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SHORT REPORT

Therapy-related acute myeloid leukaemia after successful therapy for acute promyelocytic leukaemia with t(15;17): a report of two cases and a review of the literature

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Summary. We describe two patients with positive t(15;17) acute promyelocytic leukaemia (APL) that developed into a therapy-related myelodysplasia 2–2·5 years after complete remission (CR) and then evolved into therapy-related acute myeloid leukaemia (t-AML). Both patients received anthracyclines as potential leukaemogenic drugs. In both cases, cytogenetic changes usually occurring after use of alkylating agents were noticed: monosomy 7 associated with monosomy 5 or 5q– chromosome. A review of the literature on t-AML occurring after successful therapy for APL showed only one report similar to these two cases. These observations suggest that anthracyclines can cause t-AML similar to that induced by alkylating agents.

Keywords: acute promyelocytic leukaemia, therapy-related acute myeloid leukaemia, anthracyclines, monosomy 7 and 5, 5q– chromosome.

Relapses occurring after a complete remission (CR) of acute promyelocytic leukaemia (APL) usually develop from their original APL clone (Berger *et al*, 1991). The appearance of a therapy-related leukaemia (t-AML) with clonal chromosome changes unrelated to the abnormal initial APL clone is a rare event and only single cases have been reported in the literature (Jubashi *et al*, 1993; Myazaki *et al*, 1994; Hatzis *et al*, 1995; Todisco *et al*, 1995; Bseiso *et al*, 1997; Meloni *et al*, 1997; Felice *et al*, 1999; Sawada *et al*, 1999) (Table I). We report two cases of APL that developed a t-AML preceded by a therapy-related myelodysplasia (t-MDS), one at 23 and one at 29 months after complete remission (CR).

PATIENTS AND METHODS

Karyotypes were analysed by R-banding from bone marrow samples according to the ISCN. Fluorescence *in situ* hybridization (FISH) analysis was performed during the course of the disease with commercially available probes, either using a dual whole chromosome painting (WCP) method (Cambio laboratories) or using two-colour detection of the PML–RARα fusion gene (Vysis laboratories).

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Case 1.. A 61-year-old woman presented in November 1993 with a history of bleeding tendency. Disseminated intravascular coagulation (DIC) was diagnosed associated with a mild hyperleucocytosis at $22{\cdot}9 \times 10^9$/l with 32% blast cells. Bone marrow aspiration diagnosed an APL. No myelodysplastic change was found. Karyotype analysis reported 47,XX,+8,t(15;17)(q22;q21) in 20 out of 20 metaphases.

The patient had not undergone any previous cytotoxic exposure. The patient was included in the APL91 protocol and CR was achieved in 1 month. Therapy consisted of all *trans* retinoic acid (ATRA) 45 mg/m^2/d until CR, daunorubicin 60 mg/m^2/d (days 2–4) and cytosine arabinoside (Ara-C) 100 mg/m^2 × 2/d (days 1–7). Consolidation therapy included two courses of daunorubicin plus Ara-C. Maintenance therapy with 6-mercaptopurine (6-MP) 90 mg/m^2/d, methotrexate (MTX) 15 mg/m^2/d and ATRA 45 mg/m^2/d (15 d every 3 months) started in April 1994 and was maintained for 24 months. In May 1996, 29 months after achieving CR, a pancytopenia occurred that progressively worsened. Marrow examination during July 1996 showed a refractory anaemia with a 9% excess of blasts (RAEB). No typical APL cells could be detected. Karyotype disclosed new anomalies: 45,XX,−5,−7,+11 in

Table I. Review of the literature.

