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case of the month

Malignant Lymphomas

Leukemic relapse as T-cell acute lymphoblastic leukemia in a patient with acute myeloid leukemia and a minor T-cell clone at diagnosis

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ABSTRACT

***Background and Objectives*. Lineage classification needs to be established before starting chemotherapy in acute leukemias. In some cases, mixed populations can be found and these are differentiated by antigenic expression patterns.**

***Design and Methods*. We report the case of a patient with acute myelogenous leukemia whose relapse was classified as T-acute lymphoblastic leukemia (T-ALL).**

***Results*. Flow cytometry analysis at diagnosis enabled us to identify a minor T-cell subclone which progressively increased and became dominant at relapse. There were no changes at cytogenetic and molecular levels.**

***Interpretation and Conclusions*. This case illustrates the usefulness of multiparametric flow cytometry for assessing minor leukemic populations.**

Key words: leukemia, cytometry, subclone, rearrangements, lineage

Acute leukemia is a clonal expansion of malignant cells arrested at a specific stage of differentiation. Neoplastic cells show aberrant immunophenotypic characteristics when compared with normal hematopoietic cells: they simultaneously express antigens of more than one lineage or show asynchronic expression of these antigens.[1] When blast cells co-express lymphoid and myeloid antigens the leukemia is called mixed or hybrid. When single blasts express lymphoid or myeloid antigens but not both, these leukemias are regarded as *bilineage*.[1] The co-expression of early and late differentiation antigens from the same lineage is regarded as a common aberrant leukemic feature.[1,2] Cytogenetic and molecular studies are needed to: 1) clarify the origin of a particular leukemic clone, 2) demonstrate a phenotypic switch or a clonal evolution and 3) distinguish these cases from therapy-induced leukemias.[3]

We report the case of a patient with TdT positive acute myelogenous leukemia (AML), who relapsed with T-cell acute lymphoblastic leukemia (ALL). At diagnosis, trisomy 8 was demonstrated by cytogenetic studies, and this karyotype remained constant at relapse.

Case report

A 52-year old woman was admitted to our hospital because of generalized malaise and cervical lymph node enlargement. Past medical history was not remarkable. On physical examination the patient showed bilateral cervical lymphadenopathy and there was no enlargement of the spleen or the liver. The hematocrit was 22%, and the white cell count was 44.3×10^9/L with 2% neutrophils, 19% lymphocytes, 3% monocytes, 1% erythroblasts and 76% blasts. Serum lactate dehydrogenase level was increased (2,248 U/L, normal value < 480). A bone marrow aspirate showed a hypercellular marrow with 94% atypical cells of large-medium size, 1 or 2 nucleoli, indented nuclei and the absence of Auer rods or vacuoles in the cytoplasm. Immunocytochemical stain for myeloperoxidase was positive in 60% of the blast cells, and the α-naphthyl-acetate-esterase stain was positive in 90%, and inhibited by fluoride. The patient was diagnosed as having AML, M5a according to the French-American-British classification. The immunophenotype showed a TdT positive acute leukemia with two populations of blasts: 56% were myeloblasts and 20% were T-cell lymphoblasts (Table 1, Figure 1). Cytogenetic studies showed the following karyotype: 47,XX, +8/47,XX, der(7),+8. The patient underwent induction chemotherapy consisting of idarubicin (10 mg/m^2 i.v. days 1, 3, 5), cytarabine (25 mg/m^2 i.v. day 1 and 100 mg/m^2 days 1-7 in i.v. perfusion) and etoposide (100 mg/m^2 i.v. days 1-3) plus intrathecal chemotherapy. A complete remission was achieved after 4 weeks but the patient presented persistent fever after leukocyte recovery. On the 75th day in hospital, overt leukemic relapse occurred. The patient

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Table 1. Immunophenotype at diagnosis and at relapse.

Phenotype FITC/PE/PeCy5 at diagnosis	*Predominant leukemic clone at diagnosis*	*Minor leukemic clone at diagnosis*	*Phenotype FITC/PE/PeCy5 at relapse*	*Predominant leukemic clone at relapse*
CD15/CD34/HLA-DR	+/+/+ weak	-/+/-	CD15/CD34/HLA-DR	-/+/-
CD10/CD20/CD19	-/-/-	-/-/-	CD10/CD20/CD19	-/-/-
CD61/CD41/CD45	-/-/+	-/-/+ weak	CD22/CD13/CD45	-/+ weak/+ weak
CD22/CD13/CD3	-/+/-	-/+/-	CD2/CD33/CD19	-/+/-
CD2/CD33/CD19	-/+/-	-/+/-	CD7/CD117/CD45	+/-/+ weak
CD7/CD117/CD45	+/+/+	+/-/+ weak	CD33/CD34/CD45	+/+/+ weak
CD66/CD34/CD64	+/+/-	-/+/-	CD7/CD33/CD45	+/+/+ weak
CD36/GA/HLA-DR	-/-/+ weak	-/-/-	CD8/CD4/CD3	-/-/-
MPO/cCD79a/cCD3	+/-/-	-/-/-	MPO/Ccd79a/cCD3	-/-/+
Tdt	+	+	Tdt	+
-			CD1a/CD5/CD3	-/+/-
-			α-β/sCD3	-/-
-			γ-δ/sCD3	-/-

