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MOLECULAR MONITORING OF MINIMAL RESIDUAL DISEASE IN ACUTE PROMYELOCYTIC LEUKEMIA BY THE POLYMERASE CHAIN REACTION ASSAY FOR THE PML/RARα (RETINOIC ACID RECEPTOR-α) FUSION TRANSCRIPT IN PATIENTS TREATED WITH ALL-TRANS RETINOIC ACID FOLLOWED BY CHEMOTHERAPY

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ABSTRACT

Five acute promyelocytic leukemia (APL) patients who achieved a complete remission (CR) with all-trans retinoic acid (ATRA) underwent residual disease monitoring through reverse transcription polymerase chain reaction (PCR) for PML/retinoic acid receptor-α (PML/RARα) fusion transcript. All received consolidation chemotherapy in CR, one in the form of autologous bone marrow transplantation (ABMT). In four of the patients PCR was positive for the PML/RARα transcript immediately after ATRA treatment and/or after the first consolidation chemotherapy course. In the patient treated with ABMT, positivity was still detected six months after ABMT. One patient given five repeated courses of chemotherapy was PCR negative for PML/RARα after 14 months in CR. Our pilot study confirmed that ATRA is a highly efficient induction therapy for APL in various stages of the disease, but ATRA alone cannot cure the disease. PCR should be considered a fundamental assay for assessing minimal residual disease in CR that will influence further treatment strategies and permit evaluation of treatment results.

Key words: acute promyelocytic leukemia, PML/RARα, PCR assay, all-trans retinoic acid

The typical translocation t(15;17) (q22; q12-21) in acute promyelocytic leukemia (APL) results in DNA rearrangements in the region for the nuclear retinoic-acid receptor-α (RAR-α) and consequently the expression of abnormal messenger RNA (mRNA), with the appearance of the PML/RARα protein in APL blasts.[1] We report the use of PCR analysis for the aberrant PML/RARα transcript in five consecutive APL patients during remission after induction with ATRA and consolidation chemotherapy.

Patients and Methods

Five consecutive patients with APL M3 type[2] (one male, four female; median age 50, range 41-52 years) entered a pilot study of ATRA induction therapy followed by chemotherapy in which residual disease was monitored by PCR assay for the aberrant RARα transcript. Two patients were newly diagnosed, two had relapsed from a previous complete remission (CR) induced by conventional chemotherapy that lasted 14 and 15 months, respectively, and one patient prior to ATRA therapy was considered refractory due to a failure to achieve CR with conventional chemotherapy.

The patients received all-trans retinoic acid (ATRA, Tretinoin, 10 mg soft gelatin capsules, kindly provided by the Hoffman-La Roche Company) 45 mg/m^2/day as single therapeutic

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agent. All patients after achieving CR with ATRA were treated with one course of intensive consolidation chemotherapy (see Table 1). Patient #5 then underwent autologous bone marrow transplantation (ABMT) after conditioning with busulfan 16 mg/kg b.w. and cyclophosphamide 120 mg/kg b.w. in the sixth month of CR. Patients #2 and 3 received 5 additional courses of chemotherapy consisting of daunorubicin and cytosine arabinoside in a 1+5 schedule, while patient #4 refused further chemotherapy.

Reverse transcription polymerase chain reaction assay (PCR) for PML/RARα fusion mRNA. Total RNA was prepared by the acid guanidium/phenol/chloroform method.[3] Reverse transcription of 2 μg of total RNA to cDNA was performed for 45 minutes at 42°C in a 20 μL volume containing 200 U of Moloney murine leukemia virus (Mo-MLV) reverse transcriptase (GIBCO BRL, Eggstein, Austria) and random hexamers as primers (Boehringer, Mannheim, Germany). cDNA was amplified using the primers and under the conditions described by Pandolfi *et al.*[4]

Results

All five patients achieved CR after a median of 27 days (range 21 to 30) of ATRA treatment. One patient (#1) died during first consolidation from an intracranial hemorrhage. In three out of the five patients (#3, 4, and 5) translocation t(15;17)(q22;q11) was found at diagnosis in 100%, 92%, and 100% of the metaphases examined, respectively. Patients #1 and 2 had a normal karyotype despite morphologic findings compatible with M3 leukemia. After the ATRA-induced CR had been achieved, the karyotype was normal in all patients except #4, in whom the typical translocation was again found in one out of 25 metaphases examined. This patient relapsed early after first consolidation chemotherapy.

PML/RARα fusion mRNA expression was detected in all patients tested immediately after ATRA-induced CR and/or after first consolidation chemotherapy (Table 1 and Figure 1). No further testing was performed in patient #4 due to refusal of further therapy and early relapse.

