

REVIEW

DETECTION OF MINIMAL RESIDUAL DISEASE IN ACUTE MYELOID LEUKEMIA

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Abstract

This review summarizes recent approaches to the detection of minimal residual disease in acute myeloid leukemia. It shows advantages and disadvantages of all major methods, such as cytogenetics, fluorescence *in situ* hybridisation, flow cytometry and polymerase chain reaction, involved in minimal residual disease monitoring.

Key words

acute myeloid leukemia, minimal residual disease, remission

Abbreviations

AML, acute myeloid leukemia; FISH, fluorescence *in situ* hybridisation; MRD, minimal residual disease; RT-PCR, reverse transcription polymerase chain reaction

INTRODUCTION

Patients with acute myeloid leukemia (AML) may have a total of approximately 10^{12} malignant cells at the time of diagnosis (1). The disease is considered to be in complete remission when less than 5 % of malignant cells in bone marrow is morphologically detectable (2). These patients, however, still harbor as many as 10^{10} leukemic cells. From this point of view, the level of malignant cells is largely unknown. The sensitivity of morphologic studies can be improved by cytochemical staining, but the detection limit of these methods remains at a level of 1 leukemic cell in 10^2 cell population (2,3).

The leukemic population undetectable by morphologic methods has been called minimal residual disease (MRD). In the past 20 years, a tremendous effort has been made to describe and characterise residual leukemic cells with regard to prognosis and therapy. Leukemic cells can be distinguished from normal haematopoietic progenitors according to morphological and cytochemical properties, karyotypic or

genetic abnormalities, antigen-receptor gene rearrangements, *in vitro* cell growth requirements, and immunophenotype. Combinations of these techniques can detect small fraction of leukemic blasts among normal cells. In the following paragraphs, we will show possible clinical applications of these methods.

KARYOTYPE ABNORMALITIES

Conventional karyotyping, based on chromosomal abnormalities marked by diagnosis, has been used to monitor residual disease (4). The most common chromosomal aberrations in AML are listed in *Table 1* (1,2,4). The disappearance of an abnormal karyotype generally coincides with morphological remission but, more recently, some authors showed that abnormal metaphases, identical to those found at diagnosis, were detected in about 30% of patients with AML in morphological remission (5). All cytogenetically positive patients relapsed, thus supporting the reliability of abnormal karyotype as a good prediction marker of eventual relapse. Karyotyping by conventional banding techniques is a laborious

Table 1.
Distribution of chromosomal aberrations in acute myeloid leukemia patients.

Chromosomal aberration	Fusion gene	Relative frequency (%)
STRUCTURAL		
t (15;17)	PML-RARA	12
t (8;21)	AML1-ETO	8
inv (16)	CBFB-MYH11	4
11q23	MLL	4
t (3;v)	EVI	3
t (6;9)	DEK-KAN	1
t (3;5)	NPM-MLF1	1
Other	-	15
NUMERICAL		
+8	-	9
-7, -7q	-	6
-5, -5q	-	4
+22	-	1
Other	-	11
NOT IDENTIFIED	-	21

procedure and its success depends on the number of metaphases that can be examined. Unfortunately, only 70–80% of patients with AML can be evaluated by karyotyping.

The problem of small numbers of metaphases can be overcome by *in situ* hybridisation techniques. Fluorescence detection of *in situ* hybridisation (FISH) permits karyotypic analyses of non-dividing cells and can recognize numeric and structural chromosomal abnormalities (6,7). The FISH method is suitable for detection and quantitation of MRD because much larger numbers of cells can be evaluated than with the use of using classical cytogenetic techniques. The presence of chromosomal abnormalities detected by FISH during a follow-up highly correlates with leukemia relapse, with the detection limit being 0,5–1% leukemic cells among normal cells (7,8).

