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Prognostic factors for hematotoxicity of chemotherapy in aggressive non-Hodgkin's lymphoma

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On behalf of the German High-Grade Non-Hodgkin's Lymphoma Study Group (DSHNHL)

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Background: Little is known on the heterogeneity of hematotoxicity in patients receiving multicycle chemotherapy.
Patients and methods: We analyzed data of 1399 patients with aggressive lymphoma from trials using CHOP (combination chemotherapy with cyclophosphamide, doxorubicin, vincristine and prednisone)-like therapies. Multivariate modeling was carried out for leukocytopenia, thrombocytopenia and anemia and the models were validated by two large independent datasets from trials with/without usage of the CD20-antibody rituximab.
Results: On the basis of these models, we are able to predict the remarkable heterogeneity of hematotoxicity and propose to use risk groups. Regarding leukocytopenia, the low toxicity risk group experienced World Health Organization grade 4 in <10% of the cycles while the high toxicity risk group in almost all cycles. For thrombocytopenia, groups were detectable with almost no grade 3 or 4 toxicity and others where two out of three cycles were affected. In a separate set of models, the first cycle toxicity was the strongest predictor for later hematotoxicity. The risk for leukocytopenia was associated with infections, antibiotic use, hospitalization and treatment-related mortality, indicating the clinical usefulness of the models. For the first time, a Web-based tool is made available to easily predict the hematotoxicity in clinical practice (www.toxcalculator.com).
Conclusion: This analysis has implications for patient management and prophylaxis.
Key words: aggressive lymphoma, hematotoxicity, prognostic factors, prophylaxis

introduction

It is a common practise that chemotherapy for cancer treatment is dosed proportional to the body surface. This practise is, however, not ideal [1–3]. Dose-limiting hematotoxicity (DLHT) regularly occurs in some of the patients who experience severe infections [4] or bleeding disorders [5]. To reduce these risks, dose reduction or postponement strategies are usually recommended in subsequent treatment cycles once DLHT has occurred. This kind of dose erosion, however, often compromises treatment outcome [6–10].

It is therefore desirable to identify prognostic factors for hematotoxicities. Many previous analyses approaching this question focussed only on one cell lineage or suffered from methodological drawbacks. Voog et al. [11] were the only group to examine all three hematopoietic lineages. Other groups provided only univariate analyses [12], relied on small datasets [13, 14] or analyzed differently treated populations [13, 15, 16]. No account was taken of cumulative toxicity. Furthermore, most of the prognostic models proposed were not validated on independent datasets [11, 13, 17–23].

Two multicenter trials of the German High-Grade Non-Hodgkin's Lymphoma Study Group [24, 25] were suitable for an extensive prognostic factor analysis on hematotoxicity. In the non-Hodgkin's lymphoma (NHL)-B1 and B2 trials, combination chemotherapy with cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP)-like treatment [26] was used. Adherence to protocol was excellent and very little dose erosion has occurred [24, 25, 27]. Hence, these trials permit an insight into the biological heterogeneity of hematotoxicity largely unconfounded by dose erosion.

patients and methods

trial design

In short, the trials started in 1993 and were closed in 2000. Patients included were aged between 18 and 60 with a lactate dehydrogenase (LDH) below the upper normal limit (UNL) (NHL-B1) [24] or patients between 61 and 75 years irrespective of the risk group (NHL-B2) [25]. Inclusion criteria were a Eastern Cooperative Oncology Group (ECOG) performance status of zero to three, leukocyte counts >3000/mm^3, thrombocytes

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>100000/mm^3, no other compromising organ dysfunction, no bone marrow involvement >25% and no lymphoma pretreatment, except a prephase treatment with vincristine 2 mg and steroids 100 mg (days 1–7).

The trial design was identical in both studies. Six cycles of CHOP-like chemotherapy were given in four treatment arms: CHOP-21 (three weekly), CHOP-14 (two weekly), CHOEP-21 (three weekly with etoposide 100 mg/m^2 on days 1–3) and CHOEP-14 (two weekly). In the two-weekly treatments, granulocyte colony-stimulating factor (G-CSF) was given from day 4 to 13 (300 μg/day if <75 kg and 480 μg/day if ≥75 kg). Hematological status had to be examined twice a week. The protocol specified that treatment should be delayed until the leukocyte recovery did exceed 2500/mm^3 and the platelet count did exceed 80 000/mm^3. If this threshold was not reached after 1 week, the next cycle should be started with a reduced dose (delays of >1 and <2 weeks: cyclophosphamide 75%, doxorubicin 75%, etoposide 75%; and 50% after delays of >2 weeks).

dataset for the prognostic factor study

This analysis was carried out on data from 1399 patients (NHL-B1: 710 patients, 4151 cycles and NHL-B2: 689 patients, 3715 cycles). We splitted the population at random into a training (two-thirds) and a validation sample (one-third). We calculated separate models for the young low-risk patients (NHL-B1) and for the elderly patients (NHL-B2) throughout. Separate models were constructed and validated for leukocytopenia, thrombocytopenia and anemia. Overall, a median of three measurements per cycle and patient was reported in all lineages. Using the pooled data from all measurements, we identified time windows for the nadir values: days 10–12 for thrombocytopenia in the groups of three- and two-weekly CHOP-like chemotherapy, days 10–12 for leukocytopenia following three-weekly regimen and days 8–10 following two-weekly regimen in the context of G-CSF support. The lowest white blood and platelet counts within these windows were transformed into a World Health Organization (WHO) grade. Cycles were only considered if cell count data were available in the nadir time windows. Regarding anemia, we used the lowest hemoglobin value available per cycle to code the WHO grade.

