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Acute Lymphoblastic Leukemia

Molecular detection of minimal residual disease is associated with early relapse in adult acute lymphoblastic leukemia

Several studies in childhood acute lymphoblastic leukemia (ALL) have documented that molecular detection of minimal residual disease (MRD) based on screening for T-cell receptor and immunoglobulin gene rearrangements can identify patients at a high risk of relapse. In our experience, evaluation of MRD in adult ALL can help to identify high risk patients.

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Several studies in childhood acute lymphoblastic leukemia (ALL) have demonstrated the strong association between minimal residual disease (MRD) level and the risk of relapse.[1] The most useful methods for MRD monitoring currently available are polymerase chain reaction (PCR) techniques, using either chromosome aberrations that result in fusion gene transcripts or patient-specific junctional regions of rearranged immunoglobulin (Ig) and T-cell receptor (TcR) genes, and flow cytometric detection of aberrant immunophenotypes.[2-4] The use of antigen-receptor gene rearrangements has allowed detection of the presence of MRD, overcoming the diagnostic problem posed by the lack of recurrent chromosomal abnormalities (such as t(9;22) and t(4;11)) in most patients with ALL.

The value of MRD detected by PCR and/or immunologic techniques has been extensively evaluated in childhood ALL but less studied in adult ALL.[5-9] The aim of the present study was to determine the value of molecular MRD monitoring in predicting outcome in adult ALL patients.

We evaluated 29 patients (10 males and 19 females; median age 27 years (range 15 to 61); 23 with common ALL, 2 with pro-B ALL, and 4 with T-ALL) admitted to our Institution from April 1996 to March 2001. They were treated with the multicenter co-operative GIMEMA (Gruppo Italiano Malattie Ematologiche dell'Adulto) LAL 0496 protocol;[10] all patients achieved hematologic complete remission (CR) after induction therapy.

The bone marrow samples collected at diagnosis included more than 90% of blasts. The MRD analysis was performed at the following time points: post-induction, post-consolidation, every three months for the first year and every six months thereafter. We investigated the MRD level using rearrangements of the genes for T-cell receptors γ (TCRγ), δ (TCRδ) and Ig κ (Kde) as molecular markers for the leukemic clone and heteroduplex PCR analysis to detect the molecular marker.[3] Sensitive detection required determination of the sequence of the marker gene from material obtained at diagnosis, and development of a specific probe that recognizes the leukemic clone, with a minimal target sensitivity of 10^{-4}/10^{-5}. The concentration of leukemic cells in the bone marrow samples during follow-up was estimated by comparison of the signals with those of 10-fold dilution samples (10^{-1} to 10^{-7}) of the DNA at diagnosis. This resulted in a reproducible semiquantitative estimation of MRD. A cut-off value of $1/10^3$ was used to divide patients into two categories : MRD positive ($> 10^{-3}$) and MRD negative ($< 10^{-3}$). The former value indicated molecular relapse whereas the latter defined patients in molecular remission. In all cases evaluated for MRD at least two clonal rearrangements were found.

At the post-induction and post-consolidation points, and after three months of maintenance, 14 patients (48%) were MRD negative. Two of these cases, despite having undetectable disease during the molecular follow up, developed isolated central nervous system (CNS) relapse after 7 and 9 months; in both cases the extramedullary localization of ALL was followed by molecular and hematologic relapses after a few months (Figure 1A). Fifteen (52%) patients were MRD positive at the post induction point; among them, 14 (93%) and 1(7%) underwent hematologic and extramedullary relapse (in the CNS), respectively (Figure 1B).

Survival analysis showed a significant difference between these two groups, compared for event-free survival (from CR to hematologic/extramedullary relapse) ($p<0.0001$) (*data not* shown). In the MRD negative group the absence of MRD was confirmed except for the two cases with CNS relapse, described above at all the subsequent checkpoints during follow-up. No differences were observed in clinical and biological presenting features at diagnosis (age, gender, white blood cell count and immunophenotypic analysis) between patients with hematologic continuous complete remission and those who relapsed (*data not shown*).

