Real-time quantitative polymerase chain reaction detection of minimal residual disease in acute lymphoblastic leukemia: a single-center experience

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Background

Monitoring of minimal residual disease (MRD) has become a frontline clinical practice in the treatment of virtually all childhood acute lymphoblastic leukemia (ALL) cases and in many cases of adult patients with ALL. The MRD diagnostics has proven to be the strongest prognostic factor allowing for risk group assignment into different treatment arms. The MRD techniques need to be sensitive ($\leq 10^{-4}$), which means, the ability to detect one malignant cell among 10 000 normal cells; broadly applicable; accurate; reliable; fast; and affordable.

Aim

The objective of this study is to evaluate the analysis of immunoglobulin heavy chain (IGH) or T-cell receptor (TCR) gene rearrangements as targets for MRD assessment in ALL, allowing early detection of relapsed cases, compare with the results of morphological evaluation of the same cases and to risk stratify patients with ALL according to the MRD assessment as a prognostic marker independent and superior to other conventional risk factors.

Patients and methods

Overall, 30 patients (15 males and 15 females) with age ranged from 1 to 25 years old were included in this study. Patients were subjected to full medical history, clinical examination, laboratory examinations such as complete blood count, bone marrow aspirate smear examination, cytochemistry, and immunophenotyping. The molecular studies done by real-time PCR were performed using consensus primers and allele-specific primers for (IGH) or (TCR) gene rearrangements as targets to detect MRD. The ALL cases were assessed by real-time quantitative PCR at the time of diagnosis and at the end of induction chemotherapy, and comparative cycle threshold (C_t) relative quantification method was used for quantitative gene expression.

Results

The results showed discrepancy between the morphologic examination for ALL assessment depending on the 5% blast index as an indicator of remission or nonremission; morphological nonremissions (>5% blast) were detected in eight (26.7%) cases whereas 22 (73.3%) cases showed morphological remissions (<5% blast). Real-time quantitative PCR for IGH/TCR gene rearrangements was done for 30 patients. Of the 22 cases that showed morphological remission, there were 18 (81.8%) cases which were MRD positive (i.e. not in molecular remission) and four (18.2%) cases which were MRD negative (i.e., in molecular remission). Patients who showed nonremission morphologically (right cases) were confirmed to be MRD positive. None of the patient's clinical variables such as age, sex, total leukocyte count, and ALL immunophenotype have been identified as predictors of MRD risk.

Conclusion

Approximately 81% of ALL cases previously diagnosed as being in the remission state depending on the morphologic assessment only tend to harbor MRD as evident by positive MRD assessment using real-time PCR. The molecular assessment of MRD allows early detection of relapse with chance of intervention and tailoring of treatment according to the patients' need. It was found that there was no relation between MRD results and risk stratification in ALL according to age and white blood cell at diagnosis; this confirms that detection of MRD of leukemic cells can be considered a superior prognostic marker of relapse, independent from conventional prognostic factors. The risk classification in ALL based on MRD results could be used to guide the final treatment strategy and predict early relapse; this should allow prediction of response and relapse while patients are still in the state of clinical remission and morphological remission too.

Keywords:
acute lymphoblastic leukemia, minimal residual disease, real time polymerase chain reaction

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Introduction

Current therapy protocols in acute lymphoblastic leukemia (ALL) succeeded to acheive complete clinical remission of 80–95% of adult patients with ALL and greater than 95% of pediatric patients with ALL, yet a substantial fraction of those patients eventually ends up with relapse, which is because of the presence of residual malignant cells, so-called minimal residual disease (MRD), which are below detection level when using conventional techniques only [1]. ALL risk group classification was done according to the National Cancer Institute (NCI)-sponsored workshop in 1993. Based on their age and white blood cell (WBC count at diagnosis). Patients with ALL were classified as follows: for B-cell ALL (B-ALL), the standard-risk category includes patients 1–9 years of age with a WBC count at diagnosis of less than 50×10^9/l and high-risk category includes patients with either of the criteria of WBC count greater than 50×10^9/l or children younger than 1 year or older than 9 years. For T-cell ALL (T-ALL), patients will be assigned into corresponding risk group based on the same age/WBC count criteria [2].

