Prediction of outcome by early bone marrow response in childhood acute lymphoblastic leukemia treated in the ALL-BFM 95 trial: differential effects in precursor B-cell and T-cell leukemia

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Abstract: BACKGROUND: In the ALL-BFM 95 trial for treatment of acute lymphoblastic leukemia, response to a prednisone pre-phase (prednisone response) was used for risk stratification in combination with age and white blood cell count at diagnosis, response to induction therapy and specific genetic high-risk features. DESIGN AND METHODS: Cytomorphological marrow response was prospectively assessed on Day 15 during induction, and its prognostic value was analyzed in 1,431 patients treated on ALL-BFM 95. RESULTS: The 8-year probabilities of event-free survival were 86.1%, 74.5%, and 46.4% for patients with M1, M2, and M3 Day 15 marrows, respectively. Compared to prednisone response, Day 15 marrow response was superior in outcome prediction in precursor B-cell and T-cell leukemia with, however, a differential effect depending on blast lineage. Outcome was poor in T-cell leukemia patients with prednisone poor-response independent of Day 15 marrow response, whereas among patients with prednisone good-response different risk groups could be identified by Day 15 marrow response. In contrast, prednisone response lost prognostic significance in precursor B-cell leukemia when stratified by Day 15 marrow response. Age and white blood cell count retained their independent prognostic effect. CONCLUSIONS: Selective addition of Day 15 marrow response to conventional stratification criteria applied on ALL-BFM 95 (currently in use in several countries as regular chemotherapy protocol for childhood acute lymphoblastic leukemia) may significantly improve risk-adapted treatment delivery. Even though cutting-edge trial risk stratification is meanwhile dominated by minimal residual disease evaluation, an improved conventional risk assessment, as presented here, could be of great importance to countries that lack the technical and/or financial resources associated with the application of minimal residual disease analysis.

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Prediction of Outcome by Early Response in Childhood Acute Lymphoblastic Leukemia

Das frühe Ansprechen auf die Therapie als prognostischer Faktor bei der akuten lymphoblastischen Leukämie im Kindesalter

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Key words
- childhood acute lymphoblastic leukemia
- early treatment response
- clinical trial

Schlüsselwörter
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- frühes Therapieansprechen
- klinische Studie

Abstract

Background: In the ALL-BFM studies for treatment of acute lymphoblastic leukemia, reduction of leukemic blasts in peripheral blood after a one-week prednisone pre-phase – the so-called prednisone response – has been used for risk stratification since the 1980s and has been one of the most relevant factors for identification of high-risk patients. In the trial ALL-BFM 95, early cytomorphological marrow response on day 15 of induction therapy was prospectively evaluated and its prognostic value was analyzed in comparison to the prednisone response and other established prognostic factors.

Results: Compared to prednisone response, day 15 marrow response was superior in outcome prediction – yet with differential effect depending on blast lineage. Outcome was poor in T cell leukemia patients with prednisone poor-response independent of day 15 marrow response, whereas among patients with prednisone good-response different risk groups could be identified by day 15 marrow response. In contrast, prednisone response lost prognostic significance in precursor B cell leukemia when stratified by day 15 marrow response.

Conclusions: Selective addition of day 15 marrow response to conventional stratification criteria applied on ALL-BFM 95 may significantly improve risk-adapted treatment delivery. Even though cutting-edge trial risk stratification is meanwhile dominated by minimal residual disease evaluation, an improved conventional risk assessment, as presented here, could be of great importance to countries lacking the technical and/or financial resources associated with the application of minimal residual disease analysis.

Zusammenfassung

Hintergrund: Die Blastenreduktion im peripheren Blut nach einer einwöchigen Prednison-Vorphase – der sogenannte Prednison-Response – wird in den ALL-BFM-Studien zur Behandlung von Kindern mit akuter lymphoblastischer Leukämie seit den 1980er Jahren für die Risikostratifizierung herangezogen und stellt einen der wichtigsten Faktoren für die Identifizierung von Hochrisikopatienten dar. In der Studie ALL-BFM 95 wurde zusätzlich das frühe zytomorphologische Ansprechen auf die Therapie am Tag 15 der Induktionstherapie im Knochenmark prospektiv untersucht und dessen prognostischer Wert im Vergleich zum Prednison-Response und anderen etablierten Prognosefaktoren analysiert.

