS) was calculated using the Kaplan Meier method. OS was defined as time elapsed between date of diagnosis and death. Event-free survival (EFS) was defined as time from diagnosis until death, resistant disease, relapse or second malignant neoplasm. Cumulative incidence of relapse (CIR) was calculated using time from diagnosis until relapse with all other events as competing events. Treatment-related mortality (TRM) was defined as all deaths, with relapse, resistant disease and SMN as competing events. Ninety-five percent confidence intervals (CI) for survival curves were calculated according to the method by Link (18). CIR and TRM were calculated using competing risks data analysis according to Fine and Gray (19). Estimates of EFS, OS, CIR and TRM are given at five years. All living patients were censored at time of last follow up but not later than August 31st, 2022. The log rank test was used to compare survival of subgroups. To estimate univariate and multivariate hazard ratios (HzR) for EFS and OS, Cox proportional hazards models were used. All tests were two-sided and p-values <0.05 were considered significant.

Results:

Patients: (Table 1)

One hundred and twenty-two out of 714 (17%) protocol patients had HL. They did not differ from Non-HL patients in terms of age, sex or CNS involvement, but had a higher proportion of extramedullary tumors (18% vs 11%). They had a significantly lower frequency of *RUNX1*::*RUNX1T1* fusions (2% vs. 15%), and a higher proportion of *KMT2A* rearrangements (*KMT2A*::*MLLT3 excluded*) (23% vs. 12%) and *FLT3*-ITD without *NPM1* mutation (21% vs. 8%).

HL patients: WBC, time intervals, tumor lysis prophylaxis

Median highest WBC in HL patients was 211x10^9/L (range 101-880). Sixty-nine patients (57%) had WBC >200x10^9/L (Table 2). A definite AML diagnosis could be established at the latest one day after admission in 111/118 cases (94%, information missing in four patients). Hydration was started on the day of admission or before admission, at the referring institution, in 77 of 88 reported cases (88%). In 10 cases hydration was commenced the day after (11%), and in one after two days. Hydration alone - in patients not treated with LA and/or PCT - resulted in a mean WBC drop from 228.6±11 x 10^9/L (highest value) to 192.9±11 x 10^9/L (value at start of ETO treatment). Rasburicase alone was used as tumor lysis prophylaxis or therapy in 70 cases (63%), allopurinol alone was used in 18 (16%), both drugs in 19 patients (17%), and in four patients neither allopurinol nor rasburicase was used (11/122 not reported).

PCT, LA and ET

Contrary to the recommendations, 11/122 patients (9%) were treated with PCT and/or LA/ET (Figure 1 and Table 2). Eight of 113 patients (7%, missing information in nine patients) received PCT, in some cases before the AML diagnosis had been confirmed. It consisted of typical AML-pretreatment (low dose cytarabine ± thioguanine) in three patients, steroids in three patients, and all-trans-retinoic acid/dexamethasone in one patient misdiagnosed as APL (PCT not specified in one). LA was performed in 6/119 patients (5%, information missing in three patients). In two patients, two procedures were done. The only ET was performed in a patient who also received LA. Time interval from LA to first ETO-dose was minus two in one patient (ETO start before LA), one in three patients, and two days in two patients. Median highest WBC in the six LA patients was 320 compared to 210 in patients not treated with LA (P=0.16). All six patients were admitted to the Intensive Care Unit.

ETO therapy, peripheral venous access

In 94% of 111 patients who received ETO upfront, the first dose was applied the same day or the day after the AML diagnosis was confirmed (101/107 patients, missing information in four cases). Duration of infusion was two hours in 96% of cases, in the remaining five cases it was three or four hours. The ETO dose was modified in two of 111 patients receiving ETO upfront (aborted after second dose in one patient, dose reduced to 90% in one due to TLS).

ETO was applied through a peripheral venous line in 43 of 115 reported cases (37%). The peripheral line was used for one day in eight patients, for two to four days in 14 patients, and five

days in 13 patients (missing data in eight patients). In one patient treated for five days, ETO infusion was complicated by extravasation, which was treated with local hyaluronidase leaving no later sequelae. One patient had a superficial thrombophlebitis. No other complications were reported.

Response to ETO monotherapy (Figure 2)

The 111 patients receiving ETO monotherapy upfront were included in the following analyses. In one of these patients, ETO was aborted after two doses due to increasing WBC, and he proceeded to LA. This patient is included on intention-to-treat basis. Table 2 displays characteristics of ETO patients compared to the 11 patients receiving LA or PCT as initial therapy. The latter had significantly higher WBCs.

After initiation of ETO the remaining WBC on days 2-5 was 69%, 36%, 17% and 8% of the pre-treatment level, i.e. a reduction of 50% per day from day 2 (Figure 2A). It is important to note that the interval between start of ETO and WBC value day two (mean 16 hours), is shorter than the intervals between WBCs the other days (mean 24 hours), which may explain the smaller reduction in WBC between day one and two. On day three (after two ETO doses), 89/110 patients (81%) had a WBC below $100x10^9$/L and 84/106 (80%) were below 50% of the WBC of day 1.

