

Minimal residual disease in acute lymphoblastic leukemia

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Minimal residual disease

When acute lymphoblastic leukemia is diagnosed in a patient, the total number of leukemia cells is approximated to 10^{12} to 10^{13} . A majority of patients reach complete remission (CR) after about 4 weeks of chemotherapy. Complete remission does not mean that leukemia cells are totally eradicated from the body but that their level is beyond the sensitivity level of classical cytomorphologic methods (e.g. 1 to 5%). At this time, up to 10^{10} malignant cells can still remain in the patient. They represent the minimal residual disease (MRD). Techniques which are more sensitive than cytomorphology are now available that permit to detect these residual blasts (reviewed in [1] [2]).

Detection of residual cells allows a longer follow-up of the tumor burden during chemotherapy and thus, permits to better appreciate the sensitivity of leukemia cells to treatment. It is now established that the level of MRD represents a powerful prognostic factor. Besides, the detection of an increase of the MRD level enables to anticipate impending relapse.

Techniques for the follow-up of the residual disease

Techniques aimed at studying MRD rely on the detection of a leukemia cell specific marker which enable to distinguish blasts from normal marrow cells. Such markers have to be detected with high sensitivity, to be present in all leukemia cells and to be stable during disease evolution. Two kinds of markers are currently used: genetic markers, which can be detected by PCR, and immunophenotypic markers, which can be detected by flow cytometry.

PCR-bases techniques

PCR based strategies can be directed to 2 types of genetic targets: breakpoints of leukemia-related chromosome aberrations, and antigen-receptor gene rearrangements. The extreme sensitivity of polymerase chain reaction (PCR) makes possible the consistent detection of 1 leukemia cell among 10^5 normal cells (10^{-5}).

Chromosome aberrations

Recurrent chromosome translocations are found in 30 to 40% of ALL (figure 1) [3]. The molecular counterpart of these translocations can be detected by PCR using primers located on each side of the breakpoint [3]. DNA amplification can only be used for chromosome aberrations in which breakpoints cluster in a small area, such as Tal-1 deletions or Ig-cMyc fusions. In most cases, breakpoints spread over large intronic regions, but translocations give rise to fusion transcripts suitable for PCR amplification after a reverse transcription step (RT-PCR). All fusion transcripts can be used as markers for MRD follow-up. Their detection in remission samples is achieved, as for diagnosis, by RT-PCR. The detection sensitivity depends upon both the amount of RNA studied and the level of expression of the transcript. When 1-2 ug of RNA is studied, fusion transcripts associated to ALL permit to detect residual blasts with a sensitivity of 10^{-4} to 10^{-6} .

The main advantage of such markers is to be directly involved in leukemogenesis. Accordingly, their presence is constant all over disease evolution. However, variations in their expression level during the disease and particularly during chemotherapy cannot be excluded. This makes difficult to correlate the level of detection of a transcript to the amount of leukemia cells.

Given the frequency of each translocation, each fusion transcript permits to study only a subset of patients (figure 1). Another inconvenient is that, because they are not patient specific, these PCR targets are much more prone to false positive results due to carry-over than antigen-receptor genes.



Figure 1: Frequencies of the main fusion genes which can be used as PCR targets for MRD monitoring in ALL

Antigen-receptor gene rearrangements

The use of antigen-receptor gene rearrangements for MRD detection has been developed in order to overcome the lack of recurrent chromosomal abnormalities in most of the patients with lymphoid malignancies. 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 rearrangements, each malignant clone will present a specific configuration and the sequence of the junctional region(s) (N-region) is highly clone-specific. For technical convenience, rearrangements studied for MRD follow up are those of TcRgamma-delta, Ig heavy chain (IgH) and Iggkappa. The frequency of these rearrangements in ALL is indicated in table 2. A combined study of these 4 loci permits to identify one or more rearrangement in virtually all cases of ALL.