Ergebnisse: Das zytomorphologische Ansprechen im Knochenmark am Tag 15 war als prognostischer Faktor dem Prednison-Response insgesamt überlegen, jedoch mit unterschiedlichem Effekt in Abhängigkeit vom Immunphänotyp der Blasten. Während bei Patienten mit B-Vorläuferzell-Leukämie der Prednison-Reponse in Kombination mit dem Ansprechen am Tag 15 seine prognostische Wertigkeit komplett verlor, war bei Patienten mit T-Zell-Leukämie ein schlechter Prednison-Response auch bei gutem Ansprechen am Tag 15 prognostisch ungünstig.

Schlussfolgerung: Die Verwendung des zytomorphologischen Therapieansprechens am Tag 15 in der Induktionstherapie für die Risikostratifizierung kann im Vergleich zur konventionellen Stratifizierung der Studie ALL-BFM 95 eine bessere Risikoadaptierung der Therapie ermöglichen. Auch wenn mittlerweile in vielen aktuellen Therapiestudien die zytomorphologische Evaluation des Therapieansprechens weitgehend durch Methoden zur Erfassung der minimalen Resterkrankung abgelöst wurde, kann insbesondere in Ländern, in denen keine ausreichenden Mittel

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für Analysen der minimalen Resterkrankung verfügbar sind, eine verbesserte konventionelle Risikoeinschätzung entscheidend zur Qualität der Therapie beitragen.

Introduction

Early treatment response is known to be of major importance for the prediction of outcome in solid and hematologic malignancies [6, 14, 15, 18]. In 1983, the Berlin-Frankfurt-Münster (BFM) study group started to evaluate the so-called prednisone response (PR) as a predictive factor for treatment outcome by measuring the peripheral blast count after a 7-day therapy of prednisone and one intrathecal (IT) dose of methotrexate (MTX) [28]. Since then, the PR has consistently been found to be one of the strongest independent prognostic factors for the prediction of treatment outcome in ALL-BFM studies [24].

In the 1970s the Children's Cancer Study Group (CCG) evaluated early bone marrow response during multi-agent induction treatment and demonstrated its predictive value for achievement of remission and ultimate outcome [11, 21, 22]. In the following trials, the CCG generated many data on the prognostic importance of marrow response on day 7 and 14, the combined impact of the 2 evaluation points, and the differential effect in patients at standard or high risk by the NCI/Rome criteria [9, 12, 32, 36]. Based on these results, early marrow response has become an integral part of risk stratification in the succeeding CCG and contemporary Children's Oncology Group (COG) ALL treatment regimens [2, 16, 19, 20, 25, 33]. The St. Jude Total Therapy Study Group showed that even the persistence of low percentages (1–4%) of BM lymphoblasts on day 15 (corresponding to day 22 of the BFM protocol without prednisone prephase) and days 22–25 of induction therapy was associated with a significantly poorer event free survival rate compared to patients without detectable BM blasts [29].

Here we will summarize the importance of cytomorphological response in bone marrow and peripheral blood at different investigation time points during induction treatment within an ALL-BFM treatment regimen. In the trial ALL-BFM 95 [23], the prognostic value of cytomorphological response in BM on day 15 (BMd15) was evaluated in comparison and combined with the clinical and treatment response parameters established in the ALL-BFM 95 risk stratification such as PR, cytomorphological BM response to induction therapy (day 33), age and white blood cell count (WBC) at diagnosis.

Response evaluation and risk stratification in trial ALL-BFM 95

Evaluation of treatment response was an integral part of risk stratification in ALL-BFM 95. PR was evaluated by the determination the absolute number of leukemic blasts/µl in the peripheral blood after 7 days of prednisone treatment and one intrathecal (IT) dose of methotrexate, regardless of the initial leukemic blast count. Prednisone good responders (PGR) were characterized by <1000 blasts/µl, whereas prednisone poor responders (PPR) had ≥1000 blasts/µl on day 8 of treatment [28]. Response in BM was categorized as M1 (<5%), M2 (5 to <25%), and M3 (≥25% lymphoblasts) and was evaluated for risk assignement at the end of induction treatment (day 33). In addition to response criteria, clinical and biological parameters were used for stratification into 3 risk groups: (i) High Risk (HR: PPR, and/or no CR on day 33, and/or evidence of t(9;22) [or BCR/ABL], and/or evidence of t(4;11) [or MLL/AF4]), (ii) Medium Risk (MR: No HR criteria, and initial WBC ≥20000/µl, and/or age at diagnosis <1 or ≥6 years, and/or T-ALL), or (iii) Standard Risk (SR: No HR criteria, and initial WBC <20000/µl, and age at diagnosis ≥1 and <6 years, and no T-ALL).

