Application of a chemiluminescent methodology for detection of minimal residual disease in childhood acute lymphoblastic leukemia

Analysis of minimal residual disease (MRD) can predict outcome in childhood acute lymphoblastic leukemia (ALL). We applied a chemiluminescent methodology in 20 children with ALL. We detected MRD at different time-points throughout the follow-up of our patients, concluding that chemiluminescent detection of MRD is a reliable, safe and sensitive method.

Recent prospective studies clearly demonstrate the prognostic value of MRD in children with ALL.1,2 Several methodologies are available for MRD analyses in ALL patients.1-5 In order to test a safe, sensitive and reliable method for those laboratories in which neither radioactive analysis nor the TaqMan strategy can be carried out,3 we present here a report on application of digoxigenin (DIG)-labeled patient-specific probes for a chemiluminescent detection of MRD in children with ALL.

Twenty children with ALL diagnosed at our institution and treated according to the ongoing protocol of the Associazione Italiana di Ematologia ed Oncologia Pediatrica (AIEOP-ALL 95) were included in this study. The childrens' characteristics are listed in Table 1. We collected samples at 5 time-points (TP): after 43 days (TP1), after three months (TP2), five (TP3), seven (TP4) and 24 months (TP5) of therapy. T-cell receptor (TCR) γ and TCR δ gene rearrangements were identified and characterized by performing diagnostic polymerase chain reactions (PCR), heteroduplex and sequencing analyses using standardized techniques.6,7 PCR of follow-up were performed using different protocols in order to amplify TCRγ and TCRδ rearrangements.7 Seven microliters of the products were spotted onto positively charged nylon membranes (NYTRAN N+, Roche Boehringer). Next, 200 pmol of each oligonucleotide were 3'-end labeled with DIG-ddUTP using a DIG oligonucleotide 3'-end labeling kit (Roche Molecular Biochemicals, Mannheim, Germany) according to manufacturer's instructions. The membranes were prehybridized at 68°C for 2 hours using 25 mL hybridization solution (Roche Molecular Biochemicals) and then hybridized in a sealed plastic bag with 3 mL hybridization solution and 20 µL of the labeling probe (200 pmol). The membranes were incubated overnight at 54°C and then washed twice in 2 × SSC, 0.1% SDS at room temperature for 5 min. The membranes were soaked with blocking solution (Roche Molecular Biochemicals) for 30 min at room temperature. Twenty-four milliliters of blocking solution containing 2.4 µL of anti-DIG-alkaline phosphatase Fab fragments (Roche Molecular Biochemicals) were added, followed by incubation for 30 min. Finally, the membranes were washed twice with washing buffer (Roche Molecular Biochemicals) for 15 min each before incubation with detection buffer (Roche Molecular Biochemicals) for 5 min. A chemiluminescent substrate (CSPD, Roche Molecular Biochemicals) was added to the surface of the membrane. The hybridization bags were sealed and incubated at room temperature for 5 min in the dark. Finally the membranes were air-dried, placed in a plastic hybridization bag and incubated at 37°C for 15 min. We exposed each membrane to an X-Omat AR film (Kodak) for a period ranging from 15 min to 4 hours, in order to increase signal detection.

The sensitivities for respective PCR targets using the chemiluminescent method are summarized in Table 1. Among our cases we designed probes which reached different sensitivities ranging from 10-3 to 10 -6. Figure 1 shows representative results regarding three paradigmatic situations of MRD analysis which might influence clinical decisions. Figure 1A shows persistence of a leukemic clone in a child with T-cell acute lymphoblastic...
leukemia (case LG) who subsequently received an allogeneic bone marrow transplantation. Figure 1B (case SR) demonstrates how reappearance of a leukemic clone at the beginning of the reinduction phase (TP3) has not been followed by a subsequent relapse (TP4).

There are several advantages in using the chemiluminescent method for detection of MRD. Firstly, the procedure is not radioactive and can, therefore, be performed in any laboratory where radioactive products are not allowed or the TaqMan strategy is too expensive or not yet applied. Secondly, because of the short exposure time (2-10 min), the whole procedure can be shortened in the future. However, end labeling of the oligonucleotide can be done at any time and the labeled probes can be stored at -20°C and used up to four times. In addition, the hybridization solution of DIG-labeled probes can be reused several times in order to analyze all time-points. We decided to use the chemiluminescent method for detection of MRD. 5 Firstly, the procedure is not radioactive and can, therefore, be performed in any laboratory and provides information important for clinical and therapeutic decisions. This procedure is faster and safer than the radioactive dot-blot technique, reducing biological hazards for the operator.

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Key words: ALL, MRD, chemiluminescent method, T-cell receptor gene, childhood.

References

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