Współczesna Onkologia (2005) vol. 9; 4 (178–182)

At the time of achieving clinical remission, patients with acute lymphoblastic leukemia (ALL) may harbor up to 10^{10} residual leukemic blasts. Detection of these cells is beyond the sensitivity level of classical cytomorphologic methods; they represent Minimal Residual Disease (MRD). Sensitive techniques developed for MRD detection can better define the leukemic burden and detect the residual blasts at the 0.1-0.0001% level. The most promising techniques for MRD monitoring are flow cytometric detection of aberrant immunophenotypes and polymerase chain reaction analysis of clonal antigen-receptor gene rearrangements. These techniques allow monitoring of MRD in almost all patients with pediatric ALL (up to 95%). In published clinical trials, MRD detection proved to be an excellent prognostic factor in the outcome of children with ALL. Evaluation of early treatment response allows for precise risk stratification that may lead to the tailoring of the treatment intensity and the reduction of long-term toxicities. Additionally, the detection of an increase in the MRD level enables one to anticipate an impending relapse. In this review, I discuss techniques used for MRD detection as well as the prognostic value of MRD monitoring during front-line treatment of childhood leukemia, during treatment of relapsed disease, and prior to bone marrow transplantation for ALL.

Key words: review, pediatric acute lymphoblastic leukemia, minimal residual disease, flow cytometry, polymerase chain reaction.

Detection of minimal residual disease in childhood acute lymphoblastic leukemia: technology and clinical applications

Wykrywanie choroby resztkowej u dzieci z ostrą białaczką limfatyczną: metodologia i zastosowanie kliniczne

Anna B. Pawlowska

City of Hope National Medical Center, Duarte, CA, USA

Introduction

The cure rate of children with acute lymphoblastic leukemia (ALL) has improved significantly during the last three decades. The 80% long-term survival achieved with the current chemotherapy is due to the intensification of the treatment regimens based on patient stratification according to up-front risk criteria [1, 2]. However, this intensification of treatment carries a risk of clinically significant late effects. To better define the risk groups with the possibility of appropriate chemotherapy tailoring, a variety of clinical and biological features are incorporated into the risk group definition. Early response to the therapy is one of the most powerful predictors of the risk of relapse [3, 4].

At the time of leukemia diagnosis, a patient harbors around 10^{12}-10^{13} leukemic cells. At the end of 4-6 week induction, 95-98% of patients achieve morphological remission. The leukemia burden is reduced to 10^{10} cells and this cell detection is beyond the sensitivity level of classical cytomorphologic methods (1-5%); they represent Minimal Residual Disease (MRD). The newly developed techniques permit detection of these residual blasts at the 0.1-0.0001% level.

MRD detection is an excellent prognostic factor in the outcome of children with ALL. It allows assessing the early response to the therapy with the rapidity and the degree of the disease regression [5-8]. This measurement indicates chemosensitivity since all aspects of the host, tumor and resistance impact the early response. Additionally, the detection of an increase in the MRD level enables to anticipate impending relapse [9]. MRD detection prior to the bone marrow transplantation (BMT) can predict the successful outcome of this treatment modality [10-14].

Techniques of MRD detection in ALL

The techniques used for the MRD detection must be able to distinguish leukemic blasts from normal marrow or blood cells. The specific markers need to be identified on the malignant cells. It may be a protein, RNA or DNA located on the cell surface, in the cytoplasm, or in the nucleus. Such markers need to be detected with high sensitivity and specificity, to be present in all leukemia cells and to be stable during disease evolution.

Additionally, for the test to be incorporated into the large-scale clinical trials, it needs to be simple, quick and easy to perform, cost effective and reproducible.

Two kinds of markers are currently used to measure MRD in ALL: genetic markers, which can be detected by the molecular methods utilizing polymerase chain reaction (PCR); and immunophenotypic markers, which can be detected by flow cytometry.