Bone marrow on day 15 (BMd15) was prospectively assessed without being used for risk stratification. From the 2169 patients enrolled, 1431 patients had assessable information on BM morphology on day 15 [17] and were included in the study on cytomorphological treatment response presented here. The estimated probability of 8-year EFS (8y-pEFS) of these patients was 78.8±0.9%. The data reported here shall focus on the additional value of BMd15 compared with the so far used PR and BM response on day 33 (BMd33).

Prognostic value of prednisone response and marrow response on day 15 and day 33

PR was evaluable in 1419 of the 1431 analyzed patients (99%) reflecting the very easy sampling and evaluation of the peripheral blood samples on day 8. In contrast, 15.6% of the BM aspirates on day 15 were considered not assessable due to non-representative BM morphology. Stratification by PR resulted in 1280 patients with PGR (90.2%) and 139 patients with PPR (9.8%). Using BMd15, 880 patients (61.5%) had an M1, 365 patients (25.5%) an M2 and 186 patients (13.0%) an M3 marrow. The prediction of treatment outcome was in principal possible with both response parameters PR or BMd15. The 8y-pEFS was 81.3±0.9% for patients with PGR and 55.1±3.7% for patients with PPR ($p<0.001$). For the patients with M1, M2 and M3 marrow, 8y-pEFS was 86.1±1.2%, 74.5±2.3%, and 46.4±3.7%, respectively. In view of the fact that the group with the worst outcome identified by BMd15 (M3) is slightly larger and has a somewhat worse pEFS than PPR patients, one can suspect that BMd15 allows a better prediction of outcome than PR considering the total patient population. In addition, BMd15 allowed the further stratification of the large "good risk" patient group (M1, M2). However, applying these generally used cut-off values characterizing M1, M2 and M3 marrow, each subgroup still comprises patients with a wide range of BM blasts. Analyzing the patients within narrower ranges of blasts resulted in a clear difference in pEFS between 0% and >0% to <5% (M1 category) and between 25% to <50% and ≥50% BM blasts on day 15 (M3 category) (**Fig. 1**).

Poor response in BMd15 was significantly associated with T-ALL ($p<0.001$) and the known high-risk features adolescent age ($p<0.001$), hyperleukocytosis ($p<0.001$), BCR/ABL ($p=0.003$), CNS involvement ($p=0.005$), PPR ($p<0.001$), and NRd33 ($p<0.001$).

BMd33 could be assessed in 1415 of 1431 patients. Only 42 of these patients (2.9%) did not achieve BM remission on day 33 (NRd33). Although these patients had a strikingly poor 8y-pEFS of only 36.3±6.9%, cytomorphological marrow response at this time point has limited prognostic value for the vast majority of

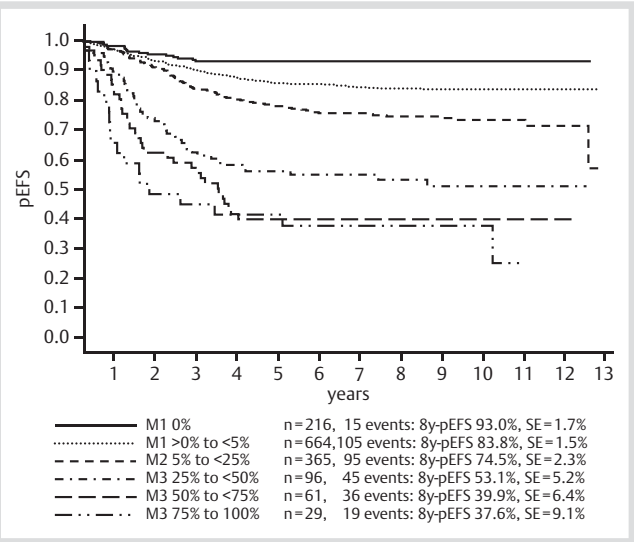

Fig. 1 Kaplan-Meier estimate of event-free survival according to percentage of blast counts in the bone marrow on day 15 [17]. Log-Rank test (pair-wise comparisons): M1 (0%) vs. M1 (>0≤5%) $p=0.001$; M3 (25≤50%) vs. M3 (50≤75%) $p=0.102$; M3 (25≤50%) vs. M3 (75–100%) $p=0.025$; M3 (50≤75%) vs. M3 (75–100%) $p=0.381$. 8y-pEFS indicates probability of event-free survival at 8 years; SE, standard error.

patients. However, using BMd33 in addition to BMd15 enables a further stratification of patients with M3 BMd15. Among these patients 8y-pEFS was 52.5±4.2% (n=146) for those who achieved complete cytomorphological remission (CR) by day 33 and 25.4±7.2% (n=38) for those, who did not (p<0.001).

