Minimal residual disease in peripheral blood at day 15 identifies a subgroup of childhood B-cell precursor acute lymphoblastic leukemia with superior prognosis

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The online version of this article has a Supplementary Appendix.

ABSTRACT

Background

Most minimal residual disease-directed treatment interventions in current treatment protocols for acute lymphoblastic leukemia are based on bone marrow testing, which is a consequence of previous studies showing the superiority of bone marrow over peripheral blood as an investigational material. Those studies typically did not explore the prognostic impact of peripheral blood involvement and lacked samples from very early time points of induction.

Design and Methods

In this study, we employed real-time quantitative polymerase chain reaction analysis to examine minimal residual disease in 398 pairs of blood and bone marrow follow-up samples taken from 95 children with B-cell precursor acute lymphoblastic leukemia treated with the ALL IC-BFM 2002 protocol.

Results

We confirmed the previously published poor correlation between minimal residual disease in blood and marrow at early treatment time points, with levels in bone marrow being higher than in blood in most samples (median 7.9-fold, range 0.04-8,293-fold). A greater involvement of peripheral blood at diagnosis was associated with a higher white blood cell count at diagnosis (P=0.003) and with enlargement of the spleen (P=0.0004) and liver (P=0.05). At day 15, a level of minimal residual disease in blood lower than 10^{-4} was associated with an excellent 5-year relapse-free survival in 78 investigated patients (100% *versus* 69±7%; P=0.0003). Subgroups defined by the level of minimal residual disease in blood at day 15 (high-risk: ≥10^{-2}, intermediate-risk: <10^{-2} and ≥10^{-4}, standard-risk: <10^{-4}) partially correlated with bone marrow-based stratification described previously, but the risk groups did not match completely. No other time point analyses were predictive of outcome in peripheral blood, except for a weak association at day 8.

Conclusions

Minimal residual disease in peripheral blood at day 15 identified a large group of patients with an excellent prognosis and added prognostic information to the risk stratification based on minimal residual disease at day 33 and week 12.

Key words: acute lymphoblastic leukemia, peripheral blood, minimal residual disease, day 15, childhood.

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Introduction

Minimal residual disease (MRD) testing demonstrated great prognostic impact in childhood acute lymphoblastic leukemia (ALL) as early as a decade ago.[1-3] Since then, it has been used in risk stratification for most progressive childhood and adult ALL treatment protocols.[4-8]

Bone marrow aspirates are consistently used for MRD evaluation based on both the bone marrow location of the disease and previous reports exploring the level of MRD in the peripheral blood compared to that in the bone marrow. Several studies have proven that the levels of MRD in peripheral blood and bone marrow correlate well in T-ALL, but that the correlation is weak in B-cell precursor ALL, with a generally lower peripheral blood MRD.[9-13]

Surprisingly, only a few reports have explored the prognostic impact of MRD in peripheral blood, although sampling peripheral blood is far more comfortable for patients and easier for clinicians than aspirating bone marrow. Brisco *et al.*[14] investigated whether peripheral blood MRD could be used for the early detection of bone marrow relapse and showed that MRD was detectable in the peripheral blood from all eight patients examined at least 10 weeks prior to bone marrow relapse. Coustan-Smith *et al.*[10] showed, in a limited number of patients, that patients with detectable MRD in both the peripheral blood and bone marrow at the end of induction therapy (day 46) had a significantly higher incidence of relapse than patients with MRD in the bone marrow only.

In this study, we investigated the prognostic impact of peripheral blood involvement at early time points of childhood ALL treatment using the Berlin-Frankfurt-Münster (BFM) ALL IC-BFM 2002 protocol.