patients' characteristics

Patients' characteristics are given in Table 1. The median relative dose intensities show the very good adherence to protocol within the multicenter setting. A detailed description of dose erosion is given elsewhere [27].

hematotoxicity end points

Table 2 gives a summary of the toxicity grades observed in the course of the treatment. Higher toxic effects were more common among elderly patients.

statistical methodology

To model prognostic factors for changes in the WHO grades, we used the proportional odds model [28, 29]. Separate models were formulated for each end point for leukocytopenia, thrombocytopenia and anemia in two variants (PRE- and C1-models). Modeling uses the average population approach implying that cycle effects are not modeled individually for each patient but in relation to all other cycles. The applicability of the proportional odds model was checked and showed no relevant violation. Before modeling we checked whether the factors considered show high correlations. This was not the case. We did not use automatic selection procedures but proceeded in a stepwise approach for including single factors as suggested by Collet [30]. Thereafter, we included interaction terms. The strength of the prognostic factors was estimated by odds ratios (ORs) and the corresponding 95% confidence intervals. For the final models, P values ≤0.05 were considered to be significant.

To compare the model with the observations, we constructed five toxicity risk groups (low, low/intermediate, intermediate, high/intermediate and high risk) using equidistant intervals of the fitted linear predictor separately for each model.

Descriptive statistics were calculated in SPSS/PC+ V 10.0 while the multivariate modeling was carried out in SAS V8.

Table 1. Patients' characteristics

	NHL-B1 (n = 710)	NHL-B2 (n = 689)
Gender		
Male	61.7%	50.9%
Female	38.3%	49.1%
Age (years): median (range)	48 (18–60)	67 (61–75)
Risk factors for outcome		
Age >60	–	100.0%
LDH > UNL	–	45.9%
Stage III/IV	30.6%	50.8%
ECOG >1	5.4%	18.1%
Extranodal disease >1	14.6%	25.0%
IPI		
Low (IPI 0, 1)	90.0%	29.2%
Low intermediate (IPI 2)	8.7%	27.3%
High intermediate (IPI 3)	1.3%	23.4%
High (IPI 4, 5)	–	20.1%
aaIPI ≤60 years		
Low (aaIPI 0)	66.8%	
Low intermediate (aaIPI 1)	30.6%	
High intermediate (aaIPI 2)	2.7%	
High (aaIPI 3)	–	
B symptoms	21.0%	36.7%
Bulky disease	27.7%	39.2%
Bone marrow involvement[a]	5.3%	11.6%
Histology		
Diffuse large B cell	59.8%	71.0%
Other B cell/not specified B cell	26.0%	22.9%
T cell	13.8%	6.0%
Lymphoblastic, NOS	0.4%	0.1%
Blood counts before first cycle[b] (median)		
WBC, 10^3/mm^3	7.1	7.2
Platelets, 10^3/mm^3	275	282
Hemoglobin male/female, g/dl	14.3/12.8	13.5/12.4
Relative dose intensity (median)		
CHOP-21	0.98	0.97
CHOP-14	0.97	0.93
CHOEP-21	0.97	0.96
CHOEP-14	0.95	0.83

[a]Bone marrow involvement >25% exclusion criteria of the study.
[b]WBC <3 × 10^3/mm^3 or platelets <100 × 10^3/mm^3 exclusion criteria of the study.
NHL, non-Hodgkin's lymphoma; LDH, lactate dehydrogenase; UNL, upper normal limit; ECOG, Eastern Cooperative Oncology Group; IPI, International Prognostic Index; aaIPI, age-adjusted IPI; NOS, not otherwise specified; WBC, white blood cell; CHOP, combination chemotherapy with cyclophosphamide, doxorubicin, vincristine and prednisone; CHOEP, combination chemotherapy with cyclophosphamide, doxorubicin, vincristine, etoposide and prednisone.

Table 2. Hematotoxicity end points and interventions

	NHL-B1 (n = 4151 cycles)	NHL-B2 (n = 3715 cycles)
Leukocytes total/only cycle 1[a]		
WHO 0	20/14%	8/8%
WHO 1	11/12%	6/6%
WHO 2	21/21%	14/14%
WHO 3	31/34%	29/28%
WHO 4	17/19%	42/43%
Thrombocytes total/only cycle 1[b]		
WHO 0	74/79%	52/56%
WHO 1	10/11%	14/15%
WHO 2	8/5%	16/17%
WHO 3	6/3%	12/9%
WHO 4	2/1%	7/4%
Hemoglobin total/only cycle 1		
WHO 0	55/76%	32/52%
WHO 1	27/16%	30/25%
WHO 2	14/6%	28/18%
WHO 3	4/1%	8/4%
WHO 4	1/0%	1/1%
Days with G-CSF per cycle (median)[c]	10	10
Platelet transfusions	0.4%	2%
Red blood cell transfusions	4%	16%
Antibiotics	12%	20%
Days in hospital per cycle (median)[d]	1/3/2/3	3/4/2/5
Infection (WHO 3/4)	1%	3%
Mucositis (WHO 3/4)	1%	2%

WHO grades were defined as follows: leukocytopenia: grade 0, ≥4.0 (×1000/mm^3); grade 1, 3.0–3.9; grade 2, 2.0–2.9; grade 3, 1.0–1.9; grade 4, <1.0. Thrombocytopenia: grade 0, ≥100 (×1000/mm^3); grade 1, 75–99; grade 2, 50–74; grade 3, 25–49; grade 4, <25. Anemia (hemoglobin g/dl): grade 0, ≥11.0; grade 1, 9.5–10.9; grade 2, 8.0–9.4; grade 3, 6.5–7.9; grade 4, <6.5.