In clinical practice, the question often arises whether an early change or intensification of treatment is reasonable in patients with very poor early response. Of the patients with ≥75% blasts on day 15, 55% reached CR on day 33 and had an 8y-pEFS of 42.9±12.6%. Among those patients with 25 to <50% blasts on day 15, even 92% reached CR on day 33 with an 8y-pEFS of 57.5±5.5%. These data show that patients with M3 marrow on day 15 still have a good chance to achieve CR by end of induction. This suggests that just for the aim of achieving remission there is no strong evidence for the need of alternative ALL treatment at this point. However, treatment results of these patients are still poor and might be improved by early treatment intensification.

Results in immunological subgroups

More detailed analyses revealed profound differences within immunophenotypic subgroups. For this reason, results are shown for each subgroup separately.

pB-ALL

In pB-ALL (n=1196 [1187 patients with evaluable PR]), patients with PGR had an 8y-pEFS of 80.2±1.2% (n=1109) and PPR patients an 8y-pEFS of 58.4±5.6% (n=78) ($p<0.001$). Patients of the BMd15 subgroups had an 8y-pEFS of 86.5±1.3% (n=741), 74.2±2.5% (n=317), and 47.1±4.3% (n=138) for the M1, M2 and M3 group. Compared with the total patient group, it is even more obvious in this immunological subgroup that the BMd15 has a better prognostic discriminative value in identifying poor risk patients than the PR: A smaller group of pB-ALL patients could be identified by PPR compared to M3 BMd15 (6.6% vs. 11.5%), but the EFS of the patients in the M3 BMd15 group was even worse than the EFS of PPR patients. This was also reflected in the distribution of events: 12.7% of all events in pB-ALL (n=32/256) clustered in the PPR group, whereas 28.5% were detected in the BMd15 M3 group (n=72/256) (**Fig. 2a, b**). Sensitivity of PR to predict poor BM response on day 15 or day 33 was low as only 27.9% of patients with M3 BMd15 and 56.7% of patients with NRd33 had shown PPR before.

BMd15 allowed a clear separation of 3 different risk groups for patients with M1, M2 and M3 marrow within the subgroups of PGR and PPR patients (**Fig. 2a, b**). Patients of the same BMd15 subgroup had no statistically significant difference in pEFS, when analyzed according to PR (**Table 1**).

Age and WBC as well as NCI risk criteria [34,35] and risk group criteria of the trial ALL-BFM 95 – both using age at diagnosis and initial WBC – showed an additional prognostic value when analyzed in combination with BMd15 (**Table 1**).

Multivariate Cox regression analysis including NCI risk criteria, PR, BMd15 and BMd33 as covariates confirmed the univariate results. In this analysis, PR lost its prognostic significance (PPR: RR 0.92, p=0.72) whereas the NCI risk criteria (NCI HR: RR 2.17, p<0.001) as well as BM response on day 15 (M2: RR 1.96, p<0.001; M3: RR 3.93, p<0.001) and day 33 (no CRd33: RR 2.14, p=0.006) retained significance.

To summarize, BMd15 could identify 3 distinct risk groups in pB-ALL and PR had no significant additional effect in patients stratified by BMd15. Biologically, this seems highly plausible considering that the PR is measured after the administration of 7 days of prednisone and one IT dose of MTX, while the evaluation of the BM on d15 reflects the response to 14 days of prednisone, one dose of vincristine, daunorubicin and asparaginase, respectively, and 2 doses of IT MTX. This additionally might indicate that in pB-ALL, resistance to prednisone can be compensated by high sensitivity to other chemotherapeutic drugs and that high sensitivity to prednisone can be overturned by resistance to other agents.

T-ALL

In T-ALL (n=194 [191 patients with evaluable PR]), PGR patients had an 8y-pEFS of 84.6±3.3% (n=130) and patients with PPR had an 8y-pEFS of 54.1±6.4% (n=61). The 8y-pEFS of patients with M1, M2 and M3 BMd15 was 86.9±3.3% (n=107), 78.8±6.7% (n=40), and 45.0±7.7% (n=47). Sensitivity of PR to predict poor BM response on day 15 or day 33 was better in T-ALL than in pB-ALL: 72.3% of the patients with M3 BMd15 and 81.8% of the patients with NRd33 had shown PPR before.