In the era of technologic advances, which allows the detection of MRD with different techniques, it becomes possible to upgrade the assesment of response to ALL therapy and tailor therapy regimen to either intensify treatment for cases with a poor early response or deintensify therapy with concomitant reduction in toxicity for cases that show an early response, predicting a good outcome. ALL risk group assignment is done based on the MRD results [3].

Both molecular and immunophenotypic techniques can detect MRD; however, most MRD studies in ALL have used PCR techniques using immunoglobulin (Ig) and/or T-cell receptor (TCR) gene rearrangements as patient-specific targets, which has sensitivities of 10^{-4}. The existence of MRD early in the course of treatment is strongly linked to poor treatment response and an increase in the relapse rate [4].

The MRD techniques with sensitivity and detection limit as high as one malignant cell per 10^{-4}–10^{-5}, which is ~100-100 000 times more sensitive than morphological assessment, are now considered in practice [5].

The aim of the work is to evaluate the analysis of immunoglobulin heavy chain (IGH) or TCR gene rearrangements as targets for MRD assessment in ALL, allowing early detection of relapse cases with very high sensitivity, and to risk stratify patients with ALL according to the MRD assessment as a prognostic marker, which is independent and superior to other conventional risk factors in ALL.

Patients and methods

Patients

This study was conducted on 30 patients who were admitted to the South Egypt Cancer Institute, Assiut University, having ALL during the period between May 2011 to August 2012. An informed consent was obtained from patients (or guardians), and the study was approved by the Ethical Committee of Faculty of Medicine, Assiut University. Patients consists of 15 males and 15 femals, with an age ranged from 1 to 25 years old (mean ± SD = 8.17 ± 6.48), and they were diagnosed as having ALL on both clinical and laboratory studies, which include morphological, cytochemical, and immunophenotyping assessments.

Inclusion criteria

All patients included were newly diagnosed as having ALL and had not previously received any treatment for ALL. Patients' regimen was based on the modified Berlin-Frankfurt-Münster BFM 76/79 protocol [6], which consisted of the following.

Phase 1 (induction therapy) 5 weeks

(1) Prednisolone 60 mg/m^2/day postoperatively in three divided doses, days 1–28, and then gradual withdrawal
(2) Vincristine 1.5 mg/m^2 intravenously on days 0, 7, 14, and 21 (maximum 2 mg)
(3) Daunorubicin 25 mg/m^2 intravenously on days 0, 7, 14, 21
(4) l-asparaginase 6000 U/m^2 intramuscularly three times/week for 3 weeks
(5) Intrathecal therapy (age-dependent dose), Ara-C day 0 and methotrexate day 14.

Phase 2 (central nervous system intensification) 5 weeks

(1) Cyclophosphamide 1000 mg/m^2 intravenously on days 0 and14
(2) Ara-C 75 mg/m^2 intravenously four times/week for 4 weeks
(3) Methotrexate intrathecally on the fifth day of every week for 4 weeks
(4) 6-mercaptopurine 60 mg/m^2 postoperatively every day for 28 days.

Phase 3 (reinduction/reintensification) 8 weeks

(1) Dexamethasone 10 mg/m^2 postoperatively in three divided doses on days 1–28 and then gradual withdrawal
(2) Vincristine 1.5 mg/m^2 intravenously on days 0, 7, 14, and 21 (maximum 2 mg)
(3) Daunorubicin 25 mg/m^2 intravenously on days 0, 7, 14, and 21

(4) l-asparaginase 6000 U/m^2 intramuscularly three times/week for 3 weeks
(5) Intrathecal therapy (age-dependent dose), methotrexate for sixth week
(6) Cyclophosphamide 1000 mg/m^2 intravenously for sixth week
(7) 6 thioguanine 60 mg/m^2 orally for sixth and seventh week
(8) Ara-C 75 mg/m^2 intravenously four times/week for sixth and seventh week.