Figure 2B displays WBC curves for all 111 ETO patients during the first 5 days. According to our definition (WBC > 100 on day 3, i.e. after two ETO doses), we identified 21 slow responders. Compared to good responders, they had significantly higher WBCs (mean 324 vs. $169x10^9$/L, $P<0.001$), more frequently *FLT3*-ITD (10/20, 50.0%, vs. 20/88, 22.7%, $P=0.04$) and less frequently FAB type M4/M5 (8/17, 47% vs. 57/72, 79%, $P=0.007$) (different denominators due to missing data). Importantly, in all but one slow responders the WBC had dropped on day 3 (in 12/21 to <67% of the value before ETO) and continued to decrease in the following days. On day 6, after five doses of ETO, 19/21 had a WBC of $< 50x10^9$/L.

Complications (Table 2)

The most feared complications of HL are pulmonary leukostasis and cerebral hemorrhage and/or infarction. Nearly half of all HL patients required supplemental oxygen, and 17% mechanical ventilation, which usually reflects severe pulmonary leukostasis. Although only reaching borderline significance, the proportion of HL patients requiring mechanical ventilation was higher in HL patients with FAB M4/M5 (15/72, 21%) than in the other FAB groups (1/25, 4%), $P=0.06$. CNS

hemorrhage and/or infarction was seen in 5% of the patients. 17% had tumor lysis syndrome, of which one patient needed hemodialysis. Other complications were bleedings outside the CNS (7%), cardiac complications (8%) and thromboembolic events (9%). Cardiac complications included reduced ventricular function (3), pericardial effusion (2), tachycardia (2), pulmonary hypertension, and chloroma on tricuspid valve. Thromboembolic events included pulmonary thrombosis (2), thromboses in vena porta (2), vena cava inferior (2), femoral vein and some minor CVL-related thromboses. Disseminated intravascular coagulation (DIC) occurred in 5.6% of patients treated with ETO upfront, but in none of the 11 LA/PCT patients. This may represent a selection bias, since ongoing DIC could be a relative contraindication for LA. Sixty-five of 120 patients (54.2%) had no registered complications (supplemental oxygen not counted as complication).

Outcome (Figure 3)

EFS_{5y} was lower for HL patients (52.9% [CI 44.4-63.0]) than for patients without HL (64.9% [CI 60.9-69.1], *P*<.001, Hazard ratio (HzR) 1.64 [CI 1.23-2.20]), due to an increased frequency of resistant disease in HL (14/122 vs 23/592, *P*<0.001). Multivariate Cox regression, adjusting for main cytogenetic subgroups (table 1), showed that HL remained an independent adverse factor (HzR 1.55 [CI 1.14-2.10], P=0.005). There was no significant difference in CIR_{5y} (27.5% [CI 19.4-36.1] vs 24.8 [CI 21.2-28.6], *P*=0.32) or TRM_{5y} (7.4% [CI 3.6-12.9] vs 5.9 [CI 4.2-8.1], *P*=0.47). Overall survival at 5 years was 74.1% (CI 66.4-82.6) in HL patients compared to 78.9% (75.4-82.6) in Non-HL patients (*P*=0.1, HzR 1.38 [CI 0.93-2.06]). Including genetic subgroups in multivariate Cox regression showed a hazard ratio of 1.22 (CI 0.83-1.88, P=0.28) for HL.

Compared to the non-HL group, HL patients had a trend to higher six-week early death rate (5/122, 4.1% vs. 13/592, 2.2%, *P*=0.06). Details on the five early death patients are shown in Table 3. Three out of 122 HL patients (2.5%) died within two weeks. Of 69 patients with WBC >200 $x10^9$/L, 14 patients died, but only three during the first six weeks. Three of 111 patients treated with ETO upfront died within six weeks (3%), compared to 2/11 (18%) in the PCT/LA group (*P*=0.06), Table 2.

Discussion

We report on 122 paediatric AML patients with HL treated on the NOPHO-DBH AML 2012 protocol. The immediate start of full dose etoposide monotherapy, without chemo prephase or

invasive procedures regardless of WBC and FAB type, was feasible and effective. A reduction of WBC to < $100x10^9$/L was obtained after two doses of ETO in 82% of patients. Overall survival was 74.1%, which was not significantly different from patients without HL. Slow responders were significantly more often *FLT3*-ITD mutated and had a significantly lower proportion of FAB type M4/M5. Early mortality within six weeks from diagnosis was low (4.1% for all, 1.8% for patients receiving ETO upfront without chemo-prephase or LA), and reported complications were similar to previous studies.

The high number of patients in this study, the adherence to the recommended management of HL patients and the detailed documentation of early treatment and WBC counts allowed a thorough evaluation of the effectiveness of five days of upfront ETO monotherapy which was part of the first chemotherapy course. Only nine percent of patients received PCT and/or LA. However, because of the small number of these patients and a selection bias (significantly higher WBCs) a meaningful comparison with patients treated with upfront ETO chemotherapy is impossible.