[a]On the basis of measurements in the nadir windows: days 10–12 for CHOP-21/CHOEP-21 and days 8–10 for CHOP-14/CHOEP-14.

[b]On the basis of measurements in the nadir windows: days 10–12 for all treatment arms.

[c]For the treatment arms CHOP-14/CHOEP-14.

[d]Separately for the treatment arms CHOP-21/CHOEP-21/CHOP-14/CHOEP-14; 0 days included.

NHL, non-Hodgkin's lymphoma; WHO, World Health Organization; G-CSF, granulocyte colony-stimulating factor; CHOP, combination chemotherapy with cyclophosphamide, doxorubicin, vincristine and prednisone; CHOEP, combination chemotherapy with cyclophosphamide, doxorubicin, vincristine, etoposide and prednisone.

results

prognostic factors for hematotoxicity in the training sample

We subsequently describe the results of the multivariate modeling for leukocytopenia, thrombocytopenia and anemia separately for patients between 18 and 60 years (NHL-B1) and >60 years (NHL-B2), respectively (Figure 1). Two different types of models are shown. One model type (PRE-models) uses only pretreatment information including patient-related factors (gender, weight index and liver function), disease-related factors (stage, bone marrow involvement, initial LDH levels, performance status, B symptoms, pretreatment hematopoietic parameters and albumin) and treatment-related covariates (planned use of etoposide, vincristine, cycle duration, G-CSF and prephase treatment) and the prediction concerns the toxicity of all six cycles. In the second type of model (C1-models), we also include the WHO grade observed during the first treatment cycle and the prediction is valid to forecast the toxicity for cycles 2–6. Consideration of cycle 1 in the models can be viewed as an *in vivo* sensitivity test for hematotoxicity. The risk for hematotoxicity can be calculated by multiplying the ORs given in Figure 1.

models considering pretreatment factors only (PRE-models)

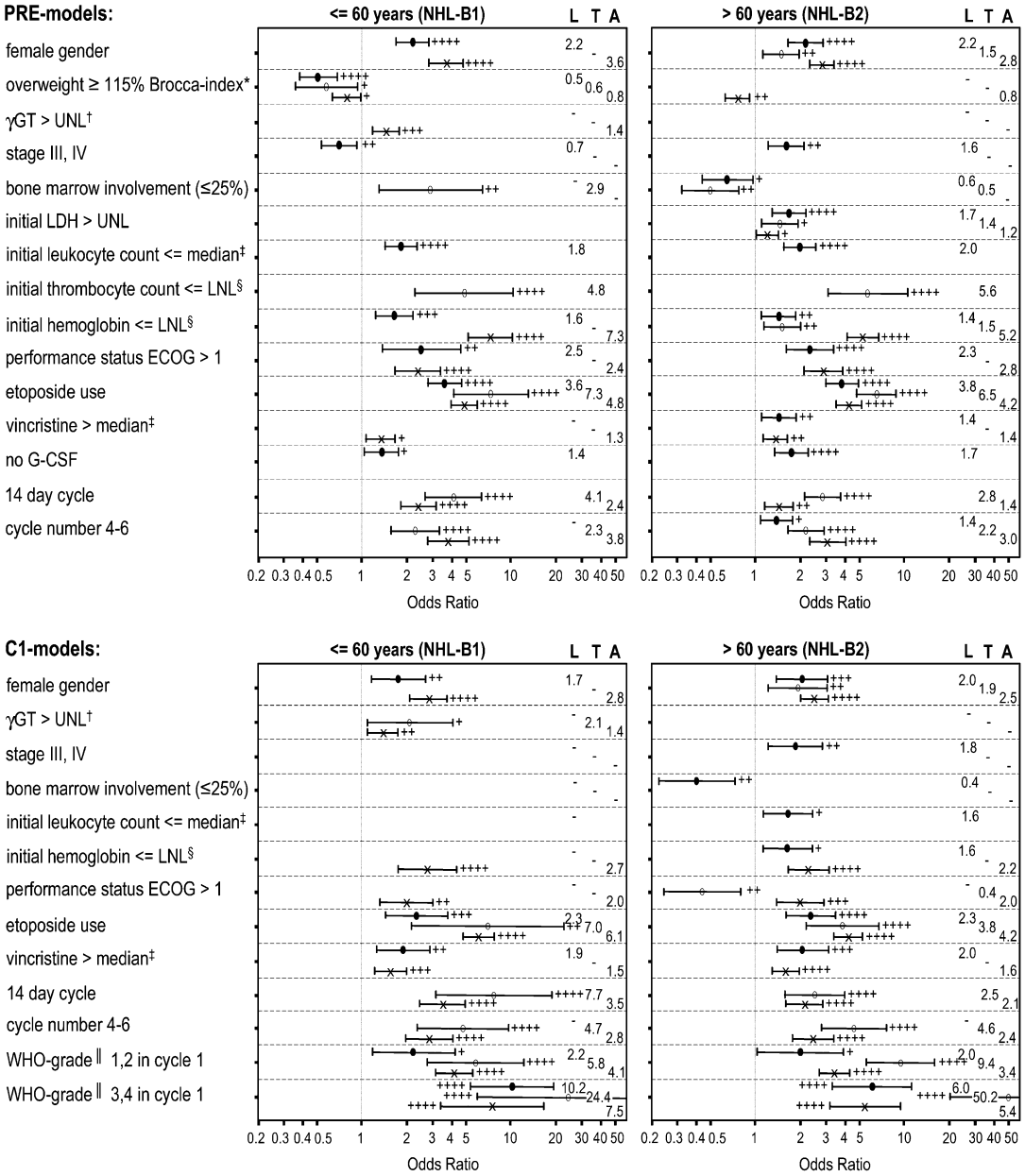
As shown in the upper panels of Figure 1, the addition of etoposide to the CHOP regimen was associated with an increased toxicity in all three lineages in both age groups. In addition, two-weekly regimen led to more thrombocytopenia and anemia while leukocytopenia was less pronounced in two-weekly schemes due to the addition of G-CSF. Low pretreatment hematological cell counts, female gender, low body weight, poor performance status and elevated LDH were generally associated with higher hematotoxicity. Cumulative toxicity could be demonstrated for thrombocytopenia and anemia but only to a very limited degree for leukocytopenia. These findings demonstrate that a series of prognostic factors can be discriminated, indicating that not only treatment regimens but also patient- and disease-related characteristics contribute to the heterogeneity of hematotoxicity.