Phase 4 (maintenance): 12 weeks (repeated for 12 times)
(1) Vincristine 1.5 mg/m^2 intravenously on days 0 and 7 (maximum 2 mg)
(2) Triple intrathecal (methotrexate, cytosar, and hydrocortisone) from days 0–6 and mercaptopurine 75 mg/m^2 postoperatively every day for 12 weeks
(3) Prednisolone 60 mg/m^2/day postoperatively in three divided doses for 2 weeks
(4) Methotrexate 20 mg/m^2/day orally once a week from the second week,

Patients assessment

Clinical assessment
Clinical assessment by physician included the following:
(1) Medical history
(2) Physical examination for symptoms and signs of ALL – for example, lymphadenopathy, splenomegally, pallor, and central nervous system involvement
(3) Radiological evaluation according to every patient's needs.

Laboratory assessment
Complete blood count was performed by Cell-Dyn 3500 (Abbott Diagnostics, Santa Clara, California, USA), and differential count was done on stained smears.

Bone marrow aspirate samples were collected from patients with ALL by clean puncture of posterior superior iliac spine and immediately delivered into EDTA tubes for further assesment. The bone marrow aspirate was done for patients twice; the first sample was collected at time of diagnosis, and the second sample was collected after induction chemotherapy. Bone marrow aspirate samples were subjected to the following:
(1) Morphologic assesment:
The diagnosis of ALL was based on morphologic assesment of Leishman stained bone marrow smears for blast cells of lymphoblast morphology.
(2) Cytochemical stain assesment (Sudan black-B (SBB) and Periodic acid–Schiff (PAS)) for the diagnosis of ALL to be included in the study.
(3) Immunophenotypic assesment using flowcytometry (FACS Calibur Flow Cytometry; South Egypt Cancer Institute, San Jose, CA) was done to confirm ALL diagnosis and to classify it into B-ALL and T-ALL. The markers used for ALL diagnosis included CD34, HLA-DR, Cytoμ, CD10, CD19, CD5, CD2, CD3, CD4, CD7, CD13, CD33, and CD45).
(4) Molecular studies for assesment of MRD by real-time PCR were as follow:
(a) DNA extraction: DNA extraction was done using a DNA isolation kit (high pure PCR template for extraction kit; Roche Diagnostics, Mannheim, Germany). This was done according to manufacturer's instructions
(b) PCR amplification.

Principle

The PCR amplification process here aims to initially identify IGH and TCR gene sequence from the whole DNA sample. This is considered the first PCR round and was done using consensus primers that contain common frequently identified DNA sequenses. To further identify and specify IGH and TCR gene rearrangments of the malignant clone, a second round of PCR was done, using the allele-specific primers (ASPs) previously designed which bind specifically to the malignant clone IGH and TCR genes [7]. LightCycler FastStart DNA masterplus SYBR Green I kit (Roche Diagnostics) was used, and the work was done in the PCR laboratory of South Egypt Cancer Institute, Assiut University. The PCR was done for each of the diagnosis samples and for follow-up samples.

Steps

First round of polymerase chain reaction (consensus primers amplification of immunoglobulin heavy chain and T-cell receptor by thermal cycler)
The first round of PCR was done using the consensus primers (forward and reverse) for IGH or TCR genes (Roche Diagnostics) to amplify variable segments (VH)-joining segments (JH) of IgH gene and TCR gene to obtain sufficient product for the second round of PCR.

The following primers are used:
(1) IgH consensus primers [8]:
Forward: 5'-GCCCAGGACTGGTGAAGC-3'.

Reverse: 5'-ACCCAGGACTGGTGAAGC-3'.