There is an ongoing debate on the benefit of LA in both adult and pediatric AML. Most authors agree that the technical aspects of LA are well established, and that the procedure can be performed safely in both adults and children/adolescents (7, 13, 20-25). Controversies exist regarding the clinical benefit of LA as an initial therapeutic measure. The main argument against LA is that a beneficial effect on early clinical outcomes has not been shown in clinical trials (7, 8, 10, 11) and that long term prognosis is not improved (26-28). Other arguments against the procedure include the requirement of a central venous catheter, the decrease in number of platelets with the procedure, the rapid rebound of blasts after LA, possible time delay before full dose chemotherapy, costs, and the fact that the procedure is not available everywhere (29, 30).

Presently, in adult AML, most authors discourage routine use of LA in HL patients, but recommend the procedure in selected cases with manifest leukostasis (10, 26, 27, 31, 32). In pediatric AML, the picture is not so clear, perhaps due to smaller patient numbers in studies on HL. A recent population-based study from the US showed that LA is still widely used in pediatric leukemias, however with decreased frequency over recent years (33). The BFM group has seen a trend towards reduced early death following LA in selected cases, and has recommended LA/ET in patients with WBC above 200 and in patients with WBC 100-200x10^9/L and FAB M4/M5 (6). Following these recommendations, 102/115 patients (88%) in our study would have been candidates for LA. The largest study on HL so far suggested that LA did not reduce induction

mortality, but data on LA were available only for a subgroup of patients (89/256), of whom 18% received LA (8). A recent study from St Jude on 49 children diagnosed between 1997 and 2017 reported no advantage for LA (N=16) compared to cytoreduction with low-dose cytarabine (N=18) or upfront protocol chemotherapy (N=14) (34). A single small pediatric study reported good results using immediate chemotherapy without invasive cytoreductive measures (12).

In our trial, the use of LA/ET was discouraged regardless of WBC and pre-existing complications. We found that immediate start of full dose chemotherapy (in our case ETO monotherapy for the first five days) has an effect on WBCs comparable to LA/ET in the majority of patients, also for very high WBCs. After one and two days of therapy (1 and 2 ETO doses), WBC was reduced to 68% and 33% compared to the value before therapy. These are numbers comparable to the effect of LA. In leukemias with HL, a single LA can reduce the WBC by 30-60% (35). In adult AML, one study reported that in 40% of cases LA did not reduce WBC counts significantly, while 60% achieved a WBC count of less than 100 x 10^9/L (36). Others reported WBC decreases of 50% (37) and even 71% (21). Three pediatric studies reported a median decrease after LA of 60% (22), an overall decrease after one or several LAs of 60% (23) and a mean 50.7% reduction after a single LA procedure and additional 17.1% reduction after a second TL procedure (25). However, patient numbers in the pediatric studies were small; the three mentioned studies include a total of 41 pediatric patients (14 of these being ALL).

The 20% slow responders to ETO monotherapy had significantly more often *FLT3*-ITD and/or non-M4/M5 FAB types. FAB M4/M5 is known to be associated with a higher risk of leukostasis, and we observed a trend towards more frequent need of mechanical ventilation in FAB M4/M5 compared to other FAB groups. Our findings support that immediate chemotherapy may be as good as or better than LA even in M4-M5 leukemias with complications due to leukostasis.

One of the 111 patients in our study receiving ETO as initial therapy was a non-responder, with increasing WBCs after two ETO doses. This patient proceeded to LA on day four but died some days later. Alternatives to LA would have been to introduce an anthracycline earlier or even just to continue ETO. However, it is impossible to conclude whether a different approach could have improved outcome.

We have used five days of ETO monotherapy to reduce initial WBCs, since this was the first part of our first induction course. It can be assumed that a different induction chemotherapy may have resulted in a similar reduction in WBC during the first days. However, an important advantage of

ETO monotherapy is its very convenient administration as a two-hour infusion once daily, which facilitates the immediate start of chemotherapy. Placing a central venous line is not necessary, since the drug can be administered via peripheral venous access and the risk of severe damage due to extravasation is low. In our study, more than one third of the patients received at least part of their ETO treatment via peripheral venous access, the only complication being one extravasation, which left no sequelae.

We discouraged the use of PCT such as low-dose cytarabine. Accordingly, only a few patients received such treatment. Three of eight patients received ALL-directed therapy (steroids) since the leukemia initially was misinterpreted as ALL. In a recent review on HL in acute and chronic leukemias it was stated that "it is increasingly important to administer 'bridging' (non-definitive) chemotherapy to mitigate the risk of leukostasis while awaiting the detailed leukemia characterization required for a definitive treatment plan" (9). In our experience, non-definitive chemotherapy is rarely necessary. In over 90% of our patients, a definitive diagnosis could be established within one day from admittance. Nonetheless, in leukemias where the diagnosis cannot be timely established, treatment with a combination of etoposide and steroids seems reasonable in urgent cases.