Design and Methods

Patients

Ninety-five children (aged 1 to 18 years) who were newly diagnosed with B-cell precursor ALL between November 2002 and April 2006 were included in this study. All children were treated in the Czech Republic according to the ALL IC-BFM 2002 protocol. The treatment and risk group stratification in this protocol (not based on MRD) have already been published.[15] In brief, age, white blood cell count at diagnosis, response to prednisone after the first week of therapy ("prednisone response"), and bone marrow morphology at day 15 (e.g., M1-M3) were used for stratification. The MRD response in the bone marrow at days 8, 15, and 33, and at week 12 was evaluated within the accompanying scientific project aimed at comparing the ALL-IC stratification with the MRD-based stratification used in the parallel AIEOP-BFM ALL 2000 study.[4,15] Of the 95 patients included in the current study, 45 were stratified into the standard-risk group, 46 into the intermediate-risk group, and 4 into the high-risk group. The patients were recruited based on the availability of paired peripheral blood and bone marrow specimens from the treatment period. The study group did not differ significantly in terms of gender, age, white blood cell count at diagnosis, bone marrow morphology at day 15, immunophenotype, presence of *TEL/AML1* fusion, hyperdiploidy, presence of the Ikaros (*IKZF1*) gene deletion, or relapse-free survival from the whole group of 166 patients with B-cell precursor ALL treated in the study period (*Online Supplementary Table S1*). We examined 398 pairs of peripheral blood and bone marrow samples taken simultaneously at the following time points: diagnosis (n=93), day 8 (n=83), and day 15 (n=78) of induction, the end of induction phase 1 - day 33 (n=53), pre-consolidation - week 12 (n=47), prior to maintenance therapy (n=6), and at the end of maintenance therapy (n=38). The median follow-up period of the study group was 6.3 years. The research was approved by the relevant institutional ethics committee. All patients or their parents or guardians gave informed consent to participation in the study. The bone marrow MRD data from this cohort, but not their impact on relapse-free survival, were published as a part of a previous study exploring the relationship between bone marrow MRD and other risk factors.[15] Enlargement of the spleen and liver was evaluated by manual palpation during a neutral respiratory phase and the results are reported in centimeters below the costal margin in the mid-clavicular line.

Flow cytometry

Flow cytometry immunophenotyping of bone marrow aspirates was performed at diagnosis using a recommended panel of monoclonal antibodies as described previously.[16]

DNA index

The DNA index was assessed routinely at diagnosis using the CycleTestPlus DNA Reagent kit (BD, San José, CA, USA) as previously described.[17,18] The DNA index was defined as a ratio of the mode of fluorescence of cells in the G0/G1 phase and the mode of fluorescence of normal peripheral blood in the G0/G1 phase. Specimens with a DNA index between 1.16 and 1.6, inclusive, were labeled as hyperdiploid in this study.

Ikaros gene deletions

The presence of *IKZF1* gene deletions was assessed by multiplex ligation-dependent probe amplification according to the manufacturer's instructions (MRC Holland, kit P335). The results were evaluated using Coffalyser v9.4 software.

Minimal residual disease monitoring

The isolation of mononuclear cells from the diagnostic and follow-up bone marrow and peripheral blood samples; the isolation of genomic DNA; the detection of Ig heavy chain (*IGH*), Ig light chain kappa (*IGK*), TCR gamma (*TCRG*), and TCR delta (*TCRD*) gene rearrangements by PCR amplification; heteroduplex analysis; and sequencing were performed as described previously.[15,19-21] The design of the patient-specific real-time quantitative PCR (RQ-PCR) systems and the immunoglobulin (*IG*)/ T-cell receptor (*TCR*) gene-based RQ-PCR MRD measurements were also described previously.[15,22-25] In all patients, at least one monoclonal *IG* or *TCR* gene rearrangement was found. Consistent with a current BFM strategy for *IG/TCR*-based MRD monitoring in the frontline protocol,[8] we regarded one patient-specific RQ-PCR assay with a minimal sensitivity of 10^{-4} as sufficient. We also previously showed a very good correlation between values measured using two independent *IG/TCR* targets during ALL IC induction treatment.[26] MRD levels were adjusted to the percentage of leukemic blasts in the bone marrow at diagnosis. The European Study Group on Minimal Residual Disease in ALL (ESG-MRD-ALL) criteria for RQ-PCR sensitivity and MRD positivity were applied.[27] MRD monitoring of patients who were included in this study started in 2002, when the ESG-MRD-ALL criteria for quantitative range were still under development. At that time the optimization of MRD assays for ALL IC studies was aimed at reaching a sensitivity of at least 10^{-4}; the requirements were later changed to a minimum quantitative range of 10^{-4}. All levels below the quantitative range should be now reported as "positive, not quantifiable", but in this study we used numerical values for the analysis.