(2) TCR consensus primers [8]:
Forward: 5'-GCATGAGGAGGAGCTGGA-3'.

Reverse: 5'-GGAAATGTTGTATTCTTCC GATA CTTAC-3'

For amplification, 100 µl of reaction mixture was prepared, containing 50 µl of Taq PCR master mix (Roche Diagnostics), 5 µl of each primer (50 pmol/µl), 30 µl of distilled water, and 10 µl of DNA template.

Amplification was done using thermal cycler (PTC100 Thermal Cycler JMR, Hague Road, Indianapolis), and the thermal profiles were as follow:
(1) Initial denaturation step 94°C for 5 min
(2) 40 cycles of amplification
(3) Denaturation 94°C for 30 s
(4) Annealing 56°C for 30 s
(5) Extension 72°C for 45 s
(6) Final extension 72°C for 10 min.

Second round of polymerase chain reaction (allele-specific primers amplification of immunoglobulin heavy chain and T-cell receptor genes by LightCycler)

The second round of PCR is done using the ASPs (forward and reverse) for IGH orTCR genes and utilizing a LightCycler program.

The following primers are used:
(1) IgH ASPs [8]:
Forward: 5'-ATCTATTATAGTGGGAGCACC-3'.

Reverse: 5'-AACCCGTACCAGCTGCCTCC-3'

(2) TCR ASPs [8]:
Forward: 5'-GGGACAGGCTCGGAGGGTATT ATAAG-3'.

Reverse: 5'-TGTGCCCCCTTAAGGGAAACTC TTTGG-3'.

Reaction mixture was prepared containing 2.0 µl of DNA master SYBR Green I kit (Roche Diagnostics), 1.6 µl of $MgCl_2$ stock solution, 0.5 µl of each primer, 1.0 µl of PCR product from the first round, and 14.4 µl of H_2O, for a total volume of 20 µl.

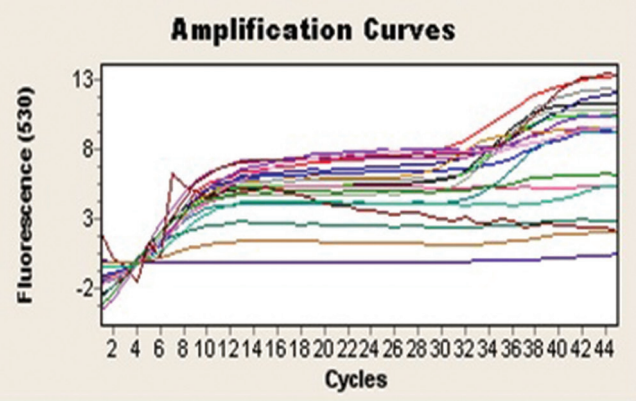
Protocol on LightCycler instrument (Roche Diagnostic, version 3.5) for the SYBR Green I detection was as follow:
(1) Denaturation for 2 min at 95°C
(2) 35 cycles of amplification
(3) Melting curve analysis
(4) Annealing temperature was 40°C for an extension time of 15 s.

Analysis of data and quantification

Amplification curves were obtained using the LightCycler FastStart DNA Masterplus SYBR Green I kit, and the LightCycler instrument; the fluorescence values versus cycle number were displayed Fig. 1.