Our six-week early mortality of 4.1% for all HL patients, and 1.8% for patients receiving ETO upfront without chemo-prephase or LA/ET compares favorably to previous studies. Death within two weeks occurred in 2.4% of all patients and 1.8% of those receiving ETO upfront. Only one of 70 patients with WBC > 200 $x10^9$/L died within the first two weeks. A previous NOPHO study reported an early death rate of 30% in patients with WBC > 200 x 10^9/L (14). In a BFM study, 2-week early mortality of bleeding and/or leukostasis was 1.8% in 1251 pediatric patients with AML. Patients with WBC > 100 x 10^9/L had an early death rate of 2.3%. Patients with WBCs > 200 x 10^9/L had a rate of 14.3%, and if they had a FAB type of M4/M5 it was 20% (6). In nine consecutive St-Jude AML studies from 1963 to 2002, early death rate in patients with HL was 22.9% in the first period before 1983, but only 2.8% in the later period (38). In their most recent study, no early death occurred in 49 HL patients from diagnosis to two weeks after initiation of protocol therapy (34). Sung et al reported an induction death rate of 1.3% for patients with WBCs < 100 x 10^9/L, and 3.4%, 1.5%, 8.0% and 10.5% for WBCs of 100-200$x10^9$/L, 200-300$x10^9$/L, 300-400$x10^9$/L, and > 400$x10^9$/L respectively (8). In all the cited studies, LP and/or ET was performed in a proportion of patients (13.4 to 37%).

We conclude that management of HL in pediatric AML with immediate chemotherapy without invasive cytoreduction is feasible, safe and results in a rapid reduction of WBC. HL patients had lower EFS due to a higher rate of resistant disease, but OS was excellent compared to other protocols using LA/ET as part of their therapy. Ideally, a very large randomized trial would be needed to provide a final answer on the possible benefit of LA. However, it seems unlikely that such a study ever will be conducted, given the rarity of the condition, the urgency for decision making, and physician preferences (10). In light of this, and based on our results, we advocate abandoning LA in HL in pediatric AML. Instead, starting induction chemotherapy as early as ever possible is crucial.

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Table 1. Characteristics and outcome of 714 patients included in the study. Patients with hyperleukocytosis (HL) compared to all other patients.

	HL (n=122) Count (%)	Non-HL (n=592) Count (%)	*p*
Male sex	63 (52)	301 (51)	.87
Age < 2 years	33 (27)	133 (22)	.71
2-9 years	42 (34)	210 (36)	
10-14 years	33 (27)	168 (28)	
15-18 years	14 (12)	81 (14)	
CNS involvement	22/119 (19)	75/587 (13)	.10
Extramedullary tumor	20/113 (18)	62/584 (11)	.03
Genetic subgroups			
RUNX1::*RUNX1T1*	2 (1.7)	91 (15)	<.001
CBFB::*MYH11*	13 (11)	54 (9.1)	.58
KMT2A::*MLLT3*	14 (12)	63 (11)	.76
KMT2A other	28 (23)	72 (12)	.002
FLT3-ITD no *NPM1*	25 (21)	48 (8.1)	<.001
FLT3-ITD with *NPM1*	7 (5.8)	11 (1.9)	.01
NPM1 no *FLT3*-ITD	4 (3.3)	22 (3.7)	.83
Other aberrations	28 (23)	231 (39)	<.001
Outcome			
Early death (6 weeks)	5 (4.1)	13 (2.2)	.06
Complete remission	100 (82)	552 (93)	<.001
Resistant disease	14 (12)	23 (3.9)	<.001
Treatment related mortality (5y)	7.4% (CI 3.6-12.9)	5.9% (CI 4.2-8.1)	.47
Cumulative incidence of relapse (5y)	28% (CI 19.4-36.1)	25 (CI 21.2-28.6)	.32
Event-free survival (5y)	53% (CI 44.4-63.0)	65% (CI 60.9-69.1)	<.001

Overall survival (5y)	74% (CI 66.4-82.6)	79% (CI 75.4-82.6)	.10

WBC, White blood cell count; CNS, central nervous system. WBC, White blood cell count; CNS, central nervous system.

Table 2. Clinical and biological characteristics, complications and outcome for all 122 hyperleukoctosis (HL) patients combined and split according to if they received Etoposide (ETO) monotherapy upfront or commenced therapy with prephase chemotherapy (PCT) and/or leukapheresis (LA).

