T-cell lymphoblastic lymphoma associated with the t(6;11)(q27;q23)

We report the case of a 9-year old girl presenting with mediastinal mass and pleural effusion. Flow cytometric analysis from a pleural aspirate revealed a T-cell neoplasia with an uncommon phenotype (CD34, CD38, CD45, CD7, CD56, CD7, CD1a, cytCD79a). Cytogenetic studies performed on neoplastic cells showed the t(6;11)(q27;q23). MLL status should be investigated in cases of T-cell lymphoblastic lymphoma.

Rearrangements of the MLL gene involving the 11q23 region have been implicated in leukemogenesis. They have been described in 50-60% of infant acute lymphoblastic leukemias (ALL), in < 5% of adult ALL, in 5% of adult *de novo* acute myeloid leukemias (AML) and in 85% of topoisomerase II inhibitor-related acute leukemias.[1,2] Translocations involving the MLL gene have rarely been associated with T-cell lymphoblastic lymphoma.[3]

A 9-year old girl was admitted to hospital because of cough, dyspnea, thoracic pain and fever. Chest radiography disclosed an anterior mediastinal mass (Figure 1a) with massive pleural effusion. Laboratory findings were: hemoglobin 133 g/L, leukocytes 9.8×10^9/L (neutrophils 86%, lymphocytes 10%) platelets 435×10^9/L. Serum lactate dehydrogenase and other biochemical parameters were normal. Pleural effusion aspiration obtained a highly cellular liquid containing monomorphous cells (Figure 1b). The patient was diagnosed as having a T-cell lymphoblastic lymphoma based on the study of the cells in the effusion. The patient underwent chemotherapy with prednisolone, vincristine, cyclophosphamide, daunorubicin, asparaginase and methotrexate followed by consolidations including vincristine, cyclophosphamide, cytarabine, asparaginase and methotrexate. The patient achieved a complete remission, which is still maintained 22 months after diagnosis. Direct immunofluorescence was performed by incubating 2×10^6 cells with the specific monoclonal antibody (MoAb) for 15 min in the dark at room temperature. The cells were lysed (FACS Lysis solution, Becton Dickinson) for 3 to 5 minutes and centrifuged at 250 g for 5 minutes. The cells were washed twice with phosphate buffered saline (PBS) before being resuspended in PBS and examined.

For detection of intracellular antigens (cytoplasmic and nuclear) by flow cytometry, the cells were incubated for fixation and permeabilization with Fix & Perm solution (CALTAG, San Francisco, CA, USA). Measurements were performed on a FACScan flow cytometer (Becton Dickinson). The LYSIS -II (BD) software program was employed for data acquisition. At least 10,000 events per tube were measured. The PAINT-A-GATE PRO software program (BD) was used for further data analysis. Thresholds for positivity were based on isotype negative controls.

Chromosomes were identified by G-banding and chromosomal abnormalities were defined according to the International System for Human Cytogenetic Nomenclature (ISCN).[4] The fluorescence *in situ* hybridization (FISH) study was performed on destained G-banding metaphases using a chromosome-specific probe.

Heavy chain and T-cell receptor (TCR) rearrangements were detected by nested polymerase chain reaction (PCR).[5] Bcl- and p53 mutations were assessed using a PCR-SSCP.[6,7] The configu-

Figure 1a. Chest radiograph showing massive left pleural effusion. Figure 1b. Neoplastic cells in the pleural effusion (May-Grünwald-Giemsa stain, × 400).

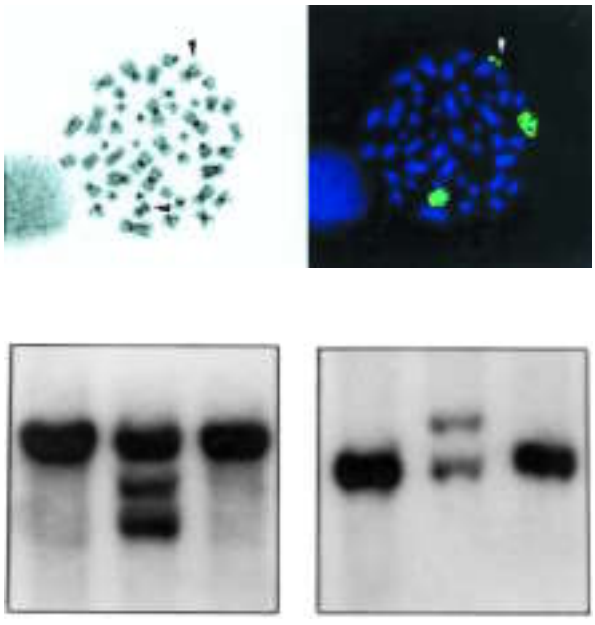

Figure 2a. A. G-banded metaphase showing der(11) and der(6) (arrowhead). B. FISH analysis of the same metaphase showing a hybridization signal on der(6)(arrowhead) and on both chromosomes 11. Figure 2b. A. Blots examined with the B859 probe. Germ-line 8.3 kb (BamHI) and 14-kb (Hind III). Rearranged bands Hind III 11 kb and 9.5 kb (lane 2, left). Rearranged for BamHI 14 kb(lane 2, right).

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ration of the MLL locus was analyzed by hybridization of *BamHI*, *Bgl II* and *Hind III*-digested DNA to the B859 probe. A Cb probe was used to investigate the status of the T-cell receptor genes (Dr. T. W. Mak, Ontario, Canada).

Neoplastic cells from the pleural effusion were positive for CD7, CD1a, CD56, CD45, CD34 CD38 and cytoplasmic CD79a. This last finding has been recently reported to be quite common in T-cell ALL.[8]

The coexistence of the IgH and TCR rearrangements and the negativity for TCR in Southern blot analysis lend support to an immature T-cell phenotype despite the negativity of TdT. Cytogenetic study revealed a t(6;11)(q27;q23) karyotype in 100% of the neoplastic cells. A chromosome 11 specific probe identified the two chromosomes 11 and the translocated material to the long arm of chromosome 6, confirming the t(6;11)(q27;q23) (Figure 2a). MLL involvement was demonstrated by a positive Southern blot (Figure 2b). Bcl-6 and p53 analysis showed no mutations in the regions analyzed.

The t(6;11) has been mainly found in acute myelomonocytic leukemias, a male predominance being observed in the initial reports. Most patients are young and mild leukocytosis is frequent. Unusual findings reported in our case were the non-leukemic presentation, the development in a young girl and the satisfactory evolution. Welborn *et al*.[3] reported a patient with T-cell acute lymphoblastic leukemia (T-ALL) whose most significant clinical features were mediastinal adenopathies. In this respect, the current case could resemble the original description although immunophenotypic findings could be different. In a recent workshop, t(6;11)(q27;q23) cases were reviewed. Most of these corresponded to M4 or M5 AML with only 3 ALL cases in children.[9]

The most frequent immunophenotype associated with the t(6;11)(q27;q23) in the cases from the workshop was: CD13, CD33 and HLA-Dr (16/17). CD34 was positive in 6/10 cases tested and TdT was always negative.

Despite the limited follow-up, the patient remains in complete remission. p53 mutations, which could be responsible for a poor evolution, were not detected in this case. These mutations have been associated with a myeloid phenotype in childhood leukemias with MLL rearrangements. Our findings suggest that MLL rearrangements should be investigated in young patients with T-cell lymphoblastic lymphomas.

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Key words: MLL, T-cell lymphoblastic lymphoma, chromosome II, immunophenotype.

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