The method used for presenting the quantitative gene expression is the relative quantification, where relative gene expression presents the data of the gene of interest relative to some calibrator or internal control gene. This method uses the cycle threshold (C_t) values. C_t is defined as the number of cycles required for the fluorescent signal to cross the threshold (i.e. exceeds background level). C_t ensures that the PCR is in the exponential phase of amplification, and it corresponds to the crossing point (CP), the time at which the fluorescence intensity is greater than the background and the curve begins to rise on the LightCycler. Here, absolute quantification is not required. Moreover standard curves are not required, instead the relative quantity of target can be obtained by calculating the difference in CPs between the samples. Results can be normalized to the housekeeping gene. The relative quantification method compares the value of one target gene to another gene (e.g., housekeeping gene) in a single sample (using the formula: $2^{\Delta\Delta C_t}$), and the result is expressed as a relative ratio of the target of interest to the housekeeping gene [9]. In this study, the glyceraldehydes 3-phosphate dehydrogenase was used as the housekeeping gene (each sample is used in two tests: one for the IgH or TCR genes and the other for glyceraldehydes 3-phosphate dehydrogenase). For each test, the CPs of the target gene and reference genes were determined, and then, the software of the LightCycler calculates the relative ratio between the target and the reference genes, which is expressed as the concentration ratio between the target gene of interest and the referance gene. The MRD status of a

Figure 1



Each sample (diagnosis and follow up) runs into amplification for the target gene and amplification for the reference gene, so each sample is represented as four amplification curves for which the C_t is calculated and analyzed by the LightCycler system and displayed as rising curve for positive cases (means that the C_t value crossed the background level).

remission in sample is determined semiquantitatively by comparing the amount of the respective PCR product with the PCR products of target DNA [10]. Although real-time quantitative PCR is a quantitative technique, it does not mean that the obtained data can be quantified in each case. A sample can be considered as positive if the C_t value of that sample is clearly outside the C_t range of the nonspecific amplification. A sample can be considered as negative if no amplification is observed at all [11].

Statistical analysis

Results were expressed as mean + SD and were analyzed using the statistical package for the social science program (version 21, SPSS, IBM Corp. in Armonk, NY). The significant level was considered as *P* valueless than 0.05 for all analyses. χ-Test was used for categorical variables and Mann–Whitney *U*-test for numerical data.

Results

Patient's characteristics were summarized in Table 1. A total of 30 patients, 15 (50%) males and 15 (50%) females, were included, and the age ranged from 1 to 25 years old, with the mean ± SD of 8.16 ± 6.48.The clinical data showed that 19 (63.3%) cases had fever as presenting features, whereas lymphadenopathy was found in 16 (53.3%), hepatomegaly and/or splenomegaly in 14 (46.7%), pallor in 15 (50%), bleeding in four (13.3%), and bone pain in two (6.7%) patients. Hemogram findings for patients with ALL at diagnosis showed that WBC count ranged from 1.3 to 400 × 10^9/l, with mean ± SD of 44.18 ± 15.30; the hemoglobin (Hb) level ranged between 5.1 and 11.6 g/dl, with mean ± SD of 7.9 ± 0.38; and the platelet count ranged from 13 to 265 × 10^9/l, with mean ± SD of 81.3 ± 14.57.The bone marrow blast percentage ranged from 24 to 98%, with mean ± SD of 76.0 ± 3.6. When patients were classified into risk groups according to NCI criteria, 50% were in the high-risk group and 50% in the standard-risk group. Immunophenotypic characteristics of ALL study group demonstrated that 25 (83.3%) patients had B-ALL phenotype, consisting of 22 (88%) cases of pre-B-ALL and three (12%) cases of common B-ALL, whereas five (16.7%) patients belonged to the T-ALL phenotype.

Table 2 illustrates the morphologic assessment of stained bone marrow aspirate smear of the ALL study group patients after receiving the induction chemotherapy in relation to the PCR results. The morphologic assessment for remission is based on the 5% blast index [12], which revealed that 22 (73.3%) patients showed morphologic remission (<5% blast cells) and eight (26.7%) patients were not in remission (>5% blast cells). Assessment of MRD using real-time PCR analysis for IGH/TCR gene rearrangement as target gene revealed that among the morphological remission group (22 patients), 18 (81.8%) patients showed positive MRD finding (i.e., not in molecular remission) and four (18.2%) patients showed negative MRD finding (i.e. in molecular remission); all patients who showed morphological nonremission (eight patients) were confirmed to be MRD positive (Table 1).