Statistical analysis

All statistical analyses were performed using StatView® version 5.0 (StatView® Software, Cary, NC, USA) or Statistica software

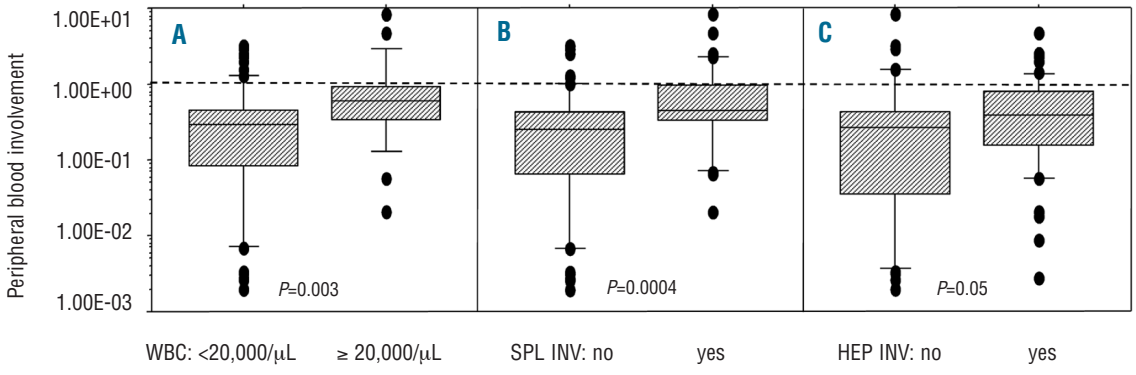

Figure 1. Peripheral blood involvement at diagnosis. Quantitative MRD levels (logarithmic scale) at diagnosis are expressed in relation to the bone marrow involvement at diagnosis, adjusted to the blast percentage at diagnosis, and related to (A) the white blood count (WBC) at diagnosis of less than or more than 20,000/μL (B) the presence of splenic involvement (SPL INV), and (C) the presence of hepatic involvement (HEP INV). Boxes indicate the 25th to 75th percentiles, medians are represented by a horizontal line, Y-error bars extend to the 5th and 95th percentiles, and individual values below the 5th and above the 95th percentiles are shown as circles.

(Statsoft, Tulsa, USA). The distribution of variables between groups was assessed using the χ^2 or Fisher's exact test. The Mann-Whitney test was used to estimate the significance of differences among continuous values. The correlation between bone marrow and peripheral blood MRD levels in paired samples was assessed using Spearman's correlation coefficient. Relapse-free survival was calculated from the date of diagnosis to the last follow-up or to relapse. Rates were calculated according to the Kaplan-Meier method and compared by the log-rank test. Cox regression was used for the multivariate analysis.

Results

Peripheral blood involvement at diagnosis

IG/TCR-based MRD monitoring uses diagnostic bone marrow samples as a standard for quantification to which all follow-up MRD values are related (the bone marrow MRD level at diagnosis is set at 1); therefore, no absolute quantification was possible. The level of relative peripheral blood disease correlated with the white blood cell count at diagnosis. Patients with a leukocyte count of more than 20×10^9/L at diagnosis, corresponding to the cut-off used in the ALL IC-BFM 2002 stratification, had a higher peripheral blood involvement at diagnosis than other patients (P=0.003, Figure 1A). A higher peripheral blood involvement at diagnosis correlated strongly with enlargement of the spleen (P=0.0004, Figure 1B) and less strongly with enlargement of the liver (P=0.05, Figure 1C).

