



# **Which technique(s) to use when studying residual disease at day 35 in childhood acute lymphoblastic leukemia**

**Katrien Swerts**



## Introduction

### Why is detection of minimal residual disease (MRD) important?

- Detection and quantification of MRD in pediatric ALL patients is important for monitoring therapeutic response and detection of early relapse.
- Studies in childhood ALL have demonstrated the prognostic significance of detection and quantification of MRD (e.g. Cavé et al. 1998; Campana et al. 1995).
- An increasing number of ALL treatment protocols include MRD parameters for risk assessment (e.g. EORTC).



## Introduction

### Which detection techniques can be used?

- Conventional cytomorphology:
  - Patients with fewer than 5% morphologically identifiable lymphoblasts in their bone marrow are considered to be in clinical remission. However, they may still harbor as many as  $10^{10}$  leukemic cells.
  - Insensitive and imprecise.
- Alternative techniques:
  - Multiparameter flow cytometry.
  - Detection of fusion genes or WT1 overexpression using real-time quantitative (RT-)PCR.
  - Competitive PCR-based quantification of clonal immunoglobulin and T-cell receptor gene rearrangements.



## Which technique(s) to use?

- Comparison of the MRD detection rates of the three detection techniques.
- The relation between the MRD results and the clinical outcome of the pediatric ALL patients was studied.



## Materials and Methods

### Patients and samples

- At the end of induction therapy (Day 35); bone marrow samples from 107 pediatric ALL patients (90 precursor B-ALL and 17 T-ALL) were analyzed using cytomorphology and at least one additional assay:
  - Flow cytometry (n = 107)
  - Real-time quantitative (RT-)PCR (n = 50; translocations (n = 36); WT1 overexpression (n = 14))
  - IgH/TCR rearrangement analysis (n = 97)
- All patients were treated according to EORTC protocol 58951.



## Materials and Methods

### Multiparameter flow cytometry

- Based on the detection of aberrant immunophenotypes.
- Ghent University Hospital:
  - 3- to 4-color assays using a FACSort Flow cytometer (Becton Dickinson).
  - 5-color analysis using a FC500 flow cytometer (Beckman Coulter).
  - Analysis are performed according to the SIHON (NVC) guidelines.



# Materials and Methods

## Real-time quantitative (RT-)PCR

- Detection of fusion genes or WT1 overexpression
- University Hospital Ghent: HemaVision Multiplex RT-PCR (BioRad)
  - 28 translocations/chromosomal rearrangements + splice variants

t(1;11)(p32;q23)  
t(1;11)(q21;q23)  
t(1;19)(q23;p13)  
t(3;21)(q26;q22)  
t(3;5)(q25.1;q34)  
t(4;11)(q21;q23)  
t(5;12)(q33;p13)  
t(5;17)(q35;q21)  
t(6;11)(q27;q23)  
t(6;9)(p23;q34)  
t(8;21)(q22;q22)  
t(9;11)(q22;q23)  
t(9;12)(q34;p13)  
t(9;22)(q34;q11)

MLL/AF1p  
MLL/AF1q  
E2A/PBX1  
AML/EAP/MDS/EVI1  
NPM/MLF1  
MLL/AF4  
TEL/PDGFRb  
NPM/RARa  
MLL/AF6  
DEK/CAN  
AML1/MGT8  
MLL/AF9  
TEL/ABL  
BCR/ABL

t(9;9)(q34;q34)  
t(10;11)(p12;q23)  
t(11;17)(q23;q21)  
t(11;17)(q23;q21)  
t(11;19)(q23;p13.1)  
t(11;19)(q23;p13.3)  
t(12;21)(p13;q22)  
t(12;22)(p13;q11)  
t(13;17)(q22;q21)  
t(16;21)(q11;q22)  
t(17;19)(q22;p13)  
inv(16)(p13;q22)  
t(0;11)(q13;q23)  
TAL1deletion(p34)