Table 1: Frequency of TcR and Ig recombination in childhood ALL (For some rearrangements, frequencies are slightly different in adult ALL)

-	B-lineage ALL	T-lineage ALL
IgH	>90%	10-15%
Igkappa*	50%	0%
TcRdelta**	55%	50%
TcRgamma	55%	90%

*Igkappa deletional rearrangements (Kde)

**The more frequent TcRdelta rearrangements are Vdelta2-Ddelta3 and Ddelta2-Ddelta3 in B-lineage ALL and Vdelta2-Ddelta3, Vdelta1-J1delta and Ddelta2-Jdelta1 in T-ALL.

All rearrangement-based PCR techniques use the same general strategy (fig.2) [5][6]. The presence of rearrangements in leukemia blasts is searched in a marrow sample obtained at diagnosis. PCR reactions are conducted using different combinations of V and D or J specific primers. When a rearrangement is present in leukemia cells, an intense and one-sized PCR signal is obtained. PCR products are then sequenced to derive either an oligonucleotidic probe or a primer specific for the junctional sequence of each specific clone. Test for MRD is conducted by PCR amplification of DNA from remission marrow cells, with the use of primer sets corresponding to the clonal rearrangement identified at the time of diagnosis. PCR products are then hybridized to the radiolabeled clono-specific probe. Alternatively, a primer specific of the junctional region can be used for nested PCR. A positive signal corresponds to the presence of residual blasts in the remission sample. This strategy permits to reach a sensitivity of 10^{-4} to 10^{-5} when 1-2 ug of DNA from the remission sample is studied. Performing PCR replicates permits to study a higher amount of DNA and thus, to reach a higher sensitivity [7].

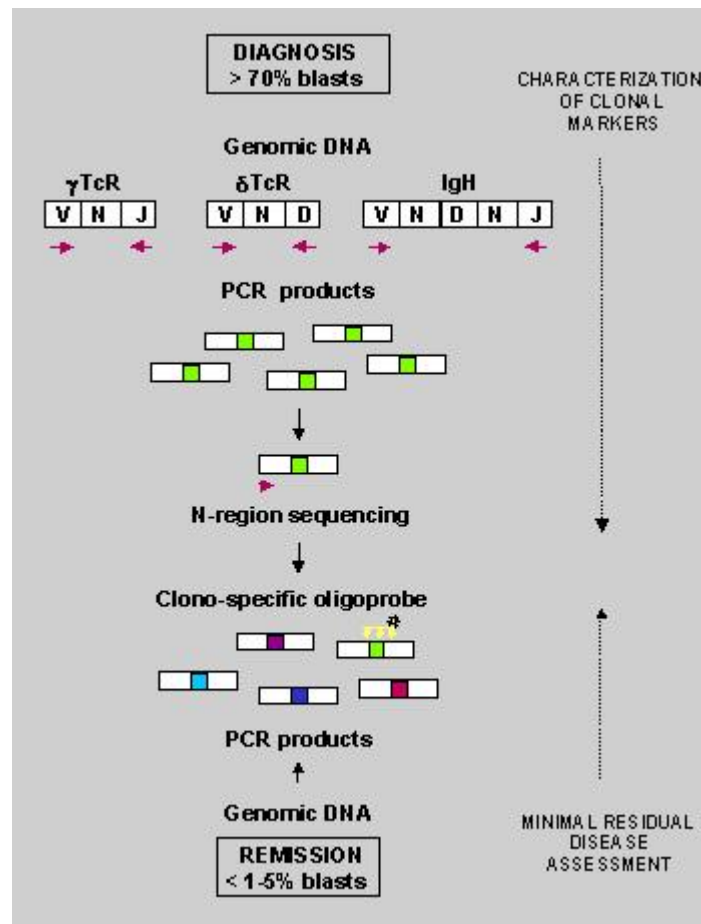


Figure 2: General strategy for MRD assessment using antigen receptor gene rearrangements as clonal markers.