Peripheral blood involvement during the course of initial treatment

As in previous studies, which were performed mostly on samples from later time points, we observed a poor correlation between peripheral blood and bone marrow MRD levels at days 8, 15 and 33 (Figure 2). Among 398 pairs of peripheral blood – bone marrow samples, MRD was double-negative in 118 pairs (30%). In 240 paired samples, MRD was detectable in both tissues; in 34 and 6 pairs, MRD was detectable in only the bone marrow and peripheral blood, respectively. The MRD levels in the peripheral blood varied enormously and were mostly lower than those in the bone marrow, with a mean difference of 206-fold (0.04- to 8,293-fold, double-positive samples only). In

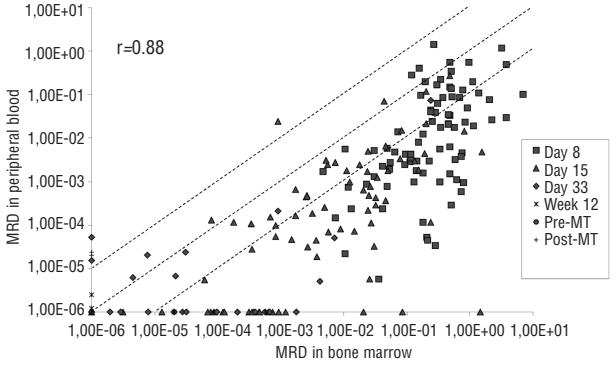

Figure 2. Correlation of MRD levels between blood and bone marrow. Quantitative MRD levels (logarithmic scale) in the bone marrow *versus* peripheral blood in patients with B-cell precursor ALL (excluding diagnostic samples). All MRD values are expressed in relation to the value in bone marrow at diagnosis, which was set as 1. MRD values below the quantitative range, which would otherwise be reported as "positive, not quantifiable", are expressed numerically for the analysis. Pre-MT: Prior to maintenance therapy; Post-MT: End of maintenance therapy; r: Spearman's correlation coefficient.

148/280 positive samples (53%), we observed more than a 1-log difference between bone marrow MRD and peripheral blood MRD. This correlation pattern did not differ among the study time points. In 34 peripheral blood-negative cases, bone marrow values were positive with a median of 2.2×10^{-4}. All six patients positive only in the peripheral blood had very low MRD levels (below 10^{-4}) and did not have any specific feature.

No obvious difference was observed between peripheral blood MRD levels regarding the genetic subtype or Ikaros status, except for the fact that patients with hyperdiploid leukemias had a trend towards lower day 15 peripheral blood MRD values than other patients, excluding the *TEL/AML1* cases (P=0.06, Mann-Whitney, *data not shown*). Peripheral blood levels in patients with common ALL were similar to those of patients with pre-B ALL. Patients with pro-B ALL seemed to have greater peripheral blood involvement, but the low number of patients precluded statistical analysis. Patients stratified to the ALL IC standard-risk and intermediate-risk groups did not differ significantly in their peripheral blood MRD response.

Minimal residual disease in peripheral blood and outcome

Figure 3 shows the levels of peripheral blood MRD at days 8, 15 and 33 with respect to the occurrence of relapse. The peripheral blood MRD at day 15 was clearly higher in patients who subsequently relapsed (P=0.0006, Figure 3B). A similar yet less significant trend was found for day 8 peripheral blood MRD (P=0.02, Figure 3A). At later time points, most of the peripheral blood samples were negative (31/53 at day 33, 42/47 at week 12, 6/6 pre-maintenance therapy, and 36/38 post-maintenance therapy) and thus precluded statistical analysis. A parallel analysis of matched bone marrow samples revealed statistically significant differences at day 15 (P=0.003) and at day 33 (P=0.003, *Online Supplementary Figure S1*).

None of the 35 patients with peripheral blood MRD less than 10^{-4} (MRD_{PB}^{low}) at day 15 relapsed, compared to 13/43 patients with a MRD higher than 10^{-4} (MRD_{PB}^{high}) at this time point (Figure 4A). The MRD_{PB}^{low} low group comprised 22 completely negative patients and 13 patients with weak positivity. The 5-year relapse-free survival was 100% for the MRD_{PB}^{low} group compared to 69±7% for the MRD_{PB}^{high} group (P=0.0003).