W momencie osiągnięcia remisji klinicznej, w organizmie pacjentów z ostrą białaczką limfatyczną może pozostać do 10^{10} resztkowych blastów białaczkowych. Klasyczne metody cytomorfologiczne nie są w stanie wykryć tych komórek, wskazujących na chorobę resztkową. Nowe, czułe metody używane do monitorowania choroby resztkowej pozwalają lepiej zdefiniować całkowitą liczbę blastów i obniżyć poziom wykrywalności do 0,1–0,0001 proc. Najbardziej obiecującą technologię wykrywania choroby resztkowej stanowi obecnie cytometria przepływowa wykrywająca fenotyp specyficzny dla białaczek oraz PCR analizujący klonalne rekombinacje genów antygenu-receptora. Nowe metody pozwalają na wykrywalność choroby resztkowej u praktycznie wszystkich dzieci z ostrą białaczką limfatyczną (do 95 proc.). Opublikowane wyniki prób klinicznych potwierdzają, że poziom choroby resztkowej jest ważnym czynnikiem prognostycznym, ściśle korelującym z przeżywalnością. Ewaluacja wczesnej odpowiedzi na chemioterapię pozwala na precyzyjne określenie ryzyka nawrotu choroby, które może pozwolić na sformułowanie efektywnej terapii i obniżenie ryzyka toksycznych powikłań. Ponadto, wykrycie choroby resztkowej pozwala przewidzieć potencjalny nawrót białaczki.
Artykuł omawia techniki używane do wykrywania choroby resztkowej oraz wartość prognostyczną monitorowania choroby resztkowej podczas leczenia białaczek u dzieci, zarówno w czasie początkowego leczenia, jak również podczas leczenia po nawrocie choroby i bezpośrednio przed przeszczepem szpiku kostnego.

Słowa kluczowe: artykuł przeglądowy, ostra białaczka limfatyczna u dzieci, choroba resztkowa, cytometria przepływowa, PCR.

PCR-based techniques

PCR technique was invented by Dr. Kary Mullins in 1983, for which he received the Nobel Prize in chemistry ten years later. PCR is a powerful technique by which repeated cycles of oligonucleotide priming and DNA polymerization allow rapid amplification of short segments of DNA taken from a very small number of cells [15]. This technique can also be used for the detection of RNA expression by the reverse transcription PCR (RT-PCR). The excellent sensitivity of this method makes it possible to consistently detect one leukemia cell among 10^4-10^6 normal cells.

PCR-based strategies can be directed to 2 types of genetic targets: breakpoints of leukemia-related chromosome aberrations; and antigen-receptor gene rearrangements.

PCR analysis of chromosome aberrations

Recurrent chromosome translocations are found in 30-40% of childhood ALL [16] (Fig. 1). They are ideal leukemia specific targets, which remain stable during the disease course as they are directly involved in leukemogenesis. In ALL, these PCR targets mainly concern fusion gene transcripts (e.g. TEL-AML1, BCR-ABL, SIL-TAL1) or aberrantly expressed specific transcripts (e.g. WT1 and HOX11L2), which can be detected via reverse transcriptase (RT) PCR analysis [17, 18]. However, because the aberrations are present in only a minority of ALL patients, it is difficult to use them in large-scale clinical trials, unless the trials are focused on distinct molecular subsets of ALL that receive unique therapies, such as Philadelphia chromosome positive ALL. Additionally, as the translocations are not patient specific, the chance of cross-contamination of PCR products can lead to false-positive results (even at diagnosis).

PCR analysis of antigen-receptor gene rearrangements

To overcome the limitations imposed by the PCR detection of chromosomal abnormalities (mainly occurrence in a limited number of patients), the use of antigen-receptor gene rearrangements became the target for MRD detection [19-21]. In ALL, T-cell receptor (TCR) and immunoglobulin (Ig) loci undergo somatic rearrangement by V(D)J recombination without strict lineage specificity. Provided the extreme diversity created by V(D)J rearrangement, (Ig – 10^{14} possible variants, TCR – 10^{17} possible variants), over 95% of malignant ALL clones will present with a highly specific clone rearrangement. Specifically, IgH rearrangements are found in more than 95% of patients, with rearrangements of TCR-δ in approximately 90% and TCR-γ and Ig-κ in approximately 60%. A combined study of these 4 loci permits identification of one or more rearrangements in virtually all cases of ALL [22].