models considering pretreatment factors and cycle 1 response (C1-models)

In the second type of models, we investigated whether the prediction for hematotoxicity in cycles 2–6 could be improved if the hematotoxic response in cycle 1 is taken into the consideration as well. We added the WHO grade toxicity found in cycle 1 into the models (assuming WHO grade 0 as baseline, WHO grade 1 or 2 as one variable and WHO grade 3 or 4 as another variable). As shown in the lower panels in Figure 1, the hematotoxicity observed in the first cycle is a very strong predictor for toxicity in later cycles with ORs ranging between 2 and 50.

heterogeneity of hematotoxicity

Figure 2 provides insight into the heterogeneity of hematotoxicity. On the basis of prognostic factors from the final models, we have defined five toxicity groups for each lineage and model by a scaling argument. The bars show the percentage of the populations found in each group. Apparently, we found a spread over all the five risk groups. This is particularly the case if cycle 1 information is used in addition. For elderly patients and leukocytopenia, we observed in our



NOTE: The Odds Ratios for leukocytopenia (**L**), thrombocytopenia (**T**) and anemia (**A**) are given within the figures on the right side. Factors which were not modeled for one of the hematopoetic lineages are missing. Not significant Odds Ratios were denoted with ‚-'.
For each significant factor the Odds Ratios with 95%-confidence interval are presented (symbols: • for **L**; ◦ for **T**; x for **A**).
Beside the bars for the Odds Ratios the significance level is plotted (+ p <= 0.05, ++ p <= 0.01, +++ p <= 0.001, ++++ p <= 0.0001).
Not significant factors for all of the models were: B-symptoms, albumin<=UNL†(=3.64 g/dl) and prephase treatment.
Interaction terms were: PRE/C1-model for anemia; NHL-B1: female*hemoglobin OR=0.4/0.5, cycle*hemoglobin OR=0.2/0.3, 14 day*cycle OR=2.3/2.2, NHL-B2: ECOG*etoposide OR=0.3/0.3, cycle*hemoglobin OR=0.3/0.5, 14 day*cycle OR=2.8/2.4

* Brocca-Index = height (cm) – 100

† UNL = Upper Normal Limit

‡ median for initial leukocyte count = 7100*10³/mm³; median for vincristine = 1.05 mg/m²

§ LNL = Lower Normal Limit;
LNL for initial thrombocyte count = 150*10³/mm³; LNL for initial hemoglobin = 12 g/dl for female and 13 g/dl for male

‖ WHO-grade leukocytopenia (10³/mm³): [0: (≥ 4) 1: (≥ 3 and < 4) 2: (≥ 2 and < 3) 3: (≥ 1 and < 2) 4: (< 1)];
WHO-grade thrombocytopenia (10³/mm³): [0: (≥ 100) 1: (≥ 75 and < 100) 2: (≥ 50 and < 75) 3: (≥ 25 and < 50) 4: (< 25)];
WHO-grade anemia (hemoglobin g/dl): [0: (≥ 11.0), 1: (≥ 9.5 and < 11.0) 2: (≥ 8.0 and < 9.5) 3: (≥ 6.5 and < 8) 4: (< 6.5)]

Figure 1. Multivariate proportional odds regression models. Odds ratios with 95% confidence intervals and significances are given according to the trial [non-Hodgkin's lymphoma (NHL)-B1/B2] and hematopoietic lineage for the 'PRE-models' (without cycle 1 information) and 'C1-models' (with cycle 1 information).