At day 8, peripheral blood MRD provided only a weak

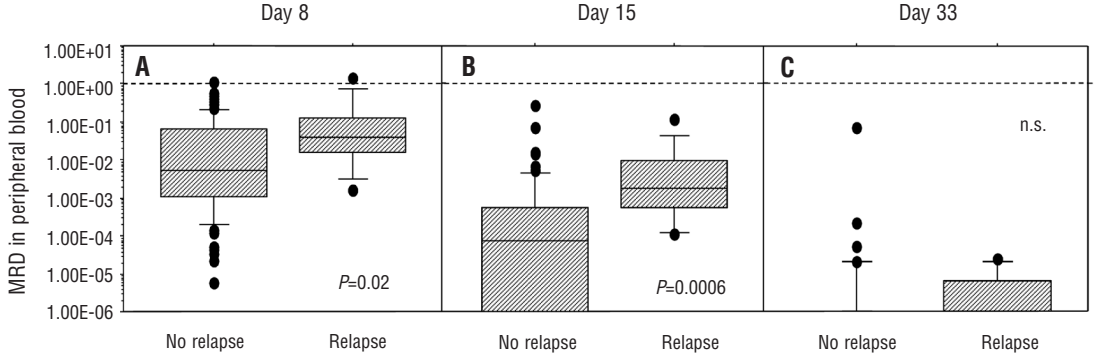

Figure 3. Relationship between MRD in peripheral blood and the occurrence of relapse. Quantitative MRD levels (logarithmic scale) in peripheral blood at days 8, 15, and 33. n.s.: non-significant.

