Minimale residuele ziekte (MRD) detectie in acute leukemie: AML/ALL

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UZ Gent

VAKB
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Overview presentation

- Introduction
- Definition of MRD – goal of MRD
- MRD techniques
- Clinical value of MRD in AML/ALL
  ✓ Prognostic significance?
  ✓ MRD directed therapy?
- Future perspectives

Incidence of AML and ALL

Overall Survival in AML patients

- Primary induction failure:
  - Young pt: 20-30%
  - Elderly pt: 40-50%
- Relapse: high %, often IF
- Early relapse: low rate of CR2 (20%)
- Survival after relapse: 18%

Survival ALL patients
Factors involved in treatment effectiveness

- Characteristics of tumor cells:
  - e.g., genetic abnormalities
  - in vivo drug sensitivity
  - gene expression profile
  - immunophenotypic/morphology

- In vivo drug distribution:
  - e.g., gastrointestinal absorption
  - distribution in body (e.g., CNS)
  - drug metabolism (e.g., polymorphisms in enzymes)
  - liver excretion

- Treatment compliance:
  - e.g., duration of fix
  - side effects (e.g., allergy, infections)

Evaluation of overall treatment effectiveness by detection of MRD

GOAL of MRD detection

- Identification/detection of small subsets residual leukemic cells
- Kinetics: tumor load reduction during and after induction treatment provides crucial information about the response to treatment
- Prognosis:
  - Predictor of outcome of patients with leukemia
  - Prediction of relapse
  - MRD-based stratification: identification of low-risk (therapy reduction) and high-risk (therapy intensification) patients
  - Prognostic relevance of patients undergoing stem cell transplantation

MRD detection with sensitive techniques

- Detection limit of PCR techniques
- Cytomorphological techniques
- Immunophenotyping

Techniques for MRD

- Identification of Leukemia Associated Immuno-Phenotype or LAIPs, based on immunophenotypic aberrations

1. Cross-lineage antigen expression
   - e.g., AML with CD20+/CD7+ /CD56+

2. Asynchronous antigen expression
   - different maturation: e.g., CD34+/CD117+ with CD14+/CD15+ or CD34+/CD19+/sIg+

3. Over- and under-expression of markers
   - ++: HLA-DR/CD117/CD13/CD33/CD34/10
   - -: CD34/CD117/CD45/24

4. Ectopic antigen expression
   - NG1 or TdT+/CD3+/CD5+
Examples of different LAIPs

R1

B-ALL: under-expression

B-ALL: over-expression

T-ALL: ectopic expression

AML: cross-lineage expression

Immunophenotypic detection of MRD

Immunophenotypic signature of leukemic cells

Advantages:

- Sensitivity between $10^{-4}$ and $10^{-5}$ with 6-color flow cytometry (multicolor analysis)
- Widely accessible
- Rapid delivery of results
- Simple; one technique
- Applicable to almost all patients
- Information about cell viability and status of normal hematopoietic cells in the sample studies
- Single-cell level, allowing recognition and characterisation of small subpopulations

Immunophenotypic detection of MRD (1)

- Heterogeneity of blast cells, especially in AML (subsets in ~75% of AML patients); preferably all subpopulations should be monitored

Major limitations of MRD by flow cytometry (1)

- Lack of ‘a priori’ (e.g. at diagnosis) of the sensitivity for each individual patient later on during FU of the disease
- Expertise and knowledge required for LAIP recognition
- Lower sensitivity ($10^{-3} – 10^{-4}$) than PCR analysis using 3-4 color flow cytometry
- Difficult to standardize (inter-laboratory)
- LAIP
- Absence of a clear LAIP at diagnosis
- Low frequency in normal marrow (regenerative marrow)

Major limitations of MRD by flow cytometry (2)

- Immunophenotypic shifts
  - Most of these changes involve Ag of cell maturation (CD10, CD22, TdT, CD34, CD13, CD33)
  - Abnormal/infrequent antigenic markers are often stable
  - Preferably ≥2 patient-specific labelings should be used per patient; in vast majority of patients at least one MRD labeling remains informative

Major limitations of MRD by flow cytometry (3)

Background of LAIP in normal bone marrow

- Absent marker expression on normal BM: % of primitive marker compartments (CD34+ or CD137)
Flow cytometry: Improvement of data analysis