	All HL patients (n=122)	Etoposide upfront (n=111)	PCT and/or LA* (n=11)	*p*
Male sex	63 (52%)	56 (51%)	7 (64%)	.53
Age mean (median/range)	7.1±0.5 (7.0/0-17)	7.3±0.5 (7.0/0-17)	4.8±2.0 (0/0-17)	.35
Highest WBC before therapy (x 10^9/L) mean (median/range)	236±11 (211/101-880)	228±11 (210/101-880)	313±32 (329/145-481)	.02
WBC > 200 x 10^9/L	69 (57%)	60 (54%)	9 (81%)	.08
CNS involvement	20/109 (18%)	18/100 (18%)	2/9 (22%)	.89
FAB-type				
M0/M1/M2	26/100 (26%)	24/89 (27%)	2/11 (18%)	.57
M4	24/100 (24%)	20/89 (23%)	4/11 (36%)	
M5	49/199 (50%)	45/89 (51%)	4/10 (40%)	
Genetic subgroups				
RUNX1::RUNXT1	0/117 (0%)	0/106 (0%)	0/11 (0%)	
CBFB::MYH11	14/117 (12%)	14/107 (13%)	0/10 (0%)	.004
KMT2A::MLLT3	11/113 (10%)	11/103 (11%)	0/10 (0%)	.21
KMT2A-other	28/114 (25%)	25/105 (24%)	3/9 (33%)	.09
FLT3-ITD no *NPM1*	33/120 (28%)	30/108 (28%)	3/12 (25%)	.81
Other aberrations	35/103 (34%)	31/88 (35%)	3/8 (38%)	.77
Therapy, complications and early death rate				
Interval from day of diagnosis to day of 1st ETO (days)	0.5±0.06 (0.0/0-3)	0.4±0.06 (0.0/0-3)	0.9±0.18 (1.0/0-3)	0.05
Care at ICU	45/120 (38%)	34/109 (31%)	11 (100%)	<0.001
Mechanical ventilation	19/120 (17%)	15/109 (14%)	4 (36%)	.07
CNS hemorrhage and/or infarction	5/121 (4.1%)	4/110 (3.6%)	1 (9.1%)	.32

Bleedings outside CNS	8/120 (6.7%)	7/109 (6.4%)	1 (9.1%)	.55
Thromboembolic event	11/121 (9.1%)	9/109 (8.3%)	1 (9.1%)	.63
DIC	6/115 (5.2%)	6/108 (5.6%)	0/8 (0%)	.49
Tumor lysis syndrome	20/121 (17%)	16/109 (15%)	3 (33%)	.24
Cardiac complications	9/120 (7.5%)	7/109 (6.4%)	2 (18%)	.06
Infection grade 3 or 4 CTCAE	50/120 (42%)	45/110 (41%)	5/10 (50%)	.58
No complications	65/120 (54%)	63/109 (58%)	2 (18%)	.01
Early death < 2 weeks	3 (2.5%)	2 (1.8%)	1 (9.1%)	.25
Early death < 6 weeks	5 (4.1%)	3 (2.7%)	2 (18%)	.06

WBC, White blood cell count; CNS, central nervous system; FAB, French-American-British classification; ICU, Intensive Care Unit; DIC, Disseminated intravascular coagulation; CTCAE, Common Terminology Criteria for Adverse Events.

** One LA patient received both LA and exchange transfusion.*

Table 3: Early deaths (during the first six weeks from diagnosis). Patient 3 (*) was included in the early death group since the secondary complications leading to death started well before the end of week 6.

	Sex, age (y)	WBC	Prephase chemo	LA	DTD	Comments
Patient 1	F, 0	164	Low dose Cytarabine, thioguanine	No	4	Never started ETO. Died of sepsis.
Patient 2	M, 5	397	No	No	3	Rapid response to ETO. Died of brain hemorrhage.
Patient 3*	M, 0	232	No	No	45	Infant, diagnosed 2 weeks after birth. Tumor lysis syndrome. Rapid response to ETO. Secondary fever with elevated CRP. Systemic fungal infection with Trichosporon asahii. In spite of antifungal treatment gradual hepatic failure, and multiorgan failure.
Patient 4	F, 9	101	No	Yes	7	Upfront ETO. After second dose, increasing WBC from 92 to $124x10^9$/L and deteriorating clinical condition. ETO therapy aborted and LA performed the day after, resulting in a WBC drop from 161 to $39x10^9$/L. Died of multiorgan failure.
Patient 5	M, 17	350	No	Yes	20	LA on the day of admission (and AML diagnosis). The following day, WBC was $215x10^9$/L and ETO therapy was commenced. Died of sepsis.

WBC White blood cell count, LA Leukapheresis, DTD Days to death (from diagnosis), ETO Etoposide, F Female, M Male.

Figure legends

Figure 1. Initial management of the 714 patients included in the study. Green color indicates patients treated according to the recommendations with ETO upfront. One of these received a leukapheresis on day 4 due to poor response to ETO. In one LA patient, both LA and Exchange transfusion were performed. HL Hyperleukocytosis, PCT Prephase chemotherapy, LA Leukapheresis, ETO Etoposide.

Figure 2. White blood cell counts (WBC) before and during etoposide monotherapy in 111 pediatric AML patients with hyperleukocytosis. Highest: Highest WBC before therapy. D1 ETO: WBC on day of first etoposide dose. The last WBC value before etoposide infusion was reported. D2-D6: WBC one to five days after first etoposide dose.

A) Average and standard deviation. Related to WBC on day 1, the remaining WBC percentage on days 2-5 was 69%, 36%, 17% and 8%.