The major advantage of antigen-receptor gene rearrangements is that they permit to study MRD in almost of cases of ALL. However, the need to sequence one or more rearrangement(s) in each patient makes the technique time-consuming and costly. Rather than by their sequence, rearrangements can be characterised by their size, and detected by resolutive electrophoresis on an automated sequence analyser. This simplification makes easier routine detection of MRD but leads to an important loss of sensitivity. It is thus mostly suited to the follow-up during the early steps of treatment. A second advantage is that DNA is less fragile than RNA and makes easier the standardisation of the amount of material studied. This is important to determine the sensitivity of the detection in case of a negative result.

However, the use of clonal markers has a main limitation: the stability of the markers during the disease course is not absolute and initial markers may be absent on blasts responsible for relapse. Marker instability is usually due to the fact that the marker identified at diagnosis was only present on a sub-clone of blasts, which is not responsible for the relapse. This phenomenon is termed "oligoclonality" [8]. Another possibility is that the clonal marker is replaced by a secondary rearrangement. This is termed "clonal evolution" [9]. Comparison of clono-specific markers present at diagnosis and at relapse reveals changes in rearrangement patterns in up to 40% of IgH rearrangements and 7-20% of TcR γ - δ rearrangements. However, at least one allele remains stable in about 90% of cases [10]. In this respect, the use of

several markers is recommended to prevent false negative results. Although MRD detection can be hampered by markers instability, it is noteworthy that the probability of changes in rearrangement pattern increases with time [10]. Thus, the risk of a false negative result is very low when MRD is studied at early stages of the treatment.

Quantitative aspects

It is well established now that quantitative MRD data have a higher predictive value than qualitative ones [11][12][13]. Moreover, sensitivity of the assay, which varies with the marker used and the specimen studied, has to be accurately determined in order to be able to interpret negative results. Quantitative techniques are thus required. Strategies such as limiting dilutions [7][11] or competitive PCR [14][15] have been used to make PCR-based assays quantitative. However, these techniques have been restricted to specialised laboratories. It is now possible to detect PCR product formation in " real-time " during amplification reaction and to determine PCR kinetics, which is related to the amount of targets in the tube. The recent development of semi-automated quantitative assays based on this technology (or " real-time PCR ") [16] encouraged many laboratories to develop quantitative MRD assays using either fusion transcripts, or antigen-receptor gene rearrangements.

Immunologicla techniques

Although leukemia cells are believed to be the malignant counterpart of normal cells at different stages of differentiation, they often display aberrant or unusual antigen expression. Multiparameter-flow cytometry permits to detect various combinations of surface membrane, cytoplasmic and nuclear molecules expressed or overexpressed by leukemia cells but not by normal bone marrow cells. Depending on the strategy used, 60 to 80% of childhood ALL present a leukemia-specific immunophenotype [17][18]. Multiparameter-flow cytometry enables to detect residual blasts with a sensitivity level of 10^{-4} [17][18], which is slightly below the sensitivity of molecular methods. Unlike molecular methods, flow cytometry discriminates between viable and dead cells. The main advantages of flow cytometry is that it directly give quantitative data and is very rapid (results can be obtained in 24h). The stability of immunological markers over the course of the disease is yet to be determined.

Clinical significance of minimal residual disease in ALL

Sequential follow-up of MRD in childhood ALL

The clinical significance of MRD assessment has remained controversial for a long time mostly because the first clinical studies were conducted retrospectively on small and heterogeneous groups of patients undergoing different treatments. More recently, large prospective studies of serial bone marrow specimen unequivocally demonstrated that MRD assessment give clinically relevant insight into the effectiveness of treatment.

Most studies investigating the value of measuring MRD in bone marrow used

antigen-receptor gene rearrangements as PCR targets. The first large study was retrospective and included 88 children enrolled in 2 therapeutic trials (Australian and New Zealand Children's Cancer Study IV and V) [11]. The level of residual blasts was quantified using limiting dilution analysis. More recently, results of two multicenter European prospective studies have been reported. One studied MRD on a semi-quantitative basis in 240 children treated according to the national protocols of the BFM (Berlin-Frankfurt-Munich) protocol [12]. The other studied MRD in 178 children treated according to the EORTC (European Organisation for research and treatment of Cancer) protocol 58 881 [13]. In this latter study, residual blasts were quantified using a competitive PCR assay. The largest study using immunological markers report the follow-up of 65 patients [17].