underwent salvage chemotherapy with mitoxantrone (12 mg/m^2 i.v. days 1-3), cytarabine (500 mg/m^2 i.v. days 1-3 and 2 g/m^2/12h days 6-8) and etoposide (100 mg/12h i.v. days 6-8), and the bone marrow aspiration 4 weeks later showed complete remission. The patient received 2 additional cycles of consolidation chemotherapy. One month after the last cycle of consolidation (10 months from diagnosis) a bone marrow aspirate showed 32% atypical cells. Morphologically, the cells were of medium size, with 1 or 2 prominent nucleoli and a basophilic cytoplasm without granulation. The immunophenotype showed a population of blast cells that were positive for CD34, CD7, CD5, CD45, CD33, CD13 and cCD3 (Table 1). Cytogenetic studies revealed the karyotype 46,XX/ 47,XX,+8. Molecular studies showed IgH and T-cell receptor (TCR) (βγ) genes in the germline configuration at diagnosis and at relapse and the Bcr-abl and the MLL rearrangements were not detected. The patient received palliative therapy and died 30 days later.

Design and Methods

Immunophenotype

Leukemic cells were analyzed using a panel of monoclonal antibodies with three markers per tube and conjugated with fluorescein-isothiocyanate (FITC), phycoerythrin (PE) and phycoerythrin-cyanine 5 (Pe/Cy5) (Table 1). The samples were analyzed on a FACScan flow cytometer (Becton Dickinson Immunocytometry Systems, San José, CA, USA). Data acquisition and analysis were performed using Lysis II and PAINT-A-GATE-PRO softwares. Gating was carried out following forward and side scatter properties of the leukemic cells.

Cytogenetic analysis

Cytogenetic studies were undertaken on bone marrow cells after short-term culture without stimulation. Chromosomes were banded using G-banding and chromosomal abnormalities were described according to the ISCN.

Southern Blot

*Bam*HI and *Hind*III restriction enzymes (New England Biolabs, Mass., USA) were used to digest 5 mg of DNA. DNA was separated on a 0.7% agarose gel (Ecogen, Barcelona, Spain) and transferred to nylon membranes (Amersham Ltd., Buckinghamshire, UK) that were hybridized with probes labeled with ^{32}P-dCTP by the random primer extension method. Filters were washed in 0.2xSSC (NaCl/Na citrate)/ 0.5% sodium dodecyl sulphate (SDS) for 2 hours at 65ºC and then autoradiographed using intensifying screens.

The following probes were used for Southern blot analysis: a Cβ probe for the T-cell receptor genes (Dr. T.W. Mak, Ontario, Canada),[4] a pH60 probe which recognizes the Jl and J2 containing fragments from the T-cell γ constant region,[5] a Jh probe (Dr. T.H. Rabbitts, Cambridge) and a B859 probe spanning the breakpoint region of the MLL locus.[6] The Bcr-abl rearrangement was investigated using well-established protocols.[7]