Our study suggests that molecular MRD monitoring in adult ALL is a better method for identifying patients with a high or low risk of relapse than other commonly described prognostic indicators. In our experience MRD monitoring failed to predict isolated CNS relapse in 2 patients. In these

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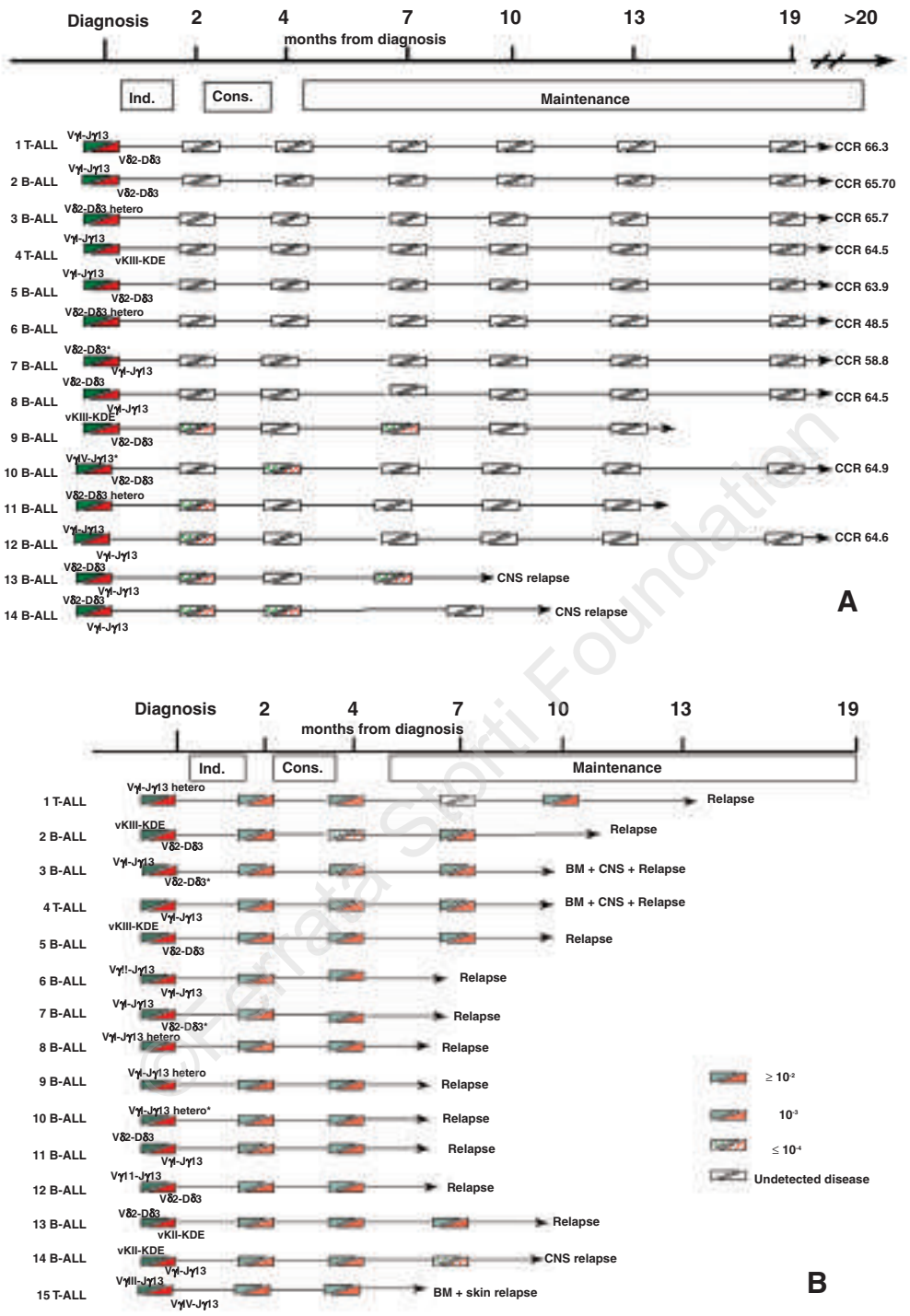

Figure 1. MRD analysis. Patients in remission and in relapse were investigated for MRD using the methods described in the paper. A cut-off value of $1/10^3$ was used to divide patients into two categories: MRD positive ($\geq 10^3$) and MRD negative ($<10^{-3}$). A. Analysis in MRD negative patients. B. Analysis in MRD positive patients. Most tested probes have a 10^{-4} sensitivity whereas those with the symbol* have a sensitivity of 10^{-5}. CCR: continuous complete remission for a longer period than the scale can indicate (>20 months).