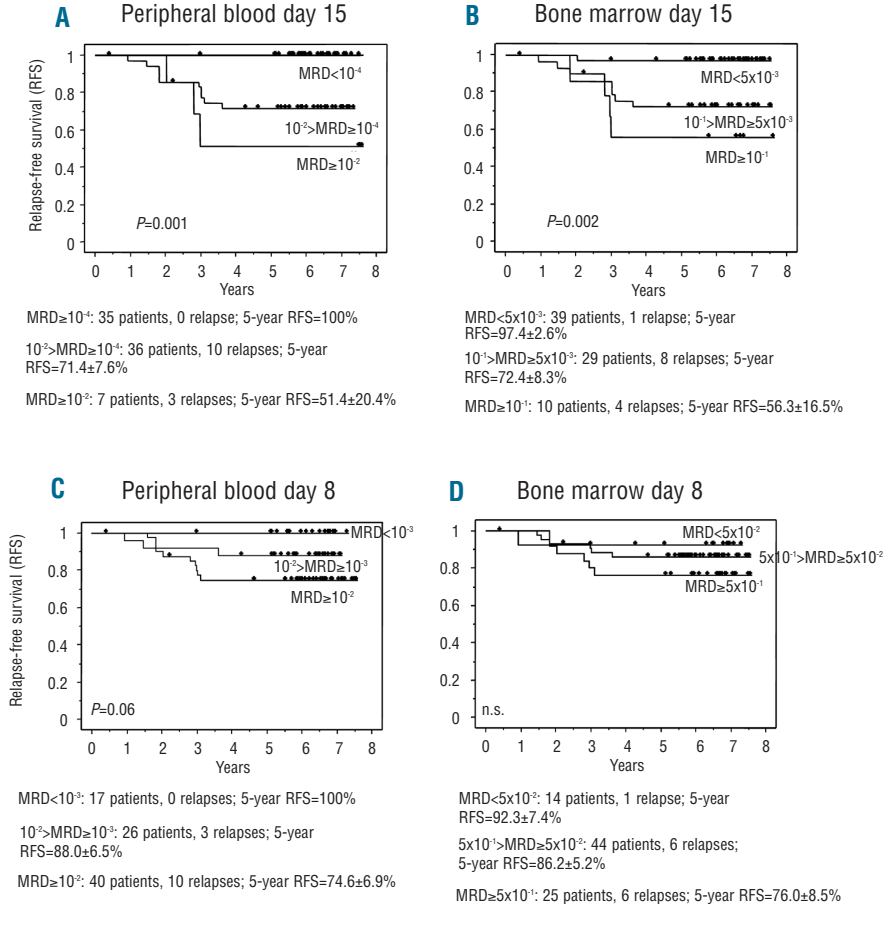

Figure 4. Prognostic significance of MRD levels in blood and bone marrow at days 8 and 15. Kaplan-Meier survival plots showing the relapse-free survival (RFS) of patients with B-cell precursor ALL based on (A) MRD in blood at day 15, as stratified into three categories using the cut-offs of 10^{-2} and 10^{-4}, (B) MRD in bone marrow at day 15, as stratified into three categories using the cut-offs of 10^{-1} and $5x10^{-3}$, (C) MRD in blood at day 8, as stratified using the cut-offs of 10^{-2} and 10^{-3}, and (D) MRD in bone marrow at day 8, as stratified using the cut-offs of $5x10^{-1}$ and $5x10^{-2}$. n.s.: non-significant.

prediction of relapse-free survival (P=0.06, using the cut-offs 10^{-3} and 10^{-2} for defining the low, intermediate and high-risk groups, Figure 4C), while bone marrow MRD was not at all predictive with any cut-off that we applied (Figure 4D).

Minimal residual disease in peripheral blood at day 15 in risk group stratification

Consistent with previous reports,[28-32] bone marrow MRD levels at day 15 were also predictive of the outcome. To define three risk groups based on day 15 bone marrow MRD, we used the cut-offs of 10^{-1} and 5×10^{-3} (Figure 4B). The size of the MRD_{PB}^{low} group (35/78, 45%) was similar to that of the bone marrow low-risk group defined by a bone marrow MRD of less than 5×10^{-3} (39/78, 50%), which comprised one relapsed patient.

As for the definition of high risk based on peripheral blood, patients with a day 15 peripheral blood MRD higher than 10^{-2} had a 5-year relapse-free survival of only 51±20% (Figure 4A), similar to that of the patients with a bone marrow MRD higher than 10^{-1} (56±17%, Figure 4B). However, the difference between the high-risk and intermediate-risk groups was not statistically significant for either bone marrow or peripheral blood.

The correlation of day 15 peripheral blood MRD stratification using the cut-off 10^{-4} with other risk factors is shown in Table 1. The group of patients with MRD_{PB}^{low} consisted of 18 standard-risk, 16 intermediate-risk, and 1 high-risk patients as stratified by the ALL IC-BFM 2002 criteria. As expected, most of the day 15 MRD_{PB}^{low} patients had M1 bone marrow morphology at day 15 (P<0.0001). The comparison of day 15 peripheral blood MRD stratification with the currently used BFM bone marrow MRD risk groups (based on combined bone marrow information from day 33 and week 12) showed only a partial overlap. Within the BFM MRD intermediate risk group, low day 15 peripheral blood MRD defined a subgroup with no relapse (P=0.008, Cox-Mantel, *data not shown*). Of note, two patients from the BFM MRD standard risk group with negative bone marrow MRD at both day 33 and week 12 relapsed, and both of them were day 15 MRD_{PB}^{high}.

The correlation with day 15 bone marrow MRD subgroups was highly significant (P<0.0001, χ^2 test), but again, the groups did not match completely (Table 2). Interestingly, the one patient in the bone marrow MRD low-risk group (less than 10^{-3}) who relapsed would have been classified as high risk (more than 10^{-2}) according to peripheral blood MRD.

To assess whether day 15 peripheral blood MRD adds information to the current risk group stratification, we performed bi-variate Cox regression analysis, first using ALL IC-BFM 2002 protocol risk groups (based on age, white blood cell count at diagnosis, prednisone response and bone marrow morphology at day 15) and then using the BFM criteria based on combined bone marrow MRD information from day 33 and week 12. Low peripheral blood MRD at day 15 ($<10^{-4}$) was prognostically significant both when added to the ALL IC criteria (HR, 0.08; 95% CI, 0.011-0.62; P=0.016) and the current BFM MRD criteria (HR, 0.12; 95% CI, 0.015-0.915; P=0.041).

Table 1. Distribution of MRD levels in peripheral blood at day 15.