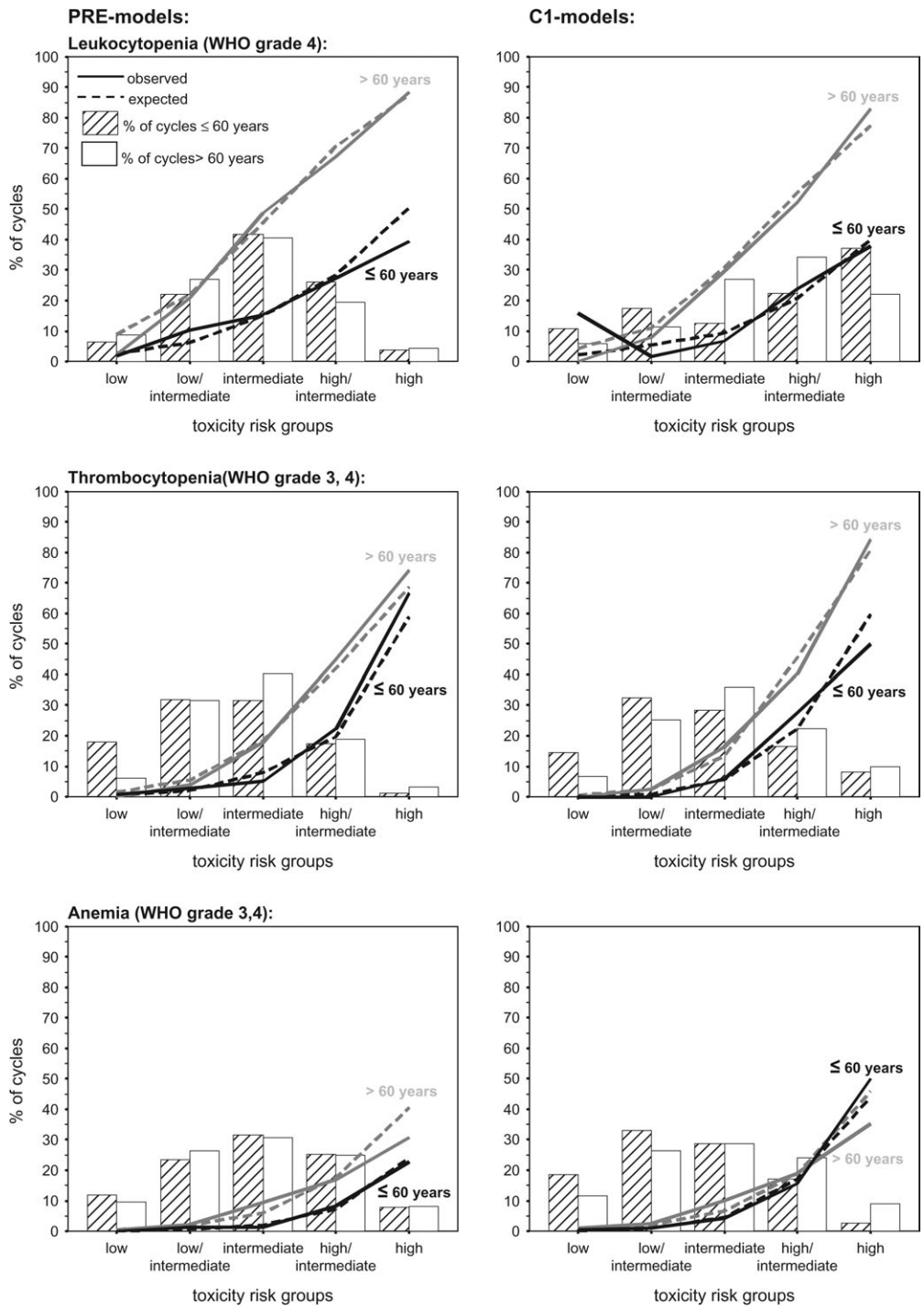


Figure 2. Heterogeneity of hematotoxicity and model checking—training sample non-Hodgkin's lymphoma (NHL)-B1/B2 trial. Comparison of observed and expected hematotoxicity within the toxicity risk groups for patients ≤60 years (NHL-B1 trial) and patients >60 years (NHL-B2 trial) according to the hematopoietic lineage for the 'PRE-models' (without cycle 1 information) and the 'C1-models' (with cycle 1 information). Number of cycles ('PRE' NHL-B1/'PRE' NHL-B2/'C1' NHL-B1/'C1' NHL-B2): leukocytopenia (873/978/357/453), thrombocytopenia (699/834/243/324) and anemia (2077/2085/1564/1560).

data the entire spectrum from only 2% of all cycles being problematic to 88% with toxicity problems (2%–39% in the young age group, respectively). This is similarly the case for thrombocytopenia where the spectrum ranges from groups with negligible toxicity to groups with >60% of the cycles with relevant decrease of the platelet counts. For anemia, we also find a remarkable heterogeneity. In addition, Figure 2 also shows the comparison between model predictions and observations. There are only minor discrepancies in groups with low case numbers.

Most importantly, the curves show that the elderly population is much more diverse than the patients <60 years; however, there are many elderly patients who can sustain similar drug doses as most of the younger patients.

model validation—NHL-B1/B2 trials

The PRE-model expectations are very consistent with the observed validation data (Figure 3). Minor discrepancies are due to small group sizes in the low- or high-risk groups. Similarly, the trend for the C1-models matches the observed data but small sample sizes affect the stability of the estimates in some groups.

model validation—RICOVER60 trial with rituximab-containing regimens

Within the RICOVER60 trial [71], patients with CD20-positive B-cell lymphoma 61–80 years of age with very similar inclusion criteria to the NHL-B1/B2 trials were randomized to six or eight cycles of CHOP-14 with or without rituximab (R). We did not observe any relevant differences concerning hematotoxicity for 6 × CHOP-14 − R, 8 × CHOP-14 − R, 6 × CHOP-14 + R and 8 × CHOP-14 + R (leukocytopenia WHO grade 4: 32.8%, 31.1%, 35.5%, 30.7%; thrombocytopenia WHO grade 3/4: 6.1%, 9.3%, 8.0%, 8.4% and anemia WHO grade 3/4: 5.5%, 5.5%, 4.9%, 6.6% over all cycles). We validated our leukocytopenia model applying it to the data from the RICOVER60 trial using an age window as for the NHL-B2 population (61–75 years). Figure 4 shows that the model fits the five toxicity risk groups in this independent trial data very well. The reason for the slight underestimation most likely is the changed G-CSF use within the RICOVER60 trial (days 6–12 instead of days 4–13 for the NHL-B1/B2 trial). The results for treatment arms with or without rituximab are nearly the same, demonstrating that the model is valid for R-CHOP treatments.