Conventional methods of manual data analysis
- Based on visualization of multiple bi-dimensional plots
- Operator’s selection of population of interest (subjective)
- Depending on the expertise of the operator

- Enormous increase in number of data by merging and calculations
- Automated method for analysis of flow cytometry immunophenotypic data
- Reference picture will facilitate MRD analysis
- Reducing expert-based data-analysis

Molecular techniques for MRD detection

PCR analysis of genetic abnormalities:
- Fusion genes
- Ig/TCR gene rearrangements

Fusion genes in B-ALL

Grimwade D, Moraek K; Hematol Oncol Clin N Am 2011

Importance of gene fusions = PROGNOSIS

Grimwade D, Moraek K; Hematol Oncol Clin N Am 2011
Overview quantitative qPCR UZ Gent

<table>
<thead>
<tr>
<th>Target (trans/mut/over expr)</th>
<th>Gene Fusions</th>
<th>Incidence</th>
<th>Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(9;22)(q34;q11) ANL/BCR: p210 en p190</td>
<td>Pre B-cell ALL, 15-30% schwereformen, 2-5% kinderen</td>
<td>Slechte prognose</td>
<td></td>
</tr>
<tr>
<td>t(8;21)(q22;q22) ETO/AML</td>
<td>AML, 5-12 %, AML, M2: 36 %</td>
<td>Goede prognose</td>
<td></td>
</tr>
<tr>
<td>t(11;22)(q13;q23) VML/MLL</td>
<td>5% pre-B-cell ALL, Mixed frequencies 11q23 aberrantie bij pre-B-ALL</td>
<td>Slechte prognose</td>
<td></td>
</tr>
<tr>
<td>t(11;12)(p13;q23) MLL-AF9</td>
<td>Precursor B-cell ALL, 11q23: 5-6 % van AML, 36-51 %</td>
<td>Intermediaire prognose</td>
<td></td>
</tr>
<tr>
<td>t(12;22)(q12;q22) TEL/AML-1</td>
<td>25% pre-B-ALL bij kinderen</td>
<td>Goede prognose</td>
<td></td>
</tr>
<tr>
<td>t(1;3)(p13;q22) MYH11/CBFbeta</td>
<td>8-12 % van AML, over hoge associatie met AML, BM eos</td>
<td>Goede prognose</td>
<td></td>
</tr>
<tr>
<td>t(11;19)(q23;p13) EZA-FK/R</td>
<td>1-5% kinderen, 3% van AML ALL</td>
<td>prognose controversieel</td>
<td></td>
</tr>
<tr>
<td>t(17;22)(p13;q11) PKN2/MLL</td>
<td>0-4 % van AML, residual</td>
<td>Goede prognose</td>
<td></td>
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Overview qualitative RT-PCR UZ Gent

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<th>Prognosis</th>
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<tbody>
<tr>
<td>t(6;9)(p23;q34) DEK/CAN</td>
<td>1% AML</td>
<td>Slechte prognose</td>
<td></td>
</tr>
<tr>
<td>t(1;22)(p13;q13) OTT-MAL</td>
<td>AML 7 (children)</td>
<td>Slechte prognose</td>
<td></td>
</tr>
<tr>
<td>NPM1 mutatie / 25-35% AML (~50% CN-AML)</td>
<td>Good</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT1-overexpressie / 5-20%</td>
<td>T controversieel</td>
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<td>t(4;11)(q21;q23) VML/MLL</td>
<td>5% pre-B-cell ALL, Mixed frequencies 11q23 aberrantie bij pre-B-ALL</td>
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PB versus BM MRD detection of fusion transcripts?

- No consensus regarding cut-off levels or most predictive threshold
- Not possible to accurately calculate MRD levels, because the number of transcripts produced by each cell is unknown
- PB versus BM MRD detection?
- Timing of PCR assessment? When?