	MRD in blood at day 15≥10^{-4}		MRD in blood at day 15<10^{-4}		
Immunophenotype					
cALL	36	62%	22	38%	
preB	6	33%	12	67%	P=0.10
proB	1	50%	1	50%	
Genetics					
TEL/AML1	16	59%	11	41%	
Hyperdiploid	9	43%	12	57%	P=0.42
Other	18	60%	12	40%	
ALL IC risk group					
SR	18	50%	18	50%	
IR	23	59%	16	41%	P=0.68
HR	2	67%	1	33%	
Bone marrow morphology at day 15					
M1	20	37%	34	63%	
M2	20	95%	1	5%	P<0.0001
M3	3	100%	0	0%	
BFM MRD risk group*					
SR	20	51%	19	49	
IR	19	70%	8	30	P=0.29
HR	1	50%	1	50	
MRD in bone marrow at day 33					
Positive	20	77%	6	23%	
Negative	22	48%	24	52%	P=0.02
Ikaros alteration					
Yes	3	60%	2	40%	
No	36	58%	26	42%	P=0.93
Relapse					
No	30	46%	35	54%	
Yes	13	100%	0	0 %	P=0.0004

*Because of missing values, the numbers do not always add up to 78. * BFM MRD risk group: SR=standard risk, day 33 and week 12 bone marrow (BM)-MRD negative; IR=intermediate risk, day 33 or week 12 BM-MRD positive; HR=high risk, week 12 ≥10^{-3} or prednisone poor response or* BCR/ABL *or* MLL/AF4 *or non-remission at day 33. MRD was evaluated by one* Ig/TCR *target.*

Table 2. Distribution of patients/relapses according to the MRD levels in bone marrow (BM) and peripheral blood (PB) at day 15.

MRD at day 15:		PB ≥10^{-2}	PB <10^{-2} and ≥ 10^{-4}	PB <10^{-4}
BM	<5×10^{-3}	1/1 rel.	11/0 rel.	27/0 rel.
	<10^{-1} and ≥ 5×10^{-3}	2/0 rel.	20/8 rel.	7/0 rel.
	≥10^{-1}	4/2 rel.	5/2 rel.	1/0 rel.

rel: relapse.

Discussion

As in previous studies exploring later treatment time points, we too showed that in the early phase of B-cell precursor ALL treatment, peripheral blood involvement is highly variable and does not always reflect the situation in the bone marrow. Despite this fact, our results showed that peripheral blood MRD at day 15 of the BFM-based treatment is related to prognosis.

Starting with a quantitative assessment of diagnostic peripheral blood involvement relative to bone marrow involvement, our study demonstrated that the level of peripheral blood disease correlates with the white blood cell count at diagnosis, which is an expected result. Taking inter-observer bias into account, the finding that a higher peripheral blood involvement at diagnosis is related to enlargement of the spleen and liver likely reflects a similar connection with the white blood cell count at diagnosis, which was observed both in our study and a previous

study.[33] Of note, the initial leukemic cell mass, calculated as a continuous variable from the blast count in the blood at diagnosis and the size of the liver and spleen, was used for stratification in the BFM-81, 83, 86, and 90 trials.[34]

One of the major goals of this work was to determine whether bone marrow aspiration could be replaced by less invasive peripheral blood sampling at some of the examined time points. Day 15 seems to be the only candidate for further consideration because most of the samples were peripheral blood MRD negative and thus did not provide any useful prognostic information at later time points (day 33, week 12, pre-maintenance therapy, post-maintenance therapy). Peripheral blood aspiration at day 8 has been established as a gold standard because of a highly predictive value of the cytomorphologically estimated "prednisone response" in defining high-risk patients.[35,36] A large study performed by the Children's Oncology Group showed that patients with high levels of peripheral blood MRD at day 8, as measured by flow cytometry, had a relatively poor outcome even if they reached bone marrow MRD negativity by day 29.[37] However, due to a different protocol without the prednisone prephase, the MRD data are not directly comparable with those of our study.