model application

Table 3 provides an example for the clinical application of the models for defining groups of patients at risk for toxicity. We defined a patient as having had true toxicity if he had a grade 4 toxicity in at least one of the reported cycles. On the basis of linear predictor for leukocytopenia from the PRE-model, one could, e.g. classify 75.4% of all patients ≤60 years as a high-risk group (toxicity risk groups 3–5). In this group, one can expect almost all patients with a toxic event (31.1% compared with 3.6% in the low-risk group). Thus, a prophylactic strategy would be almost fully effective although restricted to 75.4% of the patients. On the basis of the C1-model, one could achieve an even more cost-effective strategy for the remaining cycles, if the toxicity risk groups 4–5 are

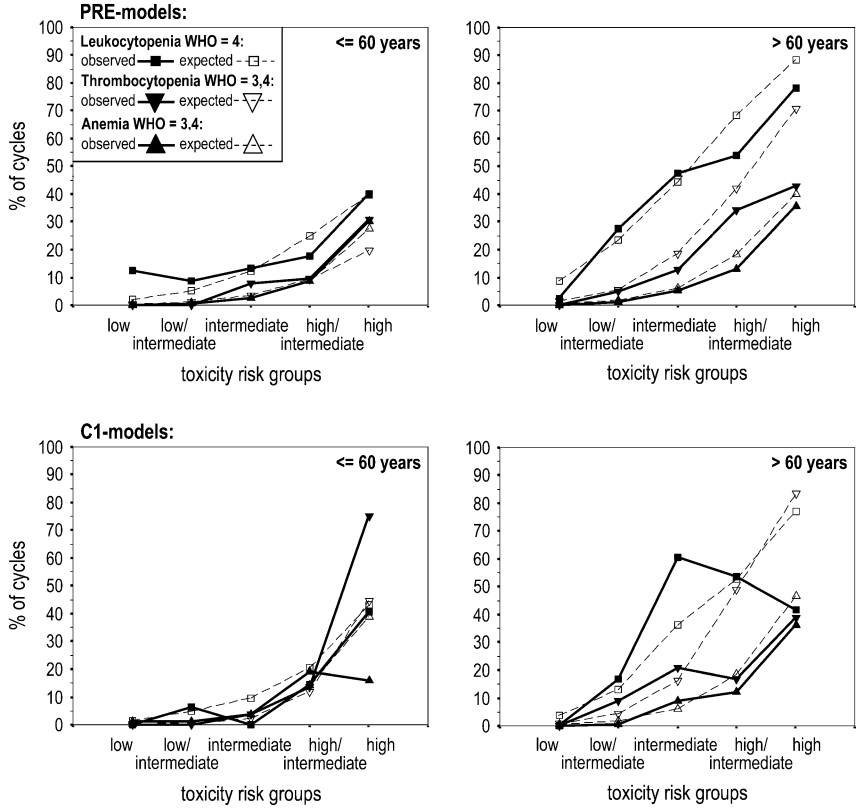


Figure 3. Model checking—validation sample non-Hodgkin's lymphoma (NHL)-B1/B2 trial. Comparison of observed and expected hematotoxicity within the toxicity risk groups for patients ≤60 years (NHL-B1 trial) and patients >60 years (NHL-B2 trial) according to the hematopoietic lineage for the 'PRE-models' (without cycle 1 information) and the 'C1-models' (with cycle 1 information). Number of cycles ('PRE' NHL-B1/'PRE' NHL-B2/'C1' NHL-B1/'C1' NHL-B2): leukocytopenia (251/362/116/153), thrombocytopenia (196/306/65/121) and anemia (647/729/501/534).

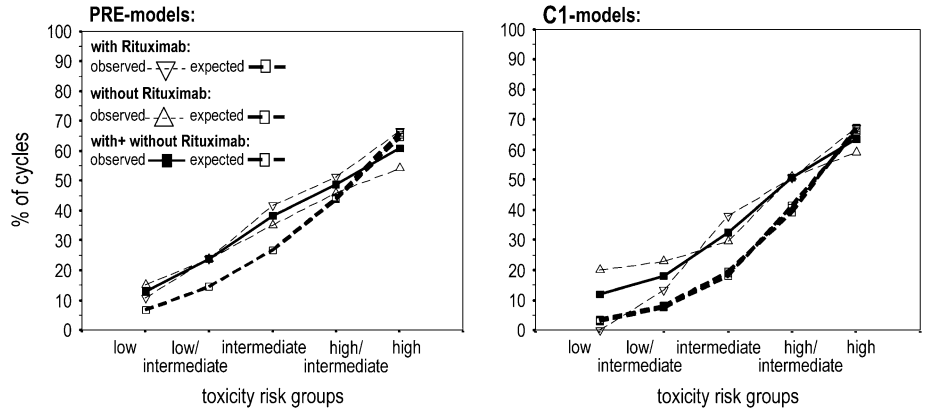


Figure 4. Model checking—validation sample RICOVER60 trial. Comparison of observed and expected leukocytopenia (World Health Organization grade 4) within the toxicity risk groups for patients with rituximab (R), without R or both for the 'PRE-models' (without cycle 1 information) and the 'C1-models' (with cycle 1 information). Number of cycles ('PRE'/'C1'): with R (819/318), without R (795/340) and both (1614/658). For the expected curves of the three plotted groups, only one symbol was used because of overlap.