FLOW CYTOMETRY

Two approaches to identification of a leukemic clone are possible with the use of flow cytometry. The first is based on the identification of aneuploidy by single-laser cytometry in cells labelled with DNA-binding fluorochromes such as propidium iodide. This technique allows us to monitor MRD in most of the hyperdiploid and hypodiploid cases. The clinical significance of this approach has been documented in several studies. A disappearance of aneuploid peaks obtained by flow cytometry coincided with an attainment of morphological remission (9) and, on the other hand, an examination of remission bone marrow samples showed the persistence of leukemic aneuploid cells that preceded overt relapse (10).

The second flow cytometry approach to the detection of MRD in patients with AML is based on the fact that leukemic cells frequently display aberrant phenotypic features that allow their distinction from normal cells. The most relevant types of phenotype changes include asynchronous antigen expression, cross-lineage antigen expression or antigen overexpression (11,12). The multiparametric flow cytometry can precisely detect these phenotypic features with detection limits at a level of one residual leukemic cell among 10^3 - 10^4 normal cells. It has been shown that patients who have more than 5×10^3 residual leukemic cells at the end of induction therapy are at a significantly increased risk of AML relapse (12). Overall, the immunophenotypical investigation of MRD strongly predicted outcome for patients with AML, and the number of residual leukemic cells also correlated with multidrug resistance (12).

POLYMERASE CHAIN REACTION

Based on the karyotype studies carried out in patients with AML, there are several targets that are good candidates for amplification of the leukemia-specific fusion genes by the polymerase chain reaction (PCR). Molecular targets can be

derived from DNA or RNA following a reverse transcription (RT) PCR step (13). For this approach, PCR primers matching conserved regions that flank the translocation junction have to be designed. The sensitivity of PCR in the detection of karyotype abnormalities is extremely high. Experiments with artificial mixtures of leukemic and normal cells detected a single leukemic cell in 10^5 – 10^6 normal cells (13–15). Another important fact is that leukemia cells can be quantified when the RT-PCR method is used (14–16).

Approximately one third of AML patients have a chromosomal aberration suitable for a PCR-based assay and long-term monitoring of MRD. In most of the studies, the PCR positivity level reaching more than 10^{-4} leukemic cells in morphologically remission samples is predictive of a clinical relapse within several months, which is in agreement with the multiparametric flow-cytometry studies (12,13). On the other hand, in a subset of patients with negative post-remission RT-PCR, relapses have also occurred (3,14,17). Thus, PCR negativity is a necessary but not a uniformly sufficient requirement for extended remission. This is especially true for the most frequent fusion genes, namely, PML-RARA, AML1-ETO and CBF β -MYH11, involved in AML which are detectable by RT-PCR (Table 1). On the other hand, it has been shown in AML1-ETO positive AML patients that low (less than 10^{-4}) numbers of AML1-ETO positive cells are detectable in long-term remissions, even in those following allogeneic bone marrow transplantation (18).

CONCLUSIONS

Extensive investigations have been carried out in the field of residual disease detection in AML. Several approaches to the residual disease detection are explored. Assays for MRD should be available and reproducible, and should meet rigorous criteria with respect to specificity, sensitivity and prediction of treatment outcome. Prospective studies on the detection of MRD in acute leukemia patients have shown that large-scale MRD studies are feasible and that clinically relevant, MRD-based risk group stratification can be achieved and used for designing new treatment protocols. Such multi-centre, international treatment protocols with MRD-based stratification need careful standardisation and quality control of the MRD techniques. In the past two years, ten European laboratories carried out such large studies (19).

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DETEKCE MINIMÁLNÍ REZIDUÁLNÍ NEMOCI U AKUTNÍ MYELOIDNÍ LEUKÉMIE

S o u h r n

Tento přehled shrnuje současné poznatky týkající se detekce minimální reziduální nemoci u akutní myeloidní leukemie. Poukazuje na výhody a nevýhody stěžejních metod, kterými jsou cytogenetika, fluorescenční *in situ* hybridizace, průtoková cytometrie a polymerázová řetězová reakce, využívaných pro sledování minimální reziduální nemoci.

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