Within the patients with PPR, BMd15 was not able to characterize subgroups with significantly different outcome (**Fig. 2d**). In PGR, however, outcome of patients with BMd15 M3 was significantly worse (M3, 8y-pEFS 43.1±14.7%) compared with the M1 and M2 subgroup with similarly favourable results (M1: 8y-pEFS 91.1±3.0%, M2: 8y-pEFS 83.4±7.7%, **Fig. 2c**). The prognostic relevance of the PR within the BMd15 subgroups in T-ALL is illustrated by the reverse analysis in **Table 1**: Within the BMd15 M1 subgroup, patients with significantly worse pEFS could be identified through PPR, whereas no difference in outcome was shown within the BMd15 M3 subgroup. Within the small BMd15 M2 subgroup, the difference between PGR and PPR did not reach statistical significance. Thus, by the combination of PR and BMd15, T-ALL patients can be stratified into 2 distinct risk groups, one of them including the patients with PGR plus M1 or M2 BMd15 (n=120, 8y-pEFS=89.5±2.9%) the other

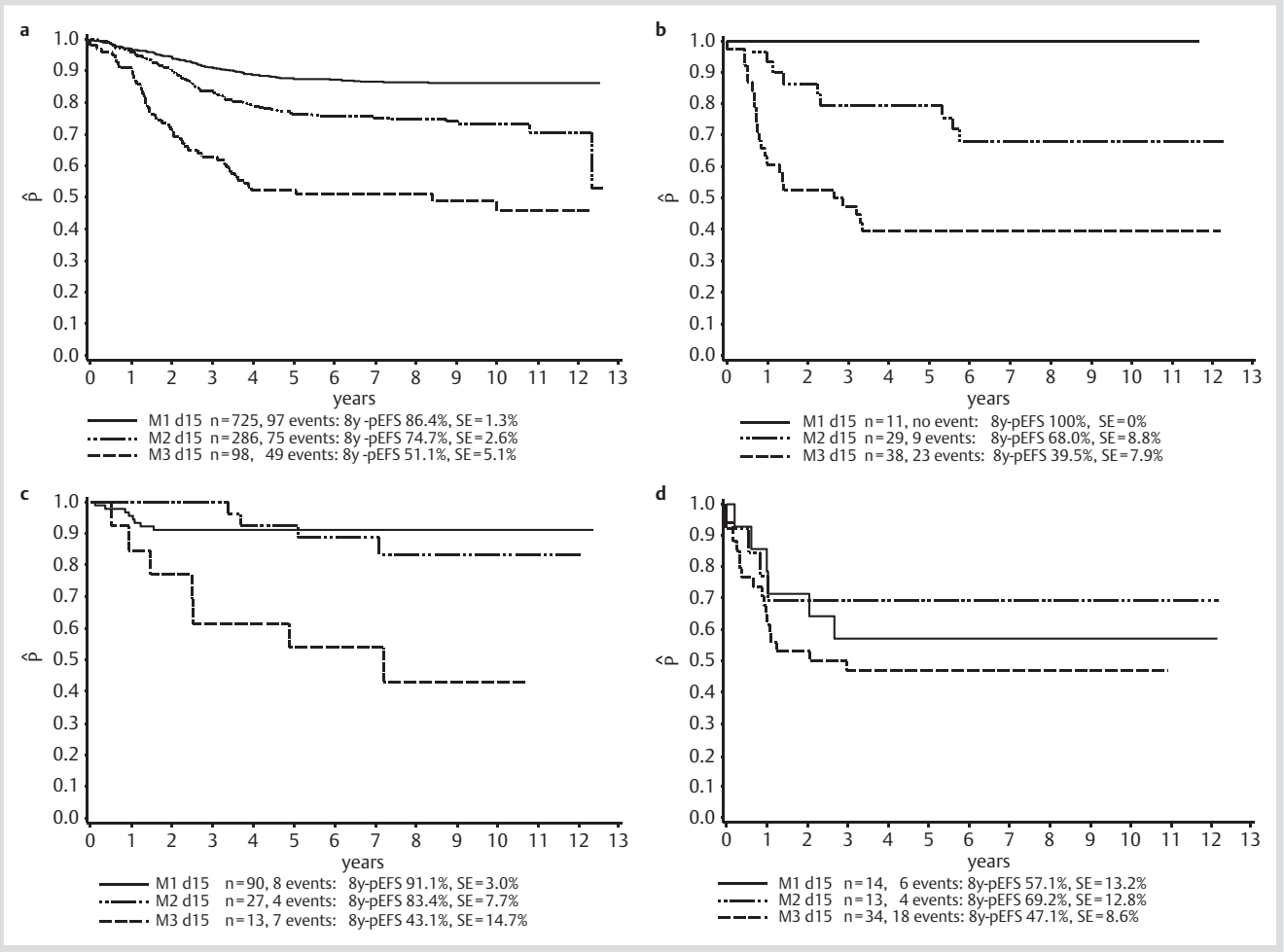

Fig. 2 Kaplan-Meier estimate of event-free survival according to bone marrow response on day 15 stratified by immunophenotypic lineage and prednisone response [17]. For definition of bone marrow M1, M2, and M3 see the Design and Methods section. **a** pB-ALL, prednisone good-response; Log-Rank test (pairwise comparisons): all *p* values <0.001. **b** pB-ALL, prednisone poor-response; Log-Rank test: M1 vs. M2 *p*=0.042; M2 vs. M3 *p*=0.007; M1 vs. M3 *p*=0.002; **c** T-ALL, prednisone good-response; Log-Rank test: M1 vs. M2 *p*=0.44; M2 vs. M3 *p*=0.005; M1 vs. M3 *p*<0.001; **d** T-ALL, prednisone poor-response; Log-Rank test: M1 vs. M2 *p*=0.65; M2 vs. M3 *p*=0.22; M1 vs. M3 *p*=0.42. 8y-pEFS indicates probability of event-free survival at 8 years; SE, standard error.

including all patients with PPR and/or M3 BMd15 (n=74, 8y-pEFS 52.1±5.9%; *p*<0.001) (**Fig. 3**).

NCI risk criteria had a borderline significant prognostic value in patients with M1 BMd15, but showed no statistic significance in patients with M2 or M3 BMd15 (**Table 1**).