Table 1 Patient's characteristics

	Cases (*N* (%))
Age (range) (years)	1-25
1-9	19 (63.3)
>9	11 (36.7)
Sex	
Male	15 (50)
Female	15 (50)
Presenting symptoms	
Fever	19 (63.3)
Pallor	15 (50)
Hepatosplenomegaly	14 (46.7)
Lymphadenopathy	16 (53.3)
Bleeding	4 (13.3)
Bone pain	2 (6.7)
Hemogram findings (mean±SD)	
TLC (×10^9/l)	44.18±15.30
Hb (×g/dl)	7.9±0.38
PLT (×10^9/l)	81.3±14.57
BM blast (%)	76±3.6
Immunophenotyping	
B-ALL	25 (83.3)
Pre-B-ALL	22
Common B-ALL	3
T-ALL	5 (16.7)
Risk stratification according to (NCI) [2]	
High risk	15 (50)
Male	9
Female	6
Standard risk	15 (50)
Male	6
Female	9

ALL, acute lymphoblastic leukemia; BM, bone marrow; Hb, hemoglobin level; NCI, National Cancer Institute (sponsored workshop); PLT, platelet count; TLC, total leukocyte count.

In the analysis of patients' data for identification of factors that predict presence of MRD, none of the patient variables, such as age, sex, immunophenotype, or total leukocyte count (TLC), have been found to be significantly associated with MRD risk (*P* = 0.241, 0.149, 0.789, and 0.432, respectively) (Table 3).

Discussion

Monitoring of MRD has become a frontline clinical practice in treatment of virtually all childhood ALL cases and in many cases of adult patients with ALL,

Table 2 Minimal residual disease detection by polymerase chain reaction and its relation to morphologic assessment of leukemic cells

MRD by PCR using IGH/TCR	Positive MRD by PCR (26) (*N* (%))	Negative MRD by PCR (4) (*N* (%))
Morphological remission group (22)	18 (81.8)	4 (18.2)
Morphological nonremission group (8)	8 (100)	0 (0)

IGH, immunoglobulin heavy chain; MRD, minimal residual disease; TCR, T-cell receptor.

Table 3 Minimal residual disease results by polymerase chain reaction in relation to patients' characteristics

	MRD by PCR (*N* (%))		*P*
	Positive (*n*=26)	Negative (*n*=4)	
Age			
<9 years	17 (56.8)	2 (6.6)	0.451 (NS)
>9 years	9 (30)	2 (6.6)	
Male	12 (40)	3 (10)	0.273 (NS)
Female	14 (46.6)	1 (3.3)	
WBC (mean±SE) ($\times 10^9$/l)			
At diagnosis	23.7±16.19	75.5±49.7	0.379 (NS)
At follow-up	12.9±3.20	44.18±15.3	
Blast% in BM (mean±SE)			
At diagnosis	73.8±3.94	91.00±3.24	0.103 (NS)
At follow-up	7.0±1.69	2.5±0.64	0.314 (NS)
Immunophenotype			
B-ALL	25 (73.07)	3 (75.0)	0.789 (NS)
Pre-B-ALL	19	3	
Common ALL	3	0	
T-ALL	4 (15.3)	1 (25.0)	

ALL, acute lymphoblastic leukemia; BM, bone marrow; MRD, minimal residual disease; WBC, white blood cell. P >0.05, NS.

allowing for risk group assignment into different treatment arms, ranging from significant treatment reduction to mild or strong intensification [13]. The most significant application of MRD for de-novo ALL is the sensitive assessment of treatment efficacy in patients reaching a complete morphological remission, thereby refining initial risk stratification. Large-scale studies have shown that initial MRD kinetics is highly predictive for outcome in ALL [14,15]. It is now widely acknowledged that MRD detection is a part of state-of-the-art diagnostics and is needed in the management of ALL. MRD detection may even replace other prognostic factors [16]. During the past few years, the debate about the sensitivity of MRD techniques has intensified. It is clear that MRD technologies should aim for 10^{-4}–10^{-5} sensitivity to define the MRD-based risk groups accurately [14,15].