The main disadvantage of using Ig/TCR rearrangements as MRD targets in ALL is the occurrence of clonal evolution during treatment which can result in the loss of the particular target with false-negative PCR results. Clonal evolution is more common when oligoclonal rearrangements are seen at diagnosis, which can occur in 20-40% of cases. It is more common in precursor-B ALL than in T-cell ALL, which is related to the fact that T-cell ALL rarely contains oligoclonal TCR gene rearrangement patterns. It is recommended that two gene targets be used for reliable and sensitive MRD detection [23, 24].

Additionally, assay of MRD using Ig/TCR targets is time consuming (mostly at diagnosis) and expensive, which limits its use in the clinical trials when fast results are needed for patient stratifications.

Flow cytometric MRD detection

Flow cytometry using a panel of monoclonal antibodies has been used extensively in the diagnosis of ALL. This technique is based on the principle that leukemic blasts express aberrant or unusual antigens that differ from the normal cells. Multi parameter-flow (using three- or four-color combinations) permits detection of various combinations of surface membrane, cytoplasmic

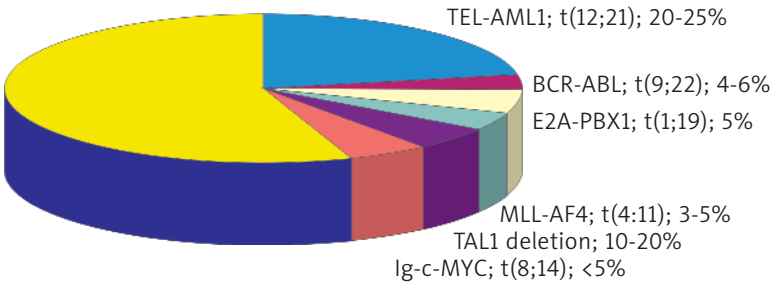

Fig. 1. Chromosome aberrations that can be used as PCR targets for childhood ALL
Ryc. 1. *Aberracje chromosomalne wykorzystywane w wykrywaniu ostrej białaczki limfatycznej u dzieci z użyciem PCRu*

and nuclear molecules expressed, overexpressed or underexpressed by leukemia cells but not by normal bone marrow cells [25]. Using a limited panel of antibodies, more than 90% of ALL cases will have one or two aberrant phenotypes that can be used for MRD detection with the sensitivity of detection in the range of 0.01% (10^{-4}) [26-28], which is slightly below the sensitivity of molecular methods. The major advantage of flow cytometry for MRD detection is that it is applicable to most patients; it is very rapid (results can be obtained within a few hours after the sample is received), and is cost effective (can be tenfold less expensive than molecular testing).

Clinical value of MRD detection in childhood ALL

MRD detection during the front-line treatment of childhood ALL

Monitoring of tumor burden by MRD during the first months of therapy provides information on the initial response to cytotoxic treatment and was shown to be a powerful and independent indicator of treatment outcome in children with ALL [5-7, 28, 29]. Virtually all recently published studies confirmed that end induction MRD levels correlate with outcome; the higher the MRD level, the worse the outcome. Patients with low levels or absent MRD at the end of induction have an excellent survival of 85-98%. High levels of MRD at the end of induction treatment identify patients with high relapse rates of 70-100%. However, the single time point data are not sufficiently precise for defining MRD based high-risk and low-risk groups. Several studies proved that combining MRD levels at the end of induction and at a second time point 3 to 4 months into therapy may be the best predictor of outcome as it provides information on the kinetics of tumor load decrease [6, 7, 28, 29]. Van Dongen et al [7] defined three risk groups: patients who were negative at both the end of induction and in week 12 had an excellent prognosis (5-year relapse rate of 2%), whereas those who were positive (more than 0.1%) at both time points had a poor one (5-year relapse rate of 84%); remaining patients had an intermediate prognosis (5-year relapse rate of 24%). This precise MRD-risk stratification allowed identifying a sizable group (approximately 15%) of patients with an exceptionally high risk of relapse (84%) as well as a large group of children (approximately 45%) with an excellent event-free survival of 97%. The results allow for therapeutic interventions with chemotherapy intensification for the poor prognosis group within the first months of the treatment as intensifications delivered later in therapy have had limited benefits [30]. In contrast, MRD-based low-risk patients with 98-100% survival might profit from the treatment de-intensification. Statistical analysis has shown that the MRD status after induction therapy is the most significant prognostic factor, independent of other biological risk factors, such as age, blast count, immunophenotype, chromosomal aberrations at diagnosis, rapidity of the response [5, 7, 31-33]. MRD allows for a precise risk stratification and it is not a surrogate substitution for other prognostic factors. A recent study from Children's Oncology Group (COG) confirmed that the different genetically defined group of patients varied in their prevalence of MRD [33]. The low level of MRD was found in the minority of Philadelphia positive ALL patients and in the sizable group of the good-prognosis TEL-AML1 patients. However, unexpectedly, 20.3% of patients with very favorable trisomies of chromosomes 4 and 10 had high MRD levels. These patients have an excellent EFS approaching 95% at 3 years. These data suggest that the clinical significance of MRD positivity at the end of induction may be dependent on the patient subgroups.