SET/CAN  
MLL/AF10  
MLL/AF17  
PLZF/RARa  
MLL/ELL  
MLL/ENL  
TEL/AML1  
TEL/MNI  
DNM1/ RARa  
TLX/ERG  
E2A/HLF  
CBFB/MYH11  
MLL/AFK  
BCR/TAL1



## Materials and Methods

### Real-time quantitative (RT-)PCR

- Once the translocation or chromosomal rearrangement is identified, follow-up samples are analyzed using real-time quantitative (RT-)PCR using translocation specific primers and probes according to the EAC guidelines.
- In case no specific translocation or chromosomal rearrangement is found, WT1 overexpression is studied using real-time quantitative RT-PCR (Ogawa et al.; Blood 2003;101:1698-1704).



## Materials and Methods

### Competitive PCR-based quantification of clonal immunoglobulin and T-cell receptor gene rearrangements

- Based on PCR reactions detecting the most common rearrangements of IGH (FRIII-JH), TCRD (Vdelta2-Ddelta3) and TCRG (Vgamma1-J1J2; Vgamma2-J1J2; Vgamma9-J1J2)
- Ghent University Hospital:
  - Samples are sent to the Belgian reference center: Marleen Bakkus, Molecular Hematology, Universitair Ziekenhuis Brussel



# Results

## MRD detection rates

	MRD positivity
Cytomorphology	2/107 (2%)
Flow cytometry	8/107 (7%)
(RT-)PCR	8/50 (16%)
IgH/TCR rearrangement analysis	3/97 (3%)



## Results

### Positive and negative predictive value

- Several studies demonstrated that the presence of MRD in bone marrow at the completion of induction therapy is associated with an increased risk of relapse in pediatric ALL.
- At the conclusion of this study, 5 patients suffered from an early relapse (< 30 months after diagnosis).
- A continuous complete remission of more than 30 months was reached in 60 children (median follow-up: 63 months).
- The MRD results of these patients (n = 65) were used to calculate the positive predictive value (PPV) and negative predictive value (NPV).



# Results

## Positive and negative predictive value

	PPV	NPV
Cytomorphology	0% (0/1)	92% (59/64)
Flow cytometry	25% (1/4)	93% (57/61)
(RT-)PCR	50% (2/4)	96% (24/25)
IgH/TCR rearrangement analysis	50% (1/2)	95% (59/59)



## Results

### Combination of techniques

- The PPV's of the individual techniques were rather low (ranging from 0-50%). In order to increase the PPV, the results of at least two test were combined. Only when flow cytometry and (RT-)PCR were combined, a PPV of 100% was reached.
- The NPV's of the individual or combined tests were at least 90%, illustrating the acceptable specificity of the applied detection techniques.



## Conclusions

- These preliminary data indicate that MRD detection rates obtained by flow cytometry and (RT-)PCR are higher than those obtained by competitive PCR-based IgH/TCR rearrangement analysis.
- (RT-)PCR and IgH/TCR rearrangements analysis are superior to flow cytometry in identifying patients at risk of early relapse.
- Only when flow cytometry and (RT-)PCR were combined, a PPV of 100% was reached.
- Pitfalls:
  - Flow cytometry has a relatively high false positive rate (PPV = 25%)
  - MRD screening based on real-time quantitative (RT-)PCR detection of fusion genes or WT1 overexpression is only possible in about 50% of pediatric ALL patients.



## Conclusions

**It is advisable to combine different detection methods in order to optimize the sensitivity and specificity of MRD testing**



## Future perspectives

### Flow cytometry:

- Compare results MRD sample to those from normal or regenerating bone marrow samples (Szczepanski et al., 2006).
- Improved instrumentation (6- to 8-colour assays).

### Real-time (RT-)PCR

- Identification of new molecular markers.

### IgH/TCR rearrangements analysis

- Use a more sensitive threshold (0.5% versus 1%).
- Allele-specific oligonucleotide primers (ASO).



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