In the present study, the clinical presentation of the ALL cases showed that 63.3% of cases have fever, 46.7% have hepatomegaly and/or splenomegaly, and ~50% of patients have lymphadenopathy and pallor. This agrees with the findings by Preethi [17] who found the main presenting symptoms were fever in six (46.1%) patients, generalized weakness in four (30.8%) patients, and backache in three (23%) patients; the physical examination showed pallor of varying degrees in all patients. Lymphadenopathy was present in eight (61.5%) of 13 patients. All the patients had localized lymphadenopathy, among which cervical lymphadenopathy was common.

In this work, the hemogram findings showed that the mean value of TLC at diagnosis was 44.18, Hb mean value was 7.9, platelet count mean value was 81.3, and bone marrow blast was 76%. This agrees with the findings by Van Der Velden *et al*. [18] who reported anemia of variable degree in all patients with ALL, as the Hb level ranged from 5.9 to 7.2 g/dl., with the mean Hb level being 6.7 g/dl, and TLC ranged from 16.9 to 210 × 10^9/l, with the mean value of the TLC being 75.8 × 10^9/l. All patients had thrombocytopenia at the time of diagnosis. Our results showed that immunophenotyping of ALL cases revealed 25 (83.3%) cases were B-ALL and five (16.7%) cases were T-ALL. This agrees with the findings by Salari *et al*. [19] who reported ~75% of ALL cases have blast cells with the B-cell phenotype, and 25% have blast cells with the T-cell phenotype. In the present study, no statistically significant difference (P > 0.05) was found when relating the WBCs at diagnosis and blast count inside bone marrow at diagnosis and at follow-up to the results of MRD by real-time PCR.). These data matched with those obtained by Zhou *et al*. [20] who reported that level of MRD did not differ by WBC count.

Our results showed that 46.2% of patients with positive MRD belong to the high-risk group, and 75.0% of patients with negative MRD belongs to the standard-risk group. This agrees with the findings by Samra *et al*. [21] who found that 27 (47%) patients were classified as high risk and showed positive MRD. This means that ALL risk classification according to NCI workshop, 1993, is not useful in expecting prognosis.

In our study, MRD evaluation was performed at the end of induction, as this time point seemed most relevant for the treatment decision-making process, allowing evaluation of treatment strategies. This is consistent with the study by Eckert *et al*. [22], who showed that MRD after induction treatment in ALL can be used to quantify the activity of different induction treatment strategies. On the contrary, Parekh *et al*. [23] reported end of induction MRD using flow cytometry alone is not a useful determinant for risk stratified therapy, with 19 patients were MRD positive at the end of induction and all remain in remission with augmented Berlin-Frankfurt Münster-based therapy. In the

present study, sensitivity and specificity were calculated for morphology as compared with gold standard PCR, which were 30 and 100%, respectively. This agrees with van Der Velden *et al*. [18] who reported the sensitivity of the real-time quantitative PCR assay.

We conclude that MRD assessment by real-time PCR using IGH or TCR gene rearrangements as targets can detect early relapse in ALL with high sensitivity. Risk classification in ALL based on real-time quantitative PCR MRD results could be used to guide the final treatment strategy and predict early relapse while patients are still in clinical and morphological remission.

Conclusion

About 81% of ALL cases previously diagnosed as being in the remission state depending on the morphologic assessment only tend to harbor MRD as evident by positive MRD assessment using Real time PCR.The molecular assessment of MRD allows early detection of relapse with chance of intervention and tailoring treatment according to patients need. It was found that no relation between MRD results and risk stratification in ALL according to age and WBC at diagnosis, this confirms that detection of MRD of leukemic cells can be considered a superior prognostic marker of relapse independent from conventional prognostic factors.

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Conflicts of interest

There are no conflicts of interest.

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