Diabetes insipidus and thrombocytosis were presenting symptoms in a case of adult ANLL-M1. Cytogenetic investigations revealed a typical 3q rearrangement, i.e. inv(3)(q21q26). A subclone with monosomy 7 was also found and documented by FISH analysis. Correlations between clinical/hematological features and cytogenetic/FISH results are discussed.

Key words: 3q rearrangement, monosomy 7, diabetes insipidus, thrombocytosis, myeloid leukemia

Case report

The patient, a 48-year-old man, was in good health until August 1992, when he developed fatigue, fever, polyuria and polydipsia. In September 1992 he was referred to our Institution from another hospital where diabetes insipidus had been diagnosed on the basis of the water deprivation test. On admission the patient was under treatment with desmopressin (DDAVP). Clinical examination revealed hepatomegaly; peripheral blood count was as follows: WBC 9.2 $\times$ 10$^9$/L, PLTS 406 $\times$ 10$^9$/L, RBC 3.7 $\times$ 10$^{12}$/L, Hb 12.2 g/dL, Hct 36%. Other laboratory data were normal, but LDH was increased (471 IU/mL, NV<250). Bone marrow smears stained with May-Grunwald Giemsa showed 60% blast cells with a high nucleus/cytoplasm ratio, rare granules, and more than one nucleolus. Residual erythroid elements showed asynchrony of maturation between nucleus and cytoplasm. Bone marrow biopsy revealed infiltrating blast cells in the interstitium and a remarkable amount of hyperlobulated micromegakaryocytes. Lineage identification of blast cells was made using standard cytochemical staining.
The findings were compatible with myeloid lineage; Sudan Black was positive in 20% of blasts, both α-naphthyl acetate esterase (ANAE) and α-naphthol AS-D chloroacetate esterase (NASDCAE) gave a very weak positivity in 10% of cells. A panel of monoclonal antibodies was tested to characterize the immunophenotype of the blast cells. The myeloid nature of the malignant clone was confirmed by the following results: CD3 18%; CD10 neg; CD19 neg; CD20 2%; CD11b 38%; CD13 47%; CD14 10%; CD15 7%; CD33 40%; HLA-DR 45%. Diagnosis of AML-M1 was formulated on the basis of the French-American-British proposals. A brain NMR before chemotherapy showed the absence of the typical hyperdense signal at the level of the neurohypophysis. A lumbar puncture also failed to evidence leukemic cells in the cerebral spinal fluid. The patient underwent chemotherapy with the AML10 protocol (idarubicin, cytosine arabinoside, VP-16). Four lumbar punctures (methotrexate and cytosine arabinoside) were also performed for CNS prophylaxis.

Complete remission was documented by morphological and cytogenetic analysis after induction as well as after consolidation. A second NMR at that time revealed considerable meningeal thickening in the frontal area. In March 1993 the patient underwent autologous bone marrow transplantation in hematological and cytogenetic remission. Three months later he died in relapse.

**Cytogenetic and FISH analysis**

Cytogenetic studies were performed on bone marrow aspirates at diagnosis and after chemotherapy. Metaphases were obtained from 24- and 48-hour methotrexate synchronized cell cultures in RPMI 1640 medium supplemented with 30% fetal calf serum, 5% glutamine and 2% penicillin-streptomycin. After ten minutes’ exposure to 0.05 μg/mL colcemid and hypotonic treatment in 0.075 M KCl, cells were fixed with methanol-acetic acid 3:1. G banding with Wright’s stain was used. At diagnosis ten out of
fifteen karyotypes were abnormal: 46,XY,inv(3)(q21q26) [6 cells], 92,XXY,inv(3)(q21q26) [1 cell], and 45,XY,inv(3)(q21q26),-7 [3 cells] (Figure 1). After treatment only nine normal metaphases could be found.

