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LONG-TERM MOLECULAR REMISSION AFTER CONVENTIONAL CHEMOTHERAPY IN A PATIENT WITH PHILADELPHIA-NEGATIVE ACUTE LYMPHOBLASTIC LEUKEMIA

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ABSTRACT

Adult Philadelphia (Ph[1]) negative acute lymphoblastic leukemia (ALL) is usually treated with conventional chemotherapy regimens. Long-term disease-free survival is often achieved. The rearrangement of immunoglobulin heavy-chain genes has been used to evaluate minimal residual disease. A novel nested-polymerase chain reaction (PCR) approach was used here to study a patient in long-term complete remission (CR) after the BFM regimen. No evidence of tumor cell contamination was found in bone marrow cells collected after 93 months of CR. This finding supports the hypothesis that conventional chemotherapy can induce long-term molecular remission and cure in Ph[1] negative ALL.

Key words: Philadelphia-negative acute lymphoblastic leukemia, long-term molecular remission, minimal residual disease

In adult acute lymphoblastic leukemia (ALL), the absence of the Philadelphia (Ph1) chromosome and the expression of an early pre-B cell phenotype, together with low leukocyte count, young age and rapid achievement of complete remission (CR), identify a group of patients with good prognosis. These patients are usually treated with intensified conventional chemotherapy and survival rates at 10 years exceed 50%.[1-4] Whether such long-term remissions are the expression of eradication of the leukemic clone is still an open question. It cannot be excluded that leukemia cells persist below the sensitivity threshold of standard morphologic, cytochemical and immunophenotypic techniques.

Several methods for detecting residual leukemia and lymphoma cells have recently been developed. These methods are based on polymerase chain reaction (PCR) and use either a specific translocation breakpoint or the rearrangement of immunoglobulin heavy-chain (IgH) and T-cell receptor genes as genetic markers. The clonal specificity in B cells is primarily derived from rearrangement of the IgH variable regions (VDJ). The VDJ contains three complementarity-determining regions (CDR) which are unique to each B-cell clone. Tumor-specific sequences derived from CDR2 and CDR3 are employed in both amplification and hybridization to evaluate minimal residual disease (MRD).[5,6]

In this report, the presence of residual leukemia cells was evaluated by a novel nested-PCR approach in a patient with Ph[1] negative ALL in long-term CR after intensified conventional chemotherapy (Berlin-Frankfurt-Munster [BFM] regimen).

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Case Report

A 47-year-old man was admitted with low grade fever, fatigue and cervical adenopathies. Complete blood cell count showed anemia (hemoglobin 9.1 g/dL), thrombocytopenia (platelets 87×10^9/L) and leukocytosis (white blood cells 13×10^9/L). A diagnosis of ALL was made on the basis of bone marrow (BM) examination, cytochemical (periodic acid-Schiff negative, Sudan black negative, naphthol AS-D chloracetate esterase negative, α-naphthyl esterase negative) and immunological studies ($CD19^+$, $CD10^+$, $HLA\text{-}DR^+$, sIg-). Cytogenetic analysis showed the absence of the t(9;22) translocation, and PCR analysis did not reveal the Bcr-Abl fusion transcript. Induction chemotherapy consisted of daunoblastine, L-asparaginase, vincristine and prednisolone, according to the BFM regimen;[2] CR was achieved at day 15. On day +21 disseminated mucormycosis occurred and was successfully treated with amphotericin B. Reinduction, consolidation chemotherapy and CNS prophylaxis were performed according to the BFM regimen. Maintenance therapy consisted of mercaptopurine 60 mg/sm daily and methotrexate 20 mg/sm weekly. Because of toxic hepatitis, treatment was discontinued 16 weeks earlier than scheduled. The patient now feels well and no evidence of leukemia has been found in peripheral blood or BM by morphological and immunophenotypical examinations. The patient has been off treatment for 61 months.

Materials and Methods

PCR amplification and sequencing

BM cells were separated on a Ficoll-Hypaque density gradient. DNA was obtained by proteinase K digestion, phenol/chloroform extraction and ethanol precipitation.[7] Amplifications were performed as previously described.[8] Briefly, 1 μg of DNA at diagnosis was amplified by using a VH3 consensus sense primer derived from the IgH leader region and an antisense primer derived from the JH region. The reaction was carried out for 33 cycles (denaturation at 94°C for 30", annealing at 65°C for 30" and extension at 72°C for 30"), with a final extension of 7 minutes. PCR products were analyzed by electrophoresis on 2% agarose gel. Direct sequencing reaction was performed using the Promega fmol sequencing system according to the manufacturer's instructions.