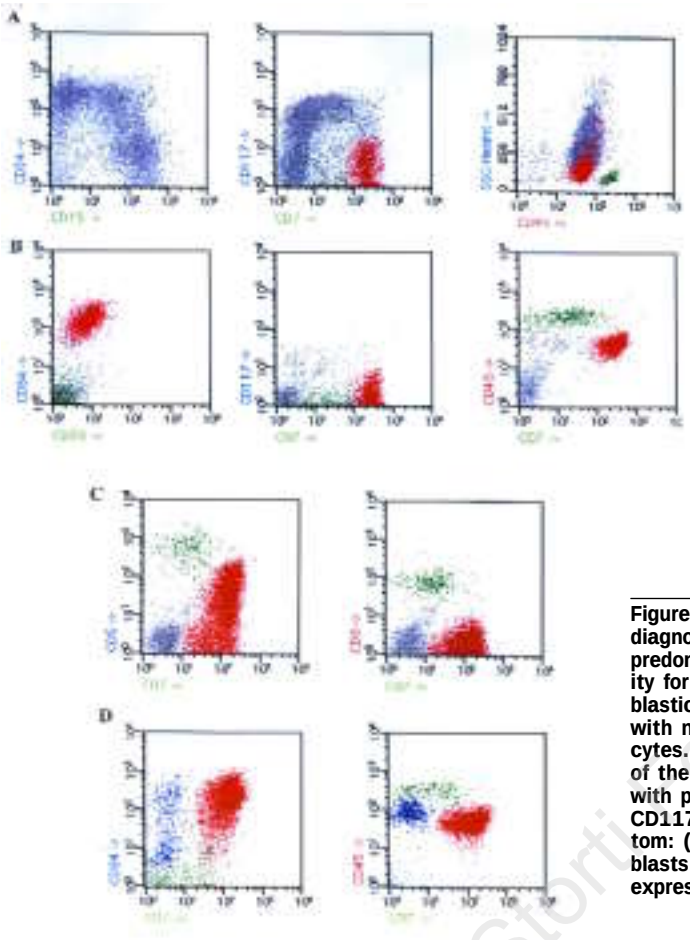

Figure 1. Top: (A). Detection of the blast population at diagnosis by multiparametric flow cytometry. In grey, predominant myeloblastic subclone (56%) with positivity for CD117 and CD45, and in red, minor T-lymphoblastic subclone (20%) which coexpressed CD7/CD45 with negativity for CD117. In green, normal lymphocytes. Middle: (B and C). The progressive appearance of the T-lymphoblastic population at relapse (in red) with positivity for CD34/CD7/CD5 and negativity for CD117 and sCD3. In green, normal lymphocytes. Bottom: (D). Overt relapse. Predominant T-lymphoblastic blasts and minor normal myeloblast population which expressed CD34/CD45 with negativity for CD7.

Results and Discussion

We present the case of a patient diagnosed with AML that relapsed as T-lineage ALL. This case could represent a biclonal leukemia which arose from the neoplastic transformation of a bipotential stem cell. This precursor cell would coexpress features of myeloid and lymphoid cells before being committed to a single cell lineage. A number of arguments support this hypothesis: 1) the different reactivity to some markers (CD117, CD7, CD5, cMPO, cCD3) clearly distinguishes two populations,[8] 2) the persistence of trisomy 8 at diagnosis and at relapse[9] and 3) the previously reported evidence of cells with $CD34^+$, $CD7^+$, $CD4^-$, $CD8^-$, $sCD3^-$ human phenotype able to differentiate as myeloid cells and T-lymphocytes.[10]

Hybrid or mixed leukemias can be due to the potential of leukemic cells to express characteristics of two lineages.[9] The clinical significance of hybrid leukemia is unclear,[3] and there is considerable confusion surrounding the prognostic significance of these leukemias.[1,3,9] Chromosomal translocations involving MLL and Bcr/abl rearrangements have been associated with hybrid leukemias.[3,11] AML with isolated trisomy 8 has been categorized in the group of leukemias having poor prognosis.[12] The bad outcome of patients carrying this chromosomal alteration may be associated with leukemogenesis in an early stem cell capable of multilineage differentiation.

A clonal selection occurred in our case: the same abnormal karyotype was found in the patient's myeloblasts at diagnosis and in her T-lymphoblasts at relapse. This observation suggests the presence of two subclones arising from the same progenitor.[11] Molecular studies did not suggest clonal evolution because all the loci investigated remained in the germline configuration at diagnosis and at relapse.

Rearranged Ig heavy-chain genes are demonstrated in about 17% of T-cell acute leukemias and in 11% of AML, especially in TdT positive leukemias.[1] Around 5% of AML and 29% and 56% of B-cell acute leukemias have rearranged TCR-β and TCR-γ genes, respectively.[1]

Some remarkable immunophenotypic features at diagnosis were the positivity for CD7 and TdT in both blast populations, suggesting that T-lymphoblasts could have the potential for myeloid

differentiation or that there could be a multipotential progenitor cell capable of both T-cell and myeloid maturation.[10,13] Acute leukemia with positivity for both markers has been studied separately from other leukemias.[14,15] In some reports, this type of leukemia has been associated with interstitial 9q deletion.[16,17] Patients with these leukemias show more chemoresistance and have a poor prognosis.[18] A biological explanation for this is that CD7+ is the most consistent marker associated with MDR1 expression in ALL and AML.[18] CD7 antigen appears early in progenitor cells within normal T-lymphocytic development, prior to entry into the thymus, suggesting that CD7+ hematopoietic cells are capable of pluri-potential differentiation.[10] There is now some evidence that the CD34+/CD7+ progenitor cells in bone marrow are able to transform into lymphocytes or myeloid cells.[10,13] In conclusion, multiparameter flow cytometry is a useful tool for identifying subclones in acute leukemias, although the clinical relevance of these findings is unknown.

Contributions and Acknowledgments

MB and JFN were responsible for the conception and discussion of this article. MJ Carnicer, ER supplied the immunophenotyping data. RM and JS provided the clinical information. OL performed the molecular studies. AA was responsible for the cytogenetic studies of this case.

Disclosures

Conflict of interest: none.

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