Consistent with these results, multivariate Cox regression analysis including NCI risk criteria, PR, BMd15 and BMd33 as covariates revealed BMd15 M3 as the strongest independent adverse risk factor (RR 2.93, p=0.008 and in addition marginal significance for PPR (RR 1.99, p=0.059) and NCI-HR (RR 2.25, p=0.092). The biological considerations which might explain the minor prognostic importance of PR compared with BMd15, seem to be less applicable for T-ALL patients. The data indicate that resistance to prednisone (i.e., PPR) in T-ALL eventually could not be overcome by the following chemotherapy even in those patients, who apparently had a reasonable response in the later course of induction treatment as reflected by M1 or M2 BMd15. The reliability of these data might be weakened due to the small patient numbers remaining in the T-ALL subgroups in this analysis. However, the results are supported by the recently published data on the prognostic impact of MRD in the AIEOP-BFM ALL 2000 trial [31]. In this study, the PR in T-ALL also retained a – though just borderline significant – prognostic value when analyzed in a multivariate model including the MRD risk groups. In pB-ALL in contrast, PPR completely lost its adverse prognostic value if compared with PGR patients with the same PCR-MRD levels [4].

Implication for risk stratification in current and future ALL trials

For more than 20 years, cytomorphological response has been the leading criterion for stratifying patients into risk groups within the ALL-BFM trials. Since ALL-BFM 86, cytomorphological response has been estimated very early during induction treatment using the PR as criterion for risk stratification. Cytomorphological treatment response in the BM, however, was evaluated only at the end of induction treatment (day 33). Poor cytomorphological response at either response evaluation point qualified a patient for high-risk treatment [5,24,27].

The prognostic significance of early reduction of leukemic blasts in BM at different points during induction treatment was shown in a number of pediatric ALL trials (for review see Gaynon et al. [12]) and was implemented as risk stratification criterion in various international trials [1,3,8,10,26,32,36,38]. Specificity of response evaluation might, nevertheless, vary depending on

Table 1 Treatment outcome in pB-ALL and T-ALL by age and WBC at diagnosis, different risk group classifications and treatment response [17].