MRD detection

A nested-PCR approach was employed to detect residual leukemia cells (Figure 1). A first PCR amplification was performed using a tumor-specific sense primer derived from CDR2 and an antisense primer derived from the 3' end of the JH region (JH3). The reaction was carried out for 30 cycles (denaturation at 94°C for 30", annealing at 58°C for 30", extension at 72°C for 30"), with a final extension of 7 minutes. Three μL of amplified DNA products were then reamplified using internal primers,

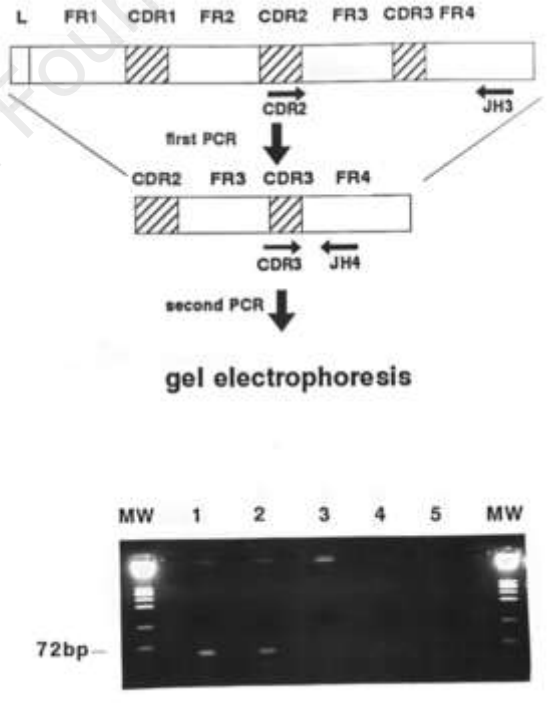

Figure 1. Panel A: schematic representation of the nested-PCR method for the evaluation of minimal residual disease using immunoglobulin heavy-chain (IgH) genes. The leader (L), variable (V), diversity (D), joining (J), constant (CH), framework (FR), and complementarity-determining (CDR) regions are represented. Arrows indicate the positions of the four primers employed. Panel B: Analysis of minimal residual disease using nested-PCR (3% metaphor agarose gel stained with ethidium bromide). MW: molecular weight marker; 1: patient DNA at diagnosis; 2: patient DNA diluted to 10^{-5} in polyclonal DNA (weak positive control); 3: remission DNA at 93 months from diagnosis; 4: polyclonal DNA; 5: No DNA lane.

the first derived from CDR3 and the second from the JH region (JH4). This second PCR reaction was carried out for 30 cycles (denaturation at 94°C for 30", annealing at 60°C for 30", extension at 72°C for 30"), with a final extension of 7 minutes. PCR products were then run on a 3% Metaphor agarose gel (FMC Bioproducts, Rockland, ME, USA) stained with ethidium bromide (0.5 μg/mL). Two polyclonal DNAs were always used as negative controls.

Discussion

In the present study, we report the results of MRD analysis in a Ph1 negative ALL patient in CR after conventional chemotherapy. The presence of residual leukemic cells was evaluated using a rearrangement of IgH genes. The leukemia IgH rearrangement was amplified with consensus primers derived from the VH leader and JH regions. Nested-PCR employing leukemia-specific primers was utilized to detect residual tumor cells (Figure 1). Nested-PCR is commonly used for the evaluation of MRD when a tumor-specific translocation is present.[5,9,10] We devised this approach for IgH genes, thus avoiding the need for a radioactively labelled probe. The first amplification was performed using a CDR2 sense primer with an antisense primer derived from the 3' end of the JH region (JH3). An aliquot of DNA was then reamplified in a second PCR using a CDR3 sense primer with another JH (JH4) antisense primer (Figure 1). The sensitivity of our method was tested by diluting 1 μg of the patient's DNA in polyclonal DNA. The nested-PCR approach was able to detect one clonal IgH in 100,000 polyclonal rearrangements.

Our patient had been treated with intensified conventional chemotherapy and had achieved CR at day +15. No leukemia cells were detectable by nested-PCR after 93 months of CR. Although PCR-negativity cannot rule out the hypothesis of leukemic cells persisting below the sensitivity threshold of the method or in localized foci within or outside hematopoietic organs, the failure to detect residual leukemic cells strongly indicates that intensified conventional regimens can induce molecular remission and perhaps cure Ph^1 negative ALL patients.

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