Fusion gene expression with a quantitative ‘real-time’ RT-PCR

LEADING ARTICLE: “Standardization and quality control studies of ‘real-time’ quantitative reverse transcriptase polymerase chain reaction of fusion gene transcripts for residual disease detection in leukemia” – A Europe Against Cancer Program

J Gabert, E Beillard, VHJ van der Velden, W Bi, D Grimwade, N Pallisgaard, G Barbany, G Cazzaniga, JM Cayuela, H Cave, F Pane, JLE Aerts, D De Micheli, X Thirion, V Pradel, M Gonzalez, S Viehmann, M Malec, G Saglio and JJM van Dongen

Leukemia 2003

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Leukemia 2003

Kwantificatie

Standardisatie
Ig/TCR gene rearrangements for MRD analysis
- Rearrangement of TCR en Ig genes: 'fingerprint-like' or unique rearranged DNA
  - Recombination of Variable (V), Diversity (D) en Joining (J) segments
  - Random insertion of nucleotides
  - Hypervariable (CDR3) regions with junctional diversity

MRD assessment based on ASO-qPCR
- Target identification

PCR analysis Ig/TCR rearrangements
- Advantages
  - High degree of standardization (ESG-MRD-ALL, since 2010 EURO-MRD): design, interpretation and guidelines
  - High sensitivity
  - Applicable for most ALL patients (90-95%)
  - Stability of DNA
- Disadvantages
  - Time consuming
  - Expensive
  - Need for preferably 2 PCR targets (clonal evolution)
  - Extensive expertise is needed

Flow cytometry vs PCR in ALL
- Study of Inaba et al (JCO 2012)
- 203 AML (children)
- 1215 compared samples
- MRD of fusion genes vs FLOW 4 colors
- Poor correlation
  - FLOW-/PCR: 99%
  - FLOW+/PCR: 19%

Flow cytometry MRD (fusion genes) vs FLOW in AML
- Study of Inaba et al (JCO 2012)
- 203 AML (children)
- 1215 compared samples
- MRD of fusion genes vs FLOW 4 colors
- Poor correlation
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Flow cytometry vs PCR in ALL
- Literature for ALL: 3/4 color FCM MRD vs PCR-based MRD
  - 70-95% concordance (cut-off 0.01%)
  - Dutch DCOG ALL10 protocol for childhood ALL: excellent concordance, over 95% with 6-color FCM and with cut-off 0.01%
Clinical significance of MRD?

- Does MRD have clinical significance?
- Does it predict relapse?
- Has it an independent prognostic factor?
- What time-points of analysis during therapy are informative?
- What cut-off levels (threshold for positivity) are informative?
- Is there any difference between AML and ALL? Between adult AML and childhood AML?
- Clinical intervention guided by MRD results?

Prognostic value of MRD in ALL

MRD prognosis in childhood ALL

“MRD after induction is most important independent prognostic factor!”

Treatment of MRD post-transplant Phi+ ALL

- Detection of MRD post-transplant = 90% probability of relapse
- Start imatinib in MRD setting
- Post-transplant imatinib group: significant better OS (86.7% vs 34.3%)

Prognostic value of MRD in AML
**Prognostic significance of MRD in AML (adult)**

- Cut-off = 0.1%
- MRD (FCM) = independent prognostic factor
- Retrospective multivariate analysis:
  - MRD+ after induction (2 cycles) = significant higher relapse rate (RR)

**MRD inv(16)/t(8;21) in AML**

Importance of PCR negativity combining consolidation samples points and first 3 months of follow-up (Corbacioglu et al, JCO 2010)

- CBF-B-MYH11 AML (n = 53)
- < 2 PCR-neg samples
- ≥ 2 PCR-neg samples
- P=0.001
- During consolidation + first 3 months FU

**MRD with NPM1 mutation-specific qPCR**

- Nucleophosmin mutations; good prognosis
- High frequency: 25-30% of AML patient (~50% CN-AML)

**Prognostic significance of MRD in AML (childhood)**
When is MRD assessment clinical relevant?

- Time point with power to provide most informative prognostic indicator!!!

Optimal sampling interval varies with molecular subgroups

- e.g. NPM1: highest relapse risk with high NPM1 transcript levels after induction and consolidation / WT overexpression: early monitoring (2-log reduction after induction) / inv(16): after consolidation

Molecular MRD levels measured at delayed rather than early time points (superior prognostic relevance) vs flow cytometry

Conclusion MRD in AML

- MRD is a strong and independent prognostic factor in AML (both childhood and adult)