Reference	Jubashi *et al* (1993)	Myazaki *et al* (1994)	Todisco *et al* (1995)	Hatzis *et al* (1995)	Bseiso *et al* (1997)	Meloni *et al* (1997)	Felice *et al* (1999)	Sawada *et al* (1999)
Sex	M	M	F	F	F	M	M	M
Age (years)	48	61	15	57	26	31	8	55
Previous cytotoxic exposition	No	No	No	No	No	No	No	No
Biology at diagnosis								
FAB subtype	M3	M3	M3	M3	M3	M3	M3	M3
Myelodysplasia	+	–	–	–	–	ND*	–	–
Cytogenetic analysis	t(15;17)	ND*	t(15;17)	t(15;17)	t(15;17)	t(15;17)	t(15;17)	t(15;17)
Molecular analysis†	ND*	ND*	+	+	ND*	+	ND*	ND*
Initial therapy‡								
ATRA§	–	–	–	–	+	–	+	–
Anthracyclines¶	+	+	+	+	+	+	+	+
Etoposide (VP16)	+	+	+	–	–	–	+	+
Alkylating agents**	–	–	–	+	–	+	+	+
Others	+	+	+	+	+	+	+	+
Latency (months)††	37	43	49	23	34	36[9]	26	43
Biology at relapse								
FAB subtype	M1	My‡‡	M4	M2	RAEBt§§	My‡‡	Biphenotypic¶¶	M2
Myelodysplasia	+	–	–	+	+	ND*	–	–
Cytogenetic analysis	t(7;21) (q31;q22)	t(3;21)	t(10;11) (q14;21)	dic(15;17) (q11;p11)	−7	−7	−7	t(10;11) (q23;p15)
Molecular analysis†	–	ND*	–	–	ND*	–	ND*	–

*Not done or not available.
†Southern blot rearrangement gene analysis or polymerase chain reaction of the fusion transcript PML–RARα.
‡Including induction, consolidation and maintenance therapy.
§All *trans*-retinoic acid.
¶Daunorubicin or idarubicin and/or adriamycin and/or mitoxantrone and/or doxorubicin and/or aclarubicine.
**Cytosine arabinoside and/or 6-mercaptopurine and/or methotrexate and/or vincristine and/or thioguanine and/or enocytabin.
††After achieving complete remission.
‡‡Myeloid leukaemia; FAB classification not possible.
§§Refractory anaemia with excess of blasts in transformation.
¶¶Lymphoblastic L2 FAB and low-differentiated myeloblast morphology with immunophenotype showing myeloid, T- and B-lineage marker.

17 out of 28 metaphases. No t(15;17) could be identified by conventional cytogenetic studies, however FISH analysis disclosed 11% of mitoses with t(15;17) (Table II). Two months later, APL cells appeared on the patient's bone marrow smear. Cytogenetic studies could detect two different clones (Table II). The patient was treated with ATRA alone between August and December 1996, when she died, in relapse, as a result of septic shock and haemorrhagic complications.

Case 2.. In July 1996, a 57-year-old woman was admitted as an emergency for haemorrhagic syndrome. Coagulation studies revealed abnormalities related to DIC. The blood count showed a hyperleucocytosis of 100×10^9/l with 97% of atypical promyelocytes. The bone marrow aspiration contained 96% of atypical promyelocytes with numerous Auer rods and bundles.

No myelodysplastic changes were noticed on this first bone marrow smear. Cytogenetic studies revealed a complex karyotype: 46,XX,del(3)(q24q26), del(5)(q23q32), t(7;11)(p11;p12), t(15;17)(q22;q21) in 21 out of 21 metaphases. The patient reported a 30-year period of exposure to radioactive iodine. The patient was included in the APL93 protocol and received the same induction therapy as for case 1. She achieved CR after 1 month. The same consolidation therapy was applied and the patient started maintenance treatment in January 1997 with 6-MP and MTX. The patient followed this course of treatment for 18 months until June 1998. In January 1998, pancytopenia appeared and progressed despite reduction of maintenance therapy. Marrow examination in July 1998, 23 months after CR, disclosed a dyserythropoiesis with no typical APL cells. In May 1999, the myelodysplasia progressed to refractory anaemia with an excess of blasts (RAEB) and the karyotype revealed a monosomy 7 (45,XX,−7). None of the initial anomalies could be detected through conventional cytogenetic studies and FISH failed to detect the t(15;17). In October 1999, the patient developed overt t-AML with multiple myelodysplastic changes. The karyotype showed, in addition to monosomy 7, a 5q− chromosome: 45,XX,del(5)(q21q34),−7. CR could not be obtained with polychemotherapy.

DISCUSSION

Two main classes of cytostatic drugs seem to induce t-MDS

Table II. Patients: evolution of karyotype.