Although differences were noted according to treatment intensity, conclusions of these studies were virtually the same:

Residual blasts are still detected after completion of induction therapy in approximately 50% of children treated for ALL. This percentage can vary according to the intensity of the chemotherapy regimen and MRD results have to be interpreted in relationship with the type of treatment [11].

MRD is closely related to the risk of relapse during all the course of chemotherapy and can be used as a prognostic factor as soon as induction therapy is completed. This suggests that the sensitivity to drugs administered during induction therapy correlates closely with the sensitivity to agents during later therapy.

The risk of relapse rises steeply with the amount of residual blasts, and the extent of MRD predict the outcome more precisely than simple presence or absence. More than 70% of children with MRD levels above 10^{-2} at the end of induction therapy relapsed, as compared to less than 10% of the children with MRD levels below 10^{-4} [12][13].

MRD is independent from other prognostic factors such as immunophenotype, white blood count, age, and corticosteroid response. Thus, MRD provides a predictive factor in virtually all subgroups of patients with ALL.

The occurrence of individual relapse is not always predicted, especially for extra-medullary and off-therapy relapses. This is not due to a lack of sensitivity of MRD detection since a large proportion of children who are not going to relapse have weakly positive signals. Other factors must be involved.

Sequential follow-up of MRD in adult ALL

MRD has been less extensively studied in adult than in childhood ALL and studies have only been conducted on few patients. As for children, the level of MRD at the end of induction therapy predicts clinical outcome. Comparison of MRD results found in children who received similar induction therapy indicates that adult ALL are more resistant to treatment even in the absence of a t(9;22) [19][20]. This is consistent with the fact that adult patients have a worse overall outcome than children do. It is not clear whether the increased resistance to chemotherapy observed in adults is age- or leukemia related.

MRD measurement in blood samples

The level of residual blasts in peripheral blood is proportional to that in bone marrow but is about ten times lower [10][15][21]. For this reason, bone marrow is considered as the best tissue source to study MRD.

MRD evaluation in bone marrow transplantation

Very few data concerns the prognostic information provided by MRD assessment for patients who underwent bone marrow transplantation (BMT). Recently, one group retrospectively studied MRD status before [22] and after [23] allo-BMT in a series of 64 patients. Their result suggests that pre-and post-transplant MRD levels correlate significantly with the risk of medullar relapse. Unfortunately, data were presented separately, which makes impossible to evaluate the individual relationship between pre- and post-BMT status of the patients. Moreover, transplantation with T-depleted unrelated donors were largely over-represented and results might not have a general significance. However, MRD assessment should be useful to monitor of emerging therapies such as donor lymphocyte infusion or immunotherapy. As in the case of first-line therapy, quantitative data might help to predict with more accuracy patient outcome after allo-BMT [22].

Conclusion

Although certain presenting features are statistically correlated with the prognosis of ALL, the outcome remains largely unpredictable for most of the patients whatever the initial seriousness of the disease. The level of MRD provides a measure of kinetics of tumor reduction, giving insights into the in-vivo resistance of the overall leukemic population to the chemotherapeutic agents used. In-vivo, drug resistance appears partly independent from the prognostic factors at presentation and probably involves additional constitutional factors determining drug metabolism for instance.

Early response predicts long-term outcome. In this respect, MRD assessment complements other methods for early evaluation of the response to chemotherapy such as corticoreistance or D15 bone marrow aspirate examination. It has been shown that MRD at the end of induction therapy provides a prognostic factor independently of corticoreistance. Probably because at this time, more drugs have been administered. The benefit of MRD assessment over D15 bone marrow aspiration has not been evaluated. D15 bone marrow aspiration is less costly but difficult to estimate reliably and reproducibly. New therapeutic trials already integrate MRD results to therapeutic decisions. The problem is now to determine what is the best to do for patients who are resistant to treatment.

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