Table 3. Scenario with risk factor groups

	Definition of high-risk groups according to the toxicity risk groups for leukocytopenia (PRE/C1-model)[a]			
	2–5	3–5	4–5	5
≤60 years (NHL-B1)				
% Patients high-risk group	95.2/**93.4**	75.4/**72.4**	33.5/**57.9**	4.8/**36.8**
% With toxicity[b]	34.7/**40.8**	31.1/**40.8**	19.2/**38.2**	3.6/**28.9**
% Without toxicity	60.5/**52.6**	44.3/**31.6**	14.4/**19.7**	1.2/**7.9**
% Patients low-risk group	4.8/**6.6**	24.6/**27.6**	66.5/**42.1**	95.2/**63.2**
% With toxicity[b]	0.0/**1.3**	3.6/**1.3**	15.6/**3.9**	31.1/**13.2**
% Without toxicity	4.8/**5.3**	21.0/**26.3**	50.9/**38.2**	64.1/**50.0**
>60 years (NHL-B2)				
% Patients high-risk group	89.8/**93.9**	62.5/**83.8**	22.2/**61.6**	5.1/**24.2**
% With toxicity[b]	55.1/**61.6**	45.4/**60.6**	16.2/**49.5**	4.6/**22.2**
% Without toxicity	34.7/**32.3**	17.1/**23.2**	6.0/**12.1**	0.5/**2.0**
% Patients low-risk group	10.2/**6.1**	37.5/**16.2**	77.8/**38.4**	94.9/**75.8**
% With toxicity[b]	0.5/**0.0**	10.2/**1.0**	39.4/**12.1**	50.9/**39.4**
% Without toxicity	9.7/**6.1**	27.3/**15.2**	38.4/**26.3**	44.0/**36.4**

[a]2, low/intermediate; 3, intermediate; 4, high/intermediate; 5, high toxicity risk group.
[b]At least one World Health Organization grade 4 for leukocytopenia during cycles 1–6 for the PRE-model and during cycles 2–6 for the C1-model.
NHL, non-Hodgkin's lymphoma.

classified as a high-risk group subjected for prophylaxis (only 57.9% of all patients ≤60 years). The risk of grade 4 toxicity in a following cycle for the low-risk group without planned prophylaxis would be only 3.9%.

The scenarios for elderly patients showed analogous results. The models would permit to spare intensive prophylaxis strategies for 10%–38% (PRE-model) or 16%–38% (C1-model) of the patients, respectively, depending on the risk of overlooking grade 4 toxic effects in the low-risk populations not included in prophylaxis strategies (1%–12%). We provide a Web-based tool to calculate prognostic scores on hematopoietic toxicity for CHOP-like regimen (www.toxcalculator.com).

association between leukocytopenia and treatment complications

Especially for elderly patients, we found a strong association between the leukocytopenia risk profile and the frequency of infection (Figure 5; infection WHO >1: 7.1% cycles, WHO 3/4: 0.0% in the low and 43.5% and 12.9% in the high toxicity risk group). The incidence of antibiotic use increased for young/elderly patients from 6.3%/10.6% in the low toxicity risk group to >22.5%/41.3% in the high toxicity risk group, respectively. The median number of days of hospitalization (0 days included) in the five toxicity risk groups were 2, 2, 3, 4 and 5 or 1, 3, 4, 5 and 8 days among young or elderly patients,

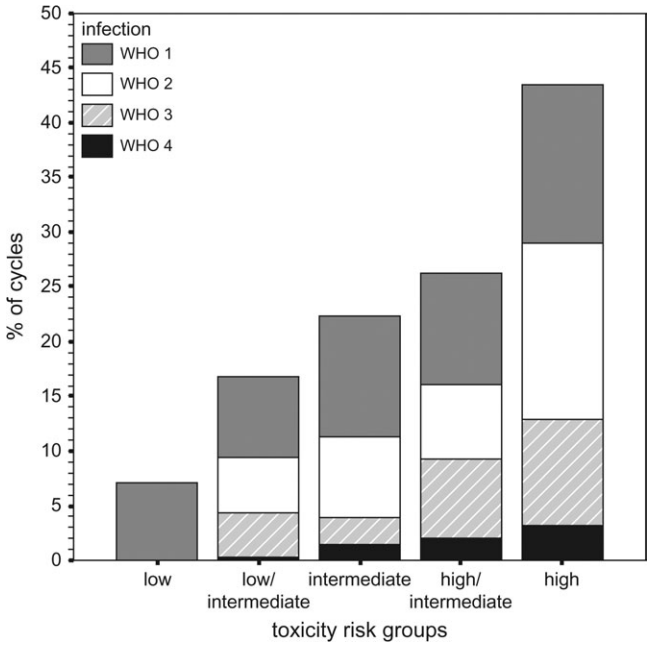


Figure 5. Leukocytopenia and infection. World Health Organization (WHO) grade for infection within the toxicity risk groups for leukocytopenia for patients >60 years [non-Hodgkin's lymphoma (NHL)-B2 trial].

respectively ($P < 0.001$ for both, Kruskal–Wallis test). Among elderly patients, there was a clear association between the treatment-related death rate and the calculated risk of leukocytopenia ($P = 0.001$, Fisher's exact test).

discussion

Regarding the scope of cell lineages, the number of prognostic factors considered and regarding the number of patients in the test and validation datasets, we here present the most comprehensive analysis of the diversity of patients with regard to chemotherapy-induced hematotoxicity reported so far. The dataset of patients with aggressive non-Hodgkin's lymphomas was particularly suited for this analysis as it covered a broad age range (18–75) and as the four CHOP-like regimens were administered with an excellent dose adherence permitting to unravel a rather unbiased picture of the heterogeneity of hematotoxicity. The prognostic factors were obtained in a test dataset and validated in two independent datasets, also with respect to rituximab.