In one patient (#3) after five courses of chemotherapy the PML/RARα transcript could not be detected, while in the remaining two (#2 and 5) PML/RARα fusion mRNA was repeatedly detected in a later phase following further consolidation chemotherapy. Patient #2 remained PCR positive and relapsed after 15 months of CR. Serial examination of the PML/RARα transcript was also positive three times in patient #5, confirming the persistence of minimal residual disease 8 months after ABMT (Figure 1).

Table 1. Outcome of patients treated with ATRA followed by chemotherapy and monitoring of minimal residual disease with the PCR assay during the course of treatment.

Pts.	ATRA therapy mq/days	Consolidation chemotherapy	CR (day)	Duration of CR (mo)	Cytogenetic findings after ATRA	PML/RARα expression during CR after ATRA	after 1st CT	after further CT	after ABMT (mo) 1	3	6	Relapse	Outcome
1	90/29	HD Ara-C+ mAMSA	28	1	46 XX, normal	ND	NE					NE	Death in CR
2	80/33	HD Ara-C	30	13	46 XX, normal	ND	+	+				YES	Alive in relapse
3	90/34	DNR+Ara-C	21	13	46 XX, normal	ND	+	–				NO	Alive in CR
4	80/30	DNR+Ara-C	27	3	46 XX/46 XX t(15;17) (24/1)	+	+					YES	Death from relapse
5	80/28	DNR+Ara-C, ABMT	22	12	46 XX, normal	+	+		+	+	+	NO	Alive in CR

DNR: daunorubicin; Ara-C: cytosine arabinoside; CR: complete remission; ND: not done; ABMT: autologous bone marrow transplantation; CT: consolidation chemotherapy; NE: not evaluable; +: positive PCR assay; –: negative PCR assay.

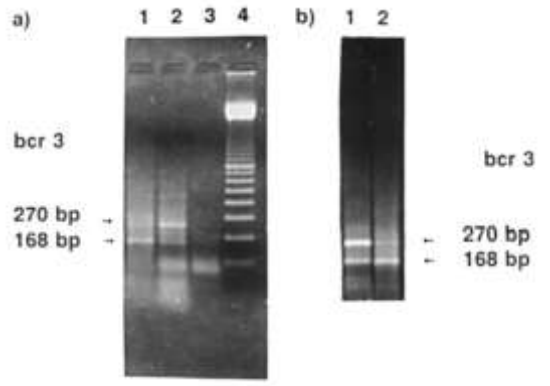

Figure 1. Detection of PML/RARα fusion by PCR in two APL patients. The gel stained with ethidium bromide contains bands corresponding to the bcr 3 PML/RARα junction.
a) 1. patient #5 after ATRA induction therapy and consolidation chemotherapy; 2. patient #2 (cytogenetically negative), after ATRA induction therapy and consolidation chemotherapy; 3. negative control, and 4. molecular marker: 100 base pairs ladder.
b) 1. patient #5 before ABMT, 2. same patient, five months after ABMT.

Discussion

Accurate diagnosis is essential for the success of ATRA treatment. Studies so far indicate that 84% of all patients with morphological evidence of APL, and 95% of patients in whom t(15;17) was documented by cytogenetic or molecular analysis achieve a CR after ATRA treatment.[5] Detection of the PML/RARα fusion transcript through PCR provides a useful diagnostic tool for APL. Although the t(15;17) translocation is a characteristic feature of APL, not all patients with this disease display this anomaly,[6] probably because of the low sensitivity of conventional cytogenetics for detecting it, even at diagnosis.

Documenting residual disease in APL is important since the CR rate is high and a relatively large proportion of patients could be cured with standard chemotherapy.[7]

Our results using molecular analysis confirm the presence of residual disease immediately after ATRA-induced CR, while in one patient we were unable to detect the PML/RARα transcript after repeated courses of chemotherapy.

The prognostic significance of PCR positivity for PML/RARα transcript in patients in CR was evaluated in some recent studies[8-10] and the results show a strong correlation between PCR positivity and the incidence of relapse. The PCR test could thus be a predictor of which patients in CR will relapse and therefore will need further intensive treatment, either with chemotherapy or some form of BMT in remission.

In conclusion, despite the efficacy of ATRA as remission induction therapy for APL, it cannot cure the disease by itself. Molecular detection of the PML/RARα transcript with the PCR technique could be a sensitive tool for assessing minimal residual disease and helping to determine further treatment strategy in patients who have already achieved CR. Further investigation is needed to establish whether PCR negativity correlates with long-term survival and cure.

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