Fixed cells were also retrospectively analyzed by using FISH with a centromeric probe for chromosome 7 (Oncor) at diagnosis, after chemotherapy, and three months after bone marrow transplantation. A patient with polycythemia vera and a normal karyotype was used as control. Fresh slides were pretreated with 10×RNAse in 2×SSC at 37°C for 1 hour and then denatured with formamide 70% 2×SSC at 70°C for 2’. Slides were incubated with 0.75 μL of probe in 15 μL of hybridization mixture (formamide 65% 2×SSC) for 16 hours. After post-hybridization washings (formamide 65% 2xSSC at 43°C for 15’ and 2×SSC at 37°C for 8’), FITC-avidin and one layer with anti-avidin antibody were used for detection. Slides were mounted with antifade medium. Fluorescent signals were evaluated in 500 interphase cells. The control showed 86.3% and 13.7% of interphase nuclei with double and single spots, respectively; patient samples revealed a gradual decrease of interphase nuclei with monosomy 7 from 88% at diagnosis, to 30% after chemotherapy, and 35% after bone marrow transplantation (Figure 2).

Discussion

The present ANLL case is unique in that the two rare clinical-hematological findings described above, i.e. thrombocytosis and diabetes insipidus, were present at diagnosis and associated with both 3q inversion and monosomy 7.

Thrombocytosis or a normal platelet count is observed in chronic myeloproliferative disorders and in some myelodysplastic syndromes. De novo and secondary adult ANLL, however, show thrombocytopenia at diagnosis in more than 90% of cases. Among the remaining 10% with normal or high platelet counts, a cytogenetic subgroup is identified by 3q rearrangements, such as t(3;3), ins(3)(q21q26), and inv(3)(q21q26). A history of exposure to cytotoxic agents, radiation, chemotherapeutic treatment, or a previous myelodysplastic phase in evolution to ANLL often characterize these cases. A subgroup of patients with rearrangements of chromosome 3 on q21 and q26 may also show monosomy 5/del(5q) and/or monosomy...
7/del(7q). Interestingly, at least two cases of 3q inversion and monosomy 7 or del(7q) with cerebral localization of the leukemia have been reported.\textsuperscript{10,12}

To the best of our knowledge, 54 cases of MDS or ANLL with diabetes insipidus at diagnosis have been reported in the literature. Only twelve of these patients underwent chromosome analysis.\textsuperscript{2-7} Three of these,\textsuperscript{2} reviewed by de la Chapelle et al., were studied before the introduction of banding techniques; one of them had 45 chromosomes and the missing chromosome was not identified. Nine cases analyzed with Q or G-banding showed the presence of monosomy 7 (8 patients) or del(7q) (1 patient): 4 were ANLL, 3 of whom with myelodysplastic features in the bone marrow smears;\textsuperscript{4,6-7} 1 was a chronic myeloid leukemia in blast phase.\textsuperscript{4} Monosomy 7 was present both as a single abnormality and in complex karyotypes.

The pathogenesis of diabetes insipidus in these cases is controversial. NMR analysis in our patient revealed the absence of neuropeptide signals in the neurohypophysis, suggesting a central origin of the disease. Clinical response to DDAVP may also support this hypothesis; in other cases, however, including that of the patient with monosomy 7 described by Sadullah et al.\textsuperscript{3} who did not respond to DDAVP, post-mortem examination revealed leukemic infiltration of both the pituitary gland and kidneys. In the present case we retrospectively applied the FISH technique in order to evaluate the leukemic clone with monosomy 7 in interphase nuclei. At diagnosis the size of the clone with monosomy 7 was much greater when the FISH method was used (88%) than when classical cytogenetics were employed (20%). Cytogenetic studies after therapy gave poor results: only nine evaluable metaphases, which proved to be normal on karyotyping. FISH investigation did not confirm cytogenetic remission since at least 30% of the cells (versus 13.7% in the normal control) still showed monosomy 7. These data illustrate how the integration of classical cytogenetics with FISH analyses may provide us with new insights in the monitoring of leukemic clones.

References