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cases no changes in clonal rearrangements were observed: in fact, cerebral spinal fluid analysis at the time of CNS relapse showed MRD positivity, although this was not observed in bone marrow samples. These observations suggest that molecular relapse might not always be evident in the marrow at the time of extramedullary disease.

In conclusion, kinetic studies relying on accurate, reliable blast quantification might help to identify patients with a high risk of relapse.

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Malignant Lymphomas

Combined seminested polymerase chain reaction and heteroduplex analysis for detecting monoclonality of *IgH* rearrangement in patients with follicular lymphoma

A new, sensitive method combining seminested polymerase chain reaction (PCR) and heteroduplex analysis was used to detect follicular lymphoma (FL) cells in peripheral blood. Based on the detection of *IgH* rearrangement in DNA from peripheral blood leukocytes, the method demonstrated the presence of monoclonal B cells in FL patients with high frequency.

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Monoclonality of *IgH* gene rearrangements has been used to distinguish malignant B cells from normal ones. The complementarity-determining region (CDR) in the variable region of the *IgH* gene is unique to each B-cell clone and the CDR III is the useful clonal signature of an individual B cell.[1,2] The main difficulty of PCR-based clonality studies of B-cell malignancies is discrimination between monoclonal and polyclonal products, especially when there is a high background of polyclonal B cells in tumor samples. To discern between them more clearly, we utilized heteroduplex analysis in which PCR products are denaturated at high temperatures and subsequently renatured to induce homoduplex or heteroduplex formation.

Genomic DNA was isolated from 24 patients (18 males, 6 females) with histologically verified FL in the Hematology and Medical Oncology Division, Department of Internal Medicine, Hasan Sadikin Hospital, Bandung, Indonesia. Consensus PCR primers used for amplifying the *IgH* gene were: FR3A (framework 3A), FR1c (framework 1c) for the 3' end of the V region, and LJH (low JH), VLJH (very low JH) for the 3' end of the J region.[3,4] The first round of amplification was performed using an upstream consensus primer FR3A or FR1c and a lowstream primer LJH that bound to all published JH gene segments. For reamplification, the lower strand primer (LJH) was replaced by a nested consensus JH primer (VLJH) and an aliquot (1%) of the first PCR was used as template.[5] The seminested PCR conditions have been described previously, but were used with some modifications.[6] For heteroduplex analysis, PCR products were denaturated at 94˚C for 10 min and subsequently cooled at 40°C to induce duplex formation. The hetero- and/or homoduplex products (10 μL) were characterized by polyacrylamide gel electrophoresis (PAGE) with 10% non-reducing gel in 0.5×TBE buffer.[7] The gel was stained with a 1:1000 dilution of SYBR Gold dye in 0.5×TBE buffer for 30 min and visualized under ultraviolet light. One or two thin clear bands within the expected size (75-140 bp for FR3A and 330-350 bp for FR1c) were judged to indicate positive clonality.[5,7] For sensitivity studies, peripheral blood mononuclear cells (PBMC) from normal subjects and DHL-4 cells (diffuse large B-cell lymphoma) were mixed to make ratios of one DHL-4 cell in 10^2, 10^3, 10^4, 10^5 and 10^6 PBMC, and the total cell number was adjusted to 5×10^6. The DNA was extracted from the mixture using a QIamp DNA Mini Kit (Qiagen).

The PCR products loaded on 4% agarose gel stained with ethidium bromide are shown in Figure 1A. TK cells (diffuse large B cell lymphoma) as the positive control (lane 2) and

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