Our analysis leads to several major conclusions. First, hematotoxic heterogeneity is particularly large among elderly patients with some subgroups exhibiting very toxic reactions while others show almost no toxicity. This is particularly true for leukocytopenia but similar findings hold for thrombocytopenia and to a lesser degree for anemia.

Secondly, we could show that hematotoxicity observed in the first treatment cycle is highly predictive for toxicity in subsequent cycles providing a means of *in vivo* sensitivity testing.

Thirdly, we showed that these factors can be used to group patients into prognostic risk groups in a model-dependent way and that these risk groups are clinically relevant. Higher risk for leukocytopenia is associated with more infections, more antibiotic use, longer hospitalization and higher treatment-related mortality, suggesting that the models can be useful for clinical decision making.

Several of these findings deserve comments. We found that treatment-related factors had a relevant effect on hematotoxicity. The effect of etoposide well conforms with available pharmacokinetic and pharmacodynamic models [31–35].

Shortening the schemes from 3 to 2 weeks with the addition of G-CSF was, however, associated with less leukocytopenia, indicating that G-CSF was effective in preventing leukocytopenia. This is in line with available knowledge [17, 19, 24, 25, 36–47].

We also found that vincristine has a small but significant contribution to hematotoxicity [48].

Remarkably, several disease-related factors are prognostic for both disease outcome and hematotoxicity as well. Among them are elevated LDH and bad performance status. LDH > UNL, ECOG >1 and stage III/IV are known prognostic factors for overall treatment outcome [49]. Elevated LDH has been described as a risk factor for hematotoxicity or early death by other authors [18, 23]. A poor performance status has been reported to be a prognostic factor for infectious complications in indolent lymphomas [50], for early death in aggressive NHL [18] and solid cancers [20, 51] and for febrile neutropenia and low platelet count in lymphoma patients [11]. Furthermore, we found that reduced initial blood counts are associated with increased risk for toxicity. Low initial absolute neutrophil count (ANC) has been shown to be prognostic for infectious complications in indolent lymphoid malignancies [50], as well as for time to first hospitalization for febrile neutropenia in intermediate-grade NHL [17]. Low hemoglobin has been reported as prognostic for red blood cell transfusion requirements in patients with solid tumors [22, 52] and in a group of patients with solid tumors or lymphoma [21, 53] or for early death in aggressive NHL by Dumontet et al. [18]. Some authors found bone marrow involvement as a factor [54–57]. In the NHL-B trial, patients with >25% bone marrow involvement were excluded and this may be the reason for our discrepant observation.

Furthermore, patient-related factors play a role in our data. Female gender is an adverse factor in all three lineages for elderly patients and among younger patients for leukocytopenia and thrombocytopenia. This observation was also made by Lyman and Delgado [17] for febrile neutropenia. Age *per se* must also be considered as an adverse factor as been previously described by us [58] and other authors [15, 17–19, 50, 59–66]. In line with others, we have furthermore observed obesity as a factor associated with a protective effect [17, 67]. A potential explanation for this observation is that the trial protocol recommended a dose reduction for obese patients. We could in fact show that more dose reduction was used in obese patients than in controls.

The first cycle effect was a very consistent finding leading to the most prominent single prognostic factor in our analysis,

while maintaining the influence of the pretreatment factors. Related observations were also made by Silber et al. [14] for the end point ANC ≤250/μl, by Rivera et al. [68] for neutropenic events and by some other authors for febrile neutropenia [13, 15, 69].

Although we have attempted a rather comprehensive approach, we need to mention some limitations. First, we used the population average approximation implying that treatment cycles were considered independent from one another. Neglecting this kind of overdispersion in odds regression models does not bias the estimates but may lead to underestimates of the standard errors. Because we only had full information on all six cycles in a limited number of patients, modeling of intraindividual effects was not feasible. Secondly, the three hematological lineages were analyzed independently from one another. Despite the formal restriction, we found for the WHO grades only a moderate pair-wise correlation (Spearman's rank correlation coefficients between 0.43 and 0.47). Thirdly, we could not adjust the models for thrombocytopenia and anemia for the use of platelet transfusion (NHL-B1: 0.4% and NHL-B2: 2%) or red blood cell (NHL-B1: 4% and NHL-B2: 16%) transfusions. Nevertheless, the results remained virtually unchanged if the analysis was restricted to cycles not influenced by transfusions.

It was the major objective of our effort to analyze the factors that explain the heterogeneity of hematotoxicity in cancer patients. The clinical implications of our results are potentially far reaching. Our data clearly showed that one can design a strategy for selecting patients at risk for hematotoxicity while controlling for the risk of overlooking risks in the low toxicity groups. This may permit to tailor prophylactic measures for patients at risk.

Furthermore, our findings may open an avenue to design more intelligent dosing schemes than the uniform schemes generally used (same dose in mg/m^2 for all patients). To help selecting appropriate drug dosing schemes for risk groups, we can use a novel biomathematical model of granulocytopoiesis recently developed by our group [70, 71].

In summary, we found a remarkable heterogeneity of hematotoxicity to conventional chemotherapy which is related not only to the regimens used but also to patient and disease characteristics. It would be interesting to see whether similar results can be found for other chemotherapy modalities and other cancer patients. Knowledge about prognostic factors for toxicity should be incorporated into more intelligent dosing and prophylaxis schedules to get closer to the treatment ideal of individual toxicity-adopted dosing.

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