B) WBCs for each of the 111 patients. Dotted line: Patient with highest WBC 880 x 10^9/L, for the sake of readability only part of the curve is displayed (not shown: day 1, WBC 862; day 2 WBC 818; day 3, WBC 626). Red line: Non-responding patient who received leukapheresis on day 4 (patient 4 in Table 3).

Figure 3. Outcome in patients with hyperleukocytosis (HL, n=122) compared to patients without HL (n=592).
3A left panel: Event-free survival HL 52.9% (CI 44.4-63.0%) vs no HL 64.9% (CI 60.9-69.1%), P<0.001. 3B right panel: Overall survival HL 74.1% (CI 66.4-82.6%) vs no HL 78.9% (CI 75.4-82.4%), P=0.1.

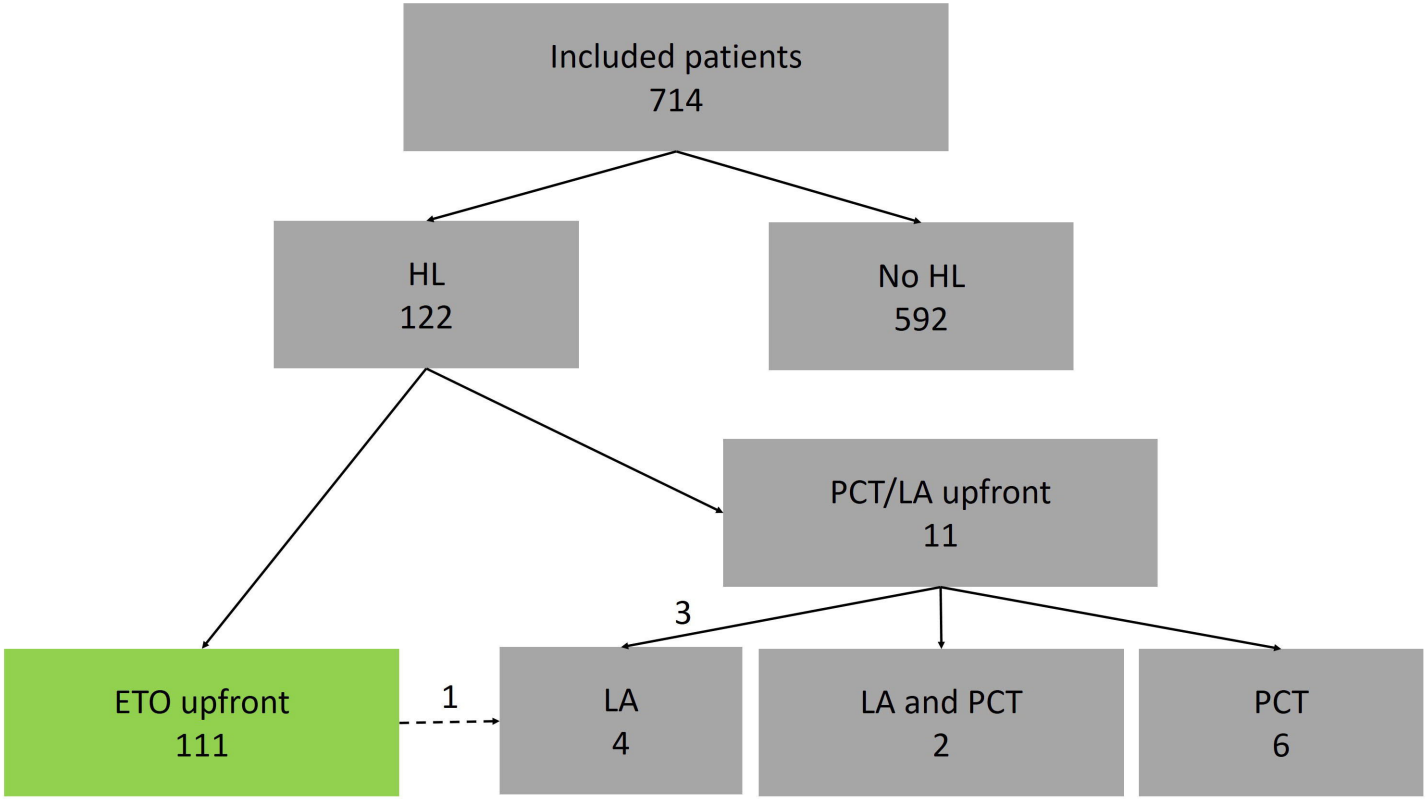

Included patients
714
HL
122
No HL
592
PCT/LA upfront
11
3
ETO upfront
111
1
LA
4
LA and PCT
2
PCT
6