		Bone marrow day 15							
		M1			M2			M3	
Variable	N (%)	8y-pEFS, % (SE)	p^1	N (%)	8y-pEFS, % (SE)	p^1	N (%)	8y-pEFS, % (SE)	p^1
pB-ALL									
Age (years)									
<1[5]	8 (1.1)	62.5 (17.1)		10 (3.2)	20.0 (12.6)		6 (4.3)	16.7 (15.2)	
1 ≤ 10	312 (82.6)	88.8 (1.3)	<0.001[4]	253 (79.8)	81.8 (2.5)	<0.001[4]	89 (64.5)	51.3 (5.3)	0.133[4]
≥ 10	121 (16.3)	75.7 (4.3)		54 (17.0)	46.3 (7.9)		43 (31.2)	43.6 (7.7)	
Initial WBC (×10^9/L)									
<50	648 (87.4)	88.2 (1.3)	<0.001	262 (82.6)	75.7 (2.7)	0.160	92 (66.7)	53.7 (5.3)	0.015
≥ 50	93 (12.6)	74.8 (4.5)		55 (17.4)	67.2 (6.3)		46 (33.3)	33.9 (7.1)	
Risk group (ALL-BFM 95)[2]									
standard	327 (44.1)	91.8 (1.5)	<0.001	109 (34.4)	84.8 (3.5)	<0.001	17 (12.3)	70.6 (11.1)	0.002
intermediate	384 (51.8)	83.7 (1.9)		162 (51.1)	72.4 (3.6)		63 (45.7)	52.9 (6.4)	
high	30 (4.0)	66.7 (8.6)		46 (14.5)	55.9 (7.4)		58 (42.0)	33.9 (6.3)	
NCI/Rome risk group[3]									
standard	537 (73.3)	90.6 (1.3)	<0.001	213 (69.4)	81.7 (2.7)	<0.001	59 (44.7)	58.8 (6.5)	0.016
high	196 (26.7)	76.0 (3.2)		94 (30.6)	62.8 (5.2)		73 (55.3)	40.7 (5.8)	
Prednisone response									
good	725 (98.5)	86.4 (1.3)	0.20	286 (90.8)	74.7 (2.6)	0.51	98 (72.1)	51.1 (5.1)	0.088
poor	11 (1.5)	100 (0.0)		29 (9.2)	68.0 (8.8)		38 (27.9)	39.5 (7.9)	
Remission day 33									
no	0 (0.0)	–	–	3 (1.0)	–	–	28 (20.6)	23.6 (8.2)	<0.001
yes	733 (100)	86.8 (1.3)		310 (99.0)	74.3 (2.5)		108 (79.4)	54.0 (4.8)	
T-ALL									
Age (years)									
<1[5]	0 (0.0)	–		1 (2.5)	–		0 (0.0)	–	
1 ≤ 10	67 (62.6)	88.1 (4.0)	0.65	26 (65.0)	80.6 (7.8)	0.76	24 (51.1)	56.8 (10.5)	0.126
≥ 10	40 (37.4)	85.0 (5.6)		13 (32.5)	67.7 (17.1)		23 (48.9)	34.2 (10.0)	
Initial WBC (×10^9/L)									
<50	55 (51.4)	92.7 (3.5)	0.069	18 (45.0)	75.7 (10.7)	0.89	17 (36.2)	20.2 (15.5)	0.46
≥ 50	52 (48.6)	80.7 (5.5)		22 (55.0)	81.8 (8.2)		30 (63.8)	53.3 (9.1)	
Risk group (ALL-BFM 95)[2]									
standard	–	–		–	–		–	–	
intermediate	93 (86.9)	91.4 (2.9)	<0.001	27 (67.5)	83.4 (7.7)	0.168	11 (23.4)	50.9 (16.3)	0.26
High	14 (13.1)	57.1 (13.2)		13 (32.5)	69.2 (12.8)		36 (76.6)	44.4 (8.3)	
NCI/Rome risk group[3]									
standard	32 (29.9)	96.6 (3.1)	0.054	12 (30.8)	82.5 (11.3)	0.62	6 (12.8)	41.7 (30.4)	0.26
high	75 (70.1)	82.6 (4.4)		27 (69.2)	76.0 (8.7)		41 (87.2)	43.8 (7.8)	
Prednisone response									
good	90 (86.5)	91.1 (3.0)	<0.001	27 (67.5)	83.4 (7.7)	0.168	13 (27.7)	43.1 (14.7)	0.58
poor	14 (13.5)	57.1 (13.2)		13 (32.5)	69.2 (12.8)		34 (72.3)	47.1 (8.6)	
Remission day 33									
no	–	–	–	1 (2.6)	–	–	10 (21.3)	30.0 (14.5)	0.129
yes	106 (100)	86.8 (3.3)		38 (97.4)	80.3 (6.8)		37 (78.7)	49.3 (8.7)	