Overview MRD studies in AML by MPFC

<table>
<thead>
<tr>
<th>Study</th>
<th>n</th>
<th>Cutoff</th>
<th>Time point</th>
<th>Survival (MRD vs MRD+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Al Marzouk et al. '03</td>
<td>25</td>
<td>0.15%</td>
<td>post-Ind</td>
<td>DFS 20% vs 36%</td>
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<tr>
<td>Maurillo et al. '04</td>
<td>66</td>
<td>variable</td>
<td>post-Ind</td>
<td>DFS 5% vs 50%</td>
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<tr>
<td>Kouwenhoven et al. '09</td>
<td>100</td>
<td>0.005%</td>
<td>post-Ind</td>
<td>DFS 10% vs 60%</td>
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<tr>
<td>Kern et al. '04</td>
<td></td>
<td></td>
<td>post-Ind</td>
<td>DFS 0% vs 50%</td>
</tr>
<tr>
<td>M REVIEW</td>
<td></td>
<td></td>
<td>post-Ind</td>
<td>DFS 26% vs 43%</td>
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<tr>
<td>San Miguel et al. '07</td>
<td>126</td>
<td>0.01-1%</td>
<td>post-Ind</td>
<td>DFS 33% vs 89%</td>
</tr>
<tr>
<td>San Miguel et al. '07</td>
<td>53</td>
<td>0.5%</td>
<td>post-Ind</td>
<td>DFS 33% vs 89%</td>
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<tr>
<td>Venditti et al. '04</td>
<td>56</td>
<td>0.005%</td>
<td>post-Ind</td>
<td>DFS 23% vs 61%</td>
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<tr>
<td>Children</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Langerholke et al. '07</td>
<td>150</td>
<td>variable</td>
<td>post-Ind</td>
<td>EFS 30% vs 70%</td>
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<tr>
<td>Sievers et al. '03</td>
<td>44</td>
<td>0.5%</td>
<td>post-Ind</td>
<td>DFS 41% vs 66%</td>
</tr>
<tr>
<td>Coyleen-Smith et al. '03</td>
<td>33</td>
<td>0.1%</td>
<td>post-Ind</td>
<td>DFS 30% vs 72%</td>
</tr>
</tbody>
</table>

Conclusion MRD in AML

- MRD is a strong and independent prognostic factor in AML (both childhood and adult)
- Relevant cut-off levels vary between 0.01% and 0.5%
- Relevant cut-off levels and time points are protocol dependent
- MRD by flow cytometry: early time points vs molecular: after completion of therapy

Clinical intervention quided by MRD results in AML?

MRD-directed therapy in APL

- Therapeutic goal = PCR negativity after consolidation (positivity results in relapse)
- MRD monitoring:

  MRD-directed therapy = significant reduction in relapse rate
  MRD monitoring = standard in APL (ELN guidelines – Sanz et al, Blood 2009)
Clinical intervention guided by MRD for other molecular targets

- No guidelines
- Lack of consistency in the scheduling of MRD assessment at any given time point in AML
- Only applicable in a minority of patients
- However, real life...

Example of MRD monitoring in AML patient with CBFB-MYH11

Prognostic significance in AML (adult)

- Cut-off = 0.1%
- MRD (FCM)= independent prognostic factor
- Retrospective multivariate analysis: MRD+ after induction (2 cycles) = significant higher relapse rate (88)

MRD-directed therapy in childhood AML

Results of the AML02 Multicenter Trial -> FIRST SUDY
MRD used to guide treatment in non-APL AML!
- MRD used to intensify therapy during induction
- MRD to identify patients eligible for HSCT

1. Reduction of refractory disease (4.6%)
2. Improved OS

NOPHO DBH AML2012 (childhood)

Response evaluation on day 22 after first course and second course with flow cytometry
Inclusion of results in risk stratification!
Integration of MRD in risk stratification

- Most important risk factors at diagnosis: cytogenetics and molecular abnormalities
- Post-treatment risk indicators = response-related factors → MRD = treatment-related therapy stratification

Pre-treatment risk factors in AML

ELN paper, Dohner et al, Blood 2010

Outcome: combinatie (cyto)genetica en MRD?

Subgroup analysis of RFS and CIR of 143 AML patients stratified according to pretreatment karyotype or FLT3 status and levels of MRD after consolidation.


Future

- Risk assessment combining pretreatment and post-treatment prognosticators (MRD included) in AML
- Improving MRD detection by flow cytometry: focus on stem cells
- Standardisation of techniques (flow and PCR)
- Improvements necessary in interpretation and data analysis (new software tools)
- Next generation sequencing
EINDE