The prognostic impact of day 15 bone marrow MRD in BFM-based protocols has already been proven in several independent studies using either an *IG/TCR*-based RQ-PCR approach[28,29] or flow cytometry.[30-32] In a flow cytometric study performed on 830 patients treated according to the AIEOP-ALL BFM 2000 protocol, Basso *et al.* defined three risk groups with significantly different outcomes. The low-risk group, defined by less than 10^{-3} (0.1%) blast cells in the bone marrow, comprised 42% of the patients and had a 5-year cumulative incidence of relapse of 7.5%. Consistent with the PCR-based study of Panzer-Grümayer *et al.*,[28] there was no relapse among patients with a day 15 bone marrow-MRD below 10^{-4} in our study, but this group comprised only 13% of patients. Using the bone marrow MRD cut-off of 5×10^{-3}, a larger low-risk group could be defined with a 5-year relapse-free survival of 97±3%. Peripheral blood MRD at the same time point in our study identified a similarly large (45%) low-risk group with a superior outcome, which only partially overlapped with the day 15 bone marrow low-risk group (Table 1). This group consisted mostly of standard-risk patients but also included intermediate-risk patients and even one high-risk patient, as stratified by the ALL IC-BFM 2002 criteria based on age, white blood cell count at diagnosis, prednisone response, and bone marrow morphology status (M1-M3) at day 15.

All previous day 15 bone marrow studies defined a high-risk group with an inferior outcome. The high-risk group defined by day 15 bone marrow MRD over 10% (10^{-1}) consisted of 11% of patients and had a 5-year cumulative incidence of relapse of 47±6% in the study by Basso *et al.* In a multivariate analysis including BFM PCR MRD stratification, the high-risk group based on day 15 flow cytometry was shown to be an independent risk factor. In the study by Sutton *et al.*,[29] performed on patients treated in the Australian ANZCCSG Study VII, all patients with a day 15 bone marrow MRD higher than 5×10^{-2} relapsed. In this study, we applied a cut-off 10^{-1} with a very similar result to that of Basso *et al.* (Figure 4B). We tried to define a similar high-risk group using peripheral blood MRD and setting 10^{-2} as a cut-off; however, neither bone marrow nor peripheral blood distinguished the high-risk group from the intermediate-risk group with statistical significance (Figure 4A, B). This result was probably caused by a relatively low number of patients in our study.

Defining the low-risk group as early as day 15 offers the possibility of early treatment de-escalation (e.g., decreasing the dose of the anthracyclines used in the induction treatment in BFM-based protocols), although there are still no published data supporting this approach. The main important technical issue for day 15 MRD evaluation lies in the methodology used in this study. The introduction of a patient-specific RQ-PCR assay based on *IG/TCR* clonal rearrangement is time-demanding and requires at least 2 weeks, but usually 4, from diagnosis, which would make an early treatment intervention based on day 15 PCR MRD difficult. Flow cytometry, with its prompt results, would overcome this problem after rigorous standardization. However, it is premature to argue that our results would also be applicable to flow cytometry. Despite satisfactory PCR-flow cytometry correlation at day 15 in our previous study, there was a substantial variation in results for individual pairs of samples, suggesting that the risk group definition based on either PCR or flow cytometry would be at least partly divergent.[31]

Similarly to day 15 bone marrow MRD studies,[28,30] also the day 15 peripheral blood MRD classification added prognostic information to the current BFM risk group stratification based on combined PCR MRD data from day 33 and week 12 in a bi-variate analysis. The large study by Basso *et al.* provided a platform for adding flow cytometric bone marrow MRD at day 15 to the current BFM stratification based on PCR MRD at day 33 and week 12. Our study demonstrated that day 15 peripheral blood would be similarly helpful as the day 15 bone marrow in defining the low-risk group of patients. However, it was not sufficiently powered to elucidate whether day 15 peripheral blood alone could also safely identify other high-risk patients on top of the day 33 and week 12 PCR classification, as did the day 15 bone marrow.[30] Larger studies are needed to determine whether peripheral blood testing alone could replace bone marrow MRD monitoring at day 15.

Authorship and Disclosures

The information provided by the authors about contributions from persons listed as authors and in acknowledgments is available with the full text of this paper at www.haematologica.org.

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