[1] The p value (log-rank test) refers to comparison within BMd15 subgroups; [2] Risk groups according to the risk criteria of trial ALL-BFM 95; [3] NCI/Rome standard risk, age 1 year or older and less than 10 years and WBC less than 50 000/µl; NCI/Rome high risk, age 10 years or older or WBC 50 000/µl or higher; Infants less than 1 year were excluded from the NCI definition; [4] The p value (log-rank test) refers to comparison of the age groups 1 to less than 10 years vs. 10 years and older; [5] Patients treated in the Interfant-99 pilot study were excluded

the time of response evaluation with regard to the therapy and the composition of the treatment [30, 36, 37].

In view of the prognostic impact of PR and BMd15 in ALL-BFM 95, one should scrutinize if the PR is still needed for risk stratification when using BMd15. In the stratification of T-ALL patients, it is obvious that combining BMd15 with PR added significant value to BMd15 alone and allowed the stratification into 2 widely separated risk groups, the better of them with an excellent 8y-pEFS of almost 90 % and another poor risk group with an 8y-pEFS near 50 % including 74 % of all T-ALL events. For patients with pB-ALL, in contrast, the data on early treatment reponse in ALL-BFM 95 may suggest that the PR could be omitted as stratification parameter. However, in ALL-BFM 95 the good outcome of pB-ALL patients with PPR and subsequently good BM response on day 15 was achieved with an intensified high-risk treatment. Whether these results could be reproduced with less intensive treatment, remains unclear. This should be carefully considered before omitting the PR as a risk stratification parameter in these patients.

While in pB-ALL the combination of PR with BMd15 failed to improve risk group separation obtained through by BMd15 alone, the use of the ALL-BFM 95 or the NCI risk criteria, both using age and initial WBC, in addition to BMd15 gave an added prognostic value. In the COG (or formerly CCG) protocols, the

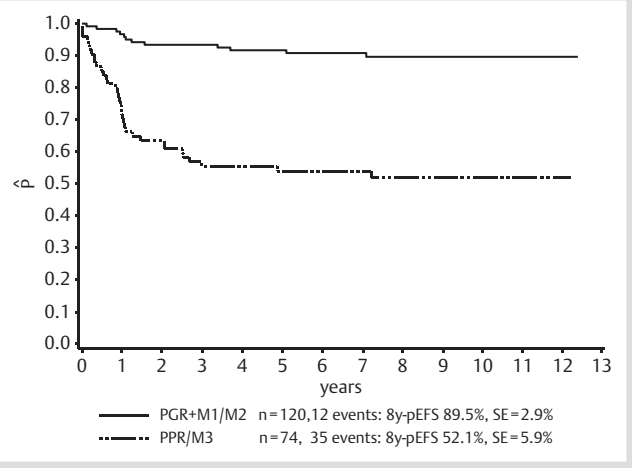

Fig. 3 Kaplan-Meier estimate of event-free survival in T-ALL comparing patients with prednisone good-response plus M1 or M2 marrow on day 15 (PGR + M1/M2) with patients with prednisone poor-response and/or M3 marrow on day 15 (PPR/M3) [17]; Log-Rank test $p < 0.001$. 8y-pEFS indicates probability of event-free survival at 8 years; SE, standard error.

combination of NCI risk criteria with early (day 7 and day 14) marrow response has been used for risk stratification for many years [13, 32]. The ALL IC-BFM study group introduced cytomorphological BM response on day 15 in the non-MRD-based protocol ALL IC-BFM 2002 for a risk stratification system which was based on the ALL-BFM 95 criteria, but shifted the patients to a higher risk group in the case of an M3 BMd15 [7]. ALL IC-BFM 2002 was performed in countries, which did not have access to MRD diagnostics, mainly due to financial burden [7]. For these countries, optimization of risk stratification by the intelligent use of clinical parameters and cytomorphological response evaluation is worthwhile. However, the prognostic relevance of cytomorphological response has always to be interpreted in the context of the specific chemotherapy regimen administered. Thus, transferring the treatment response data of ALL-BFM 95 onto other – in particular less intensive – treatment regimens has to be done with caution.

The ALL-BFM 95 protocol is currently used as regular chemotherapy protocol for childhood ALL in several countries. Our data demonstrate that the inclusion of BM response on day 15 crucially improves the ALL-BFM 95 risk stratification in the context of the ALL-BFM 95 therapy which is of particular interest in less affluent countries in which expensive laboratory techniques are not affordable.

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