MRD detection during treatment of relapsed ALL

MRD status during the first phases of re-induction treatment is a powerful predictor for the outcome in children with relapsed ALL [34]. This can be perceived as assessment of early treatment response after second induction treatment. MRD was analyzed on day 36 in 30 children treated in ALL-REZ BFM trials. Low MRD levels ($<10^{-3}$) were associated with an excellent probability of relapse-free survival of 86%, in contrast to MRD levels $\geq 10^{-3}$, which were uniformly predictive of dismal outcome (probability of relapse-free survival of 0%). Although the study includes a small number of patients, it powerfully identifies a subgroup of relapsed ALL children with chemosensitive disease. A large prospective clinical trial should define the role of chemotherapy vs. stem cell transplantation in this high-risk ALL group.

MRD detection before bone marrow transplantation in childhood ALL

Minimal residual disease status prior to bone marrow transplantation (BMT) was shown to be an important predictor for the post-BMT outcome despite the fact that most of published studies are retrospective and include a limited number of patients [10–14]. The high MRD level (10^{-2}-10^{-3}) before allogeneic BMT in patients receiving T-cell depleted graft was invariably associated with relapse after transplantation. In contrast, patients with a low MRD level (10^{-3}-10^{-5}) had 35-50% 2-year event-free survival, irrespective of graft manipulation. Significant GVHD which developed in the surviving patients receiving non-depleted grafts suggests that Graft vs. Leukemia effect can play an important role in overcoming MRD positivity, even a high MRD level. Patients with MRD-negativity before allogeneic BMT achieved an excellent outcome with a 2-year event-free survival higher than 70%.

Bone marrow transplantation is currently the most intensive therapy with significant toxicities and possible long-term sequelae available for childhood ALL. Current MRD data should play an important role in clinical BMT settings. Well designed, prospective, large clinical trials can be critical in defining patients most likely to benefit from allogeneic stem cell transplantation. Patients with high MRD burden prior to BMT can potentially undergo further cytoreductive chemotherapy in an attempt to achieve MRD-negativity, receive more intensive pre-BMT conditioning, or be offered early post-BMT immunotherapy. Alternatively, these patients can follow the protocols, which favor development of significant GVHD. In contrast, patients with negative MRD and a favorable outcome can be randomized to the stem cell transplantation vs. intensive modern chemotherapy in an attempt to better define the role of BMT in high-risk childhood ALL.

Conclusion

Sensitive MRD detection has proven to be a powerful prognostic factor in childhood ALL. Evaluation of early treatment response allows for precise risk stratification that may lead to the tailoring of the treatment intensity and the reduction of long-term toxicities. Also, the pre-BMT MRD status has a predictive value for post-BMT relapse-free survival.

The large multicenter clinical trials that aim at the MRD-based treatment intervention are the next steps in improving the childhood ALL cure rate. The standardization of the MRD techniques, quality control and the uniform approach to the initial risk-group definition, chemotherapy protocols and timing of the MRD samples is required for the success. The goal should be achieved in both approaches: the PCR analysis of Ig/PCR targets used by the European Study Group on MRD Detection on ALL as well as a four-color immunophenotyping used as a main MRD detection method by Children's Oncology Group.

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Corresponding author

Anna B. Pawlowska, M.D.
City of Hope National Medical Center
1500 Duarte Road
Duarte, CA 91010
USA