Figure 2A

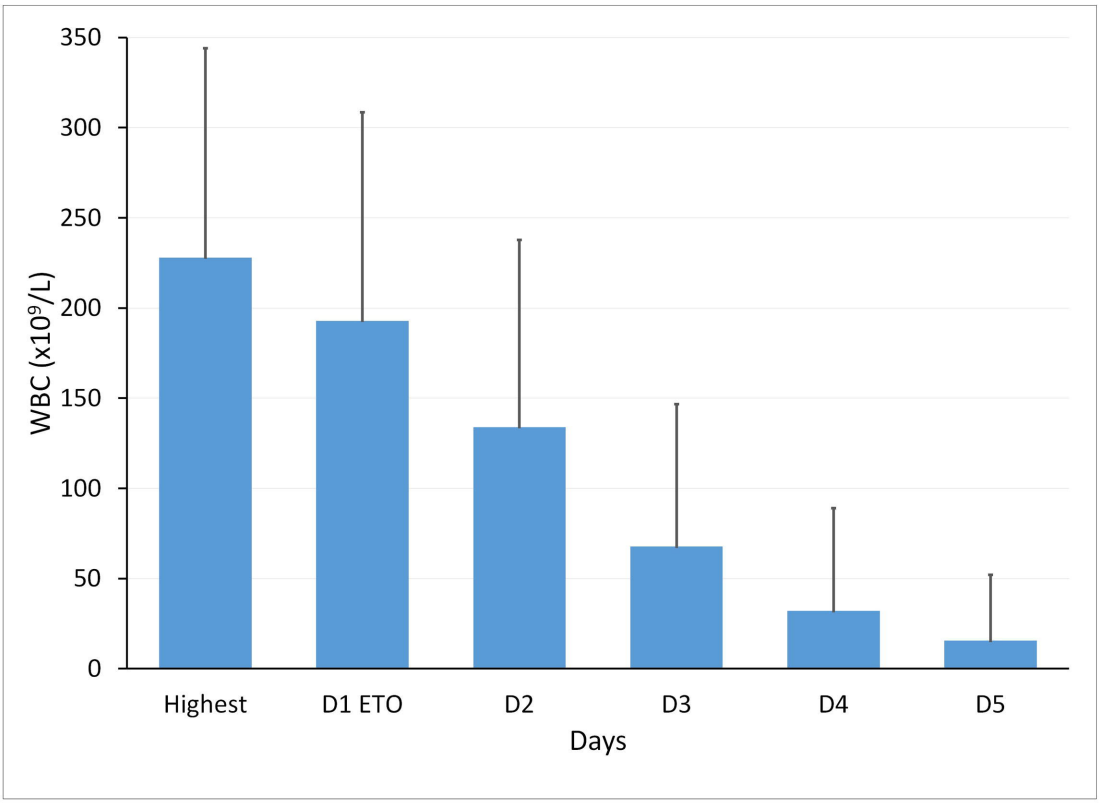
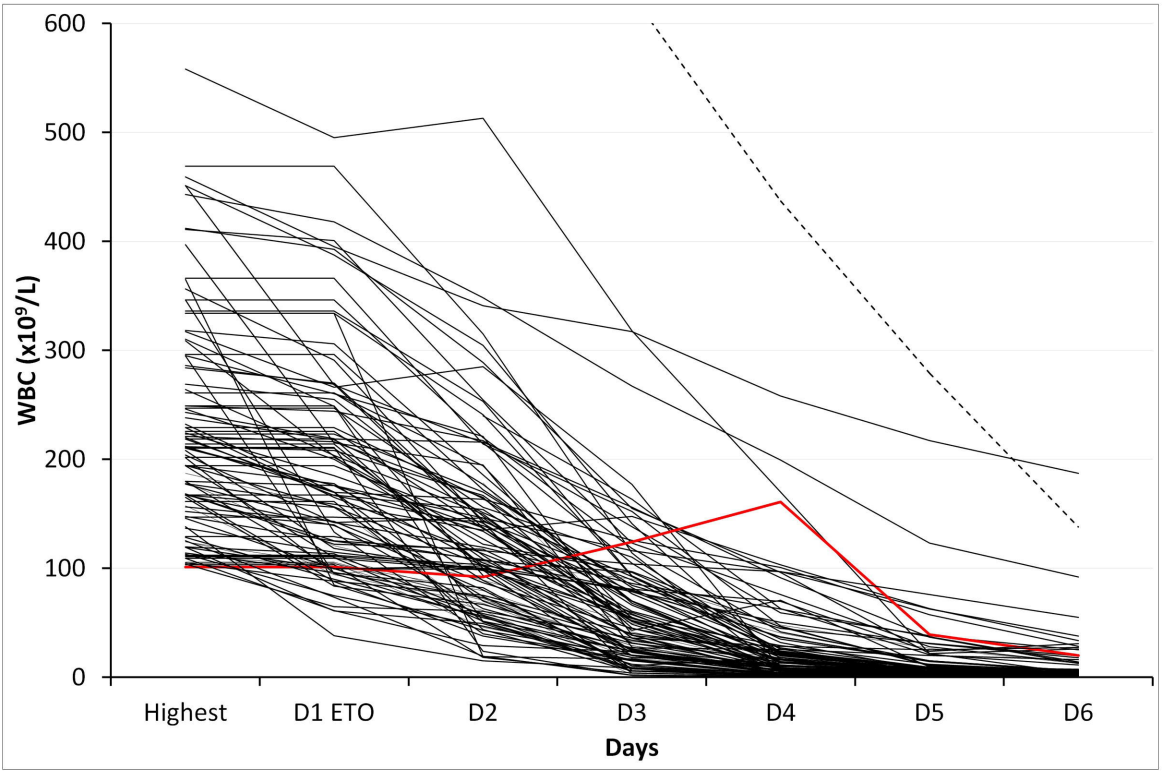
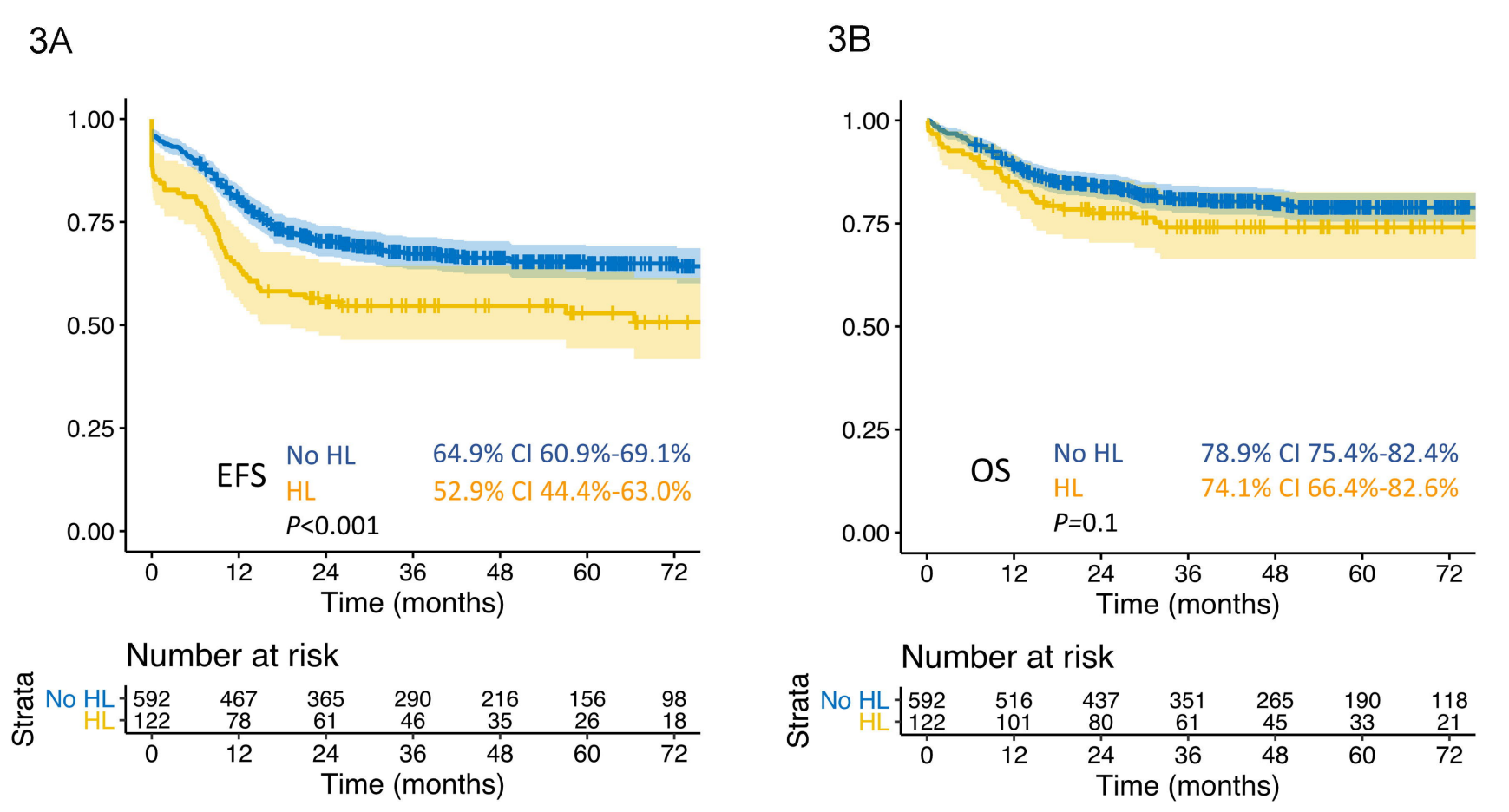


Figure 2B




3A
1.00
0.75
0.50
0.25
0.00
EFS
No HL
HL
P<0.001
64.9% CI 60.9%-69.1%
52.9% CI 44.4%-63.0%
0
12
24
36
48
60
72
Time (months)
Number at risk
Strata
No HL 592 467 365 290 216 156 98
HL 122 78 61 46 35 26 18
0
12
24
36
48
60
72
Time (months)
3B
1.00
0.75
0.50
0.25
0.00
OS
No HL
HL
P=0.1
78.9% CI 75.4%-82.4%
74.1% CI 66.4%-82.6%
0
12
24
36
48
60
72
Time (months)
Number at risk
Strata
No HL 592 516 437 351 265 190 118
HL 122 101 80 61 45 33 21
0
12
24
36
48
60
72
Time (months)