Patients	Date (month/year)	Clinical stage	Karyotype [number of metaphases]
Case 1	11/93	APL*	47, XX, +8, t(15;17)(q22;q21) [20]
	12/93	CR†	46, XX [20]
	07/96	t-AML‡	45, XX, −5, −7, +11 [17] 46, XX [11] WCP§ 11% mitoses t(15;17) +[66/603 mitoses]
	09/96	t-AML‡	45, XX, −5, −7, +11 [6]
		Relapse of APL*	47, XX,+8, t(15; 7)(q22;q21) [14] WCP§ 35% mitoses t(15;17) +[22/62 mitoses]
Case 2	07/96	Secondary APL*	46, XX, del(3)(q24q26), del(5)(q23q32), t(7;11)(p11;p12), t(15;17)(q22;q21) [21]
	08/96	CR†	46, XX [6]
	12/96	CR†	46, XX [27] WCP§ t(15;17) –[109 mitoses]
	12/97	CR†	46, XX [8] WCP§ t(15;17) –[170 mitoses]
	02/99	t-MDS¶	FISH** PML/RARα –[1000 nuclei]
	04/99	t-MDS¶	45, XX, −7
	10/99	t-AML‡	45, XX, del(5)(q21q34), −7 [20]

*Acute promyelocytic leukaemia.
†Complete remission.
‡Therapy-related acute myeloid leukaemia.
§Whole chromosome painting.
¶Therapy-related myelodysplasia.
**Fluorescence *in situ* hybridization.

and t-AML (Pedersen-Bjergaard *et al*, 1993). The first described are alkylating agents. The latent period before development of t-MDS or t-AML varies between 2 and 8 years. Cytogenetically, a 5q and/or 7q deletion or monosomy 5 and/or monosomy 7 are usually observed. Another major group of cytostatic agents targeted at DNA topoisomerase II has been proved to be leukaemogenic (Pedersen-Bjergaard *et al*, 1993). The latent period for occurrence of t-AML varies between 1 and 2·5 years. Karyotype frequently discloses anomalies of the 11q23 or the 21q22 chromosome bands (Pedersen-Bjergaard *et al*, 1993).

Our two patients had a clinical course closely related to t-AML occurring after alkylating agents: a transient period of t-MDS evolving into overt AML and characteristic karyotype anomalies involving monosomy 7 associated with a monosomy 5 (case 1) or a 5q− chromosome (case 2). However, the two patients did not receive alkylating agents. The potential leukaemogenic drugs that they received were an anthracycline, 6-MP and MTX. The second patient also reported a 30-year exposure to radioiodine. To our knowledge, neither Ara-C nor ATRA have been reported as leukaemogenic drugs. The cases of t-AML reported in the literature (Table I) in the course of APL evolution are seen either before or after the introduction of ATRA. Secondary MDS/AML are rare events after single drug treatment by 6-MP or MTX; however, these drugs may enhance the risk of developing a t-MDS/AML when used after other drugs. Indeed, after DNA damage induced by other drugs, 6-MP metabolites can interfere with DNA repair, leading to the introduction of point mutations, and both 6-MP and MTX can predispose to non-homologous recombination (Relling *et al*, 1998). Thus, in our two cases, these two drugs may have contributed to the development of secondary AML, but here the main leukaemogenic drug seems to be the anthracycline. In case 1, the karyotype also disclosed a trisomy 11 that may be compatible with previous administration of anthracyclines. A rearrangement/duplication of the MLL gene within the 11q23 band has been shown to be a recurrent molecular defect in AML with +11 (Calgiuri *et al*, 1996). The latent period observed in our two patients was of 2 years for one and 2·5 years for the other patient, which is compatible with the leukaemogenic effect of the anthracyclines. Table I shows that only the patient reported by Bseiso *et al* (1997) resembles that of our two cases.

Another hypothesis, especially for the patient exposed to radioiodine, could be that the MDS clone co-existed with the original APL clone and arose after APL-effective chemotherapy. This hypothesis cannot be ruled out as we do not have markers that are able to detect a small underlying APL clone during CR. However, against this hypothesis are the following facts: (i) all the bone marrow aspirations and cytogenetic studies carried out between diagnosis and the appearance of t-MDS were normal in both cases (Table II); (ii) the cytogenetic abnormalities found at relapse could not be detected at diagnosis, and for the second patient the del(5q) found at diagnosis and at relapse had different breakpoints; and (iii) we could not find any myelodysplastic

changes at diagnosis in the bone marrow aspiration from the two patients.

In conclusion, chromosome abnormalities usually observed after alkylating agents can also be observed after anthracycline therapy, and t-AML may occur after an anthracycline treatment even without the association of epipodophyllotoxins or alkylating agents.

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