T-cell large granular lymphocyte leukemia transfomation into aggressive T-cell lymphoma: a report of two cases with molecular characterization

T-cell large granular lymphocyte (LGL) leukemia, a clonal expansion of cytotoxic CD3$^+$ T cells, represents 5% of lymphoproliferative disorders. Clinical features include neutropenia causing recurrent bacterial infections, anemia of diverse mechanisms, and autoimmune diseases such as rheumatoid arthritis. T-LGL leukemia is an indolent disease, and related deaths are mainly due to infections.[1] We describe two cases of clonal evolution of T-LGL leukemia into aggressive T-cell lymphoma.

In 2010, a 55-year-old Caucasian woman presented with isolated lymphocytosis at 8x10^9/l. Physical examination was normal. Circulating cells were typical LGLs with a CD3$^+$, CD4$^+$, CD8$^+$, CD5$^+$, CD7$^-$, CD56$^-$, CD16$^-$, CD57$^-$ phenotype with a clonal TCR-γ gene rearrangement. Two years later, she presented with fatigue, night sweats, hepatosplenomegaly and enlarged cervical and axillary lymph nodes. The blood cell count showed: leucocytes 132x10^9/l, neutrophils 17x10^9/l, platelets 314x10^9/l, hemoglobin 13.3g/dl. Tumor lysis syndrome and lactic acidosis were detected. Blood smear revealed a lymphocyte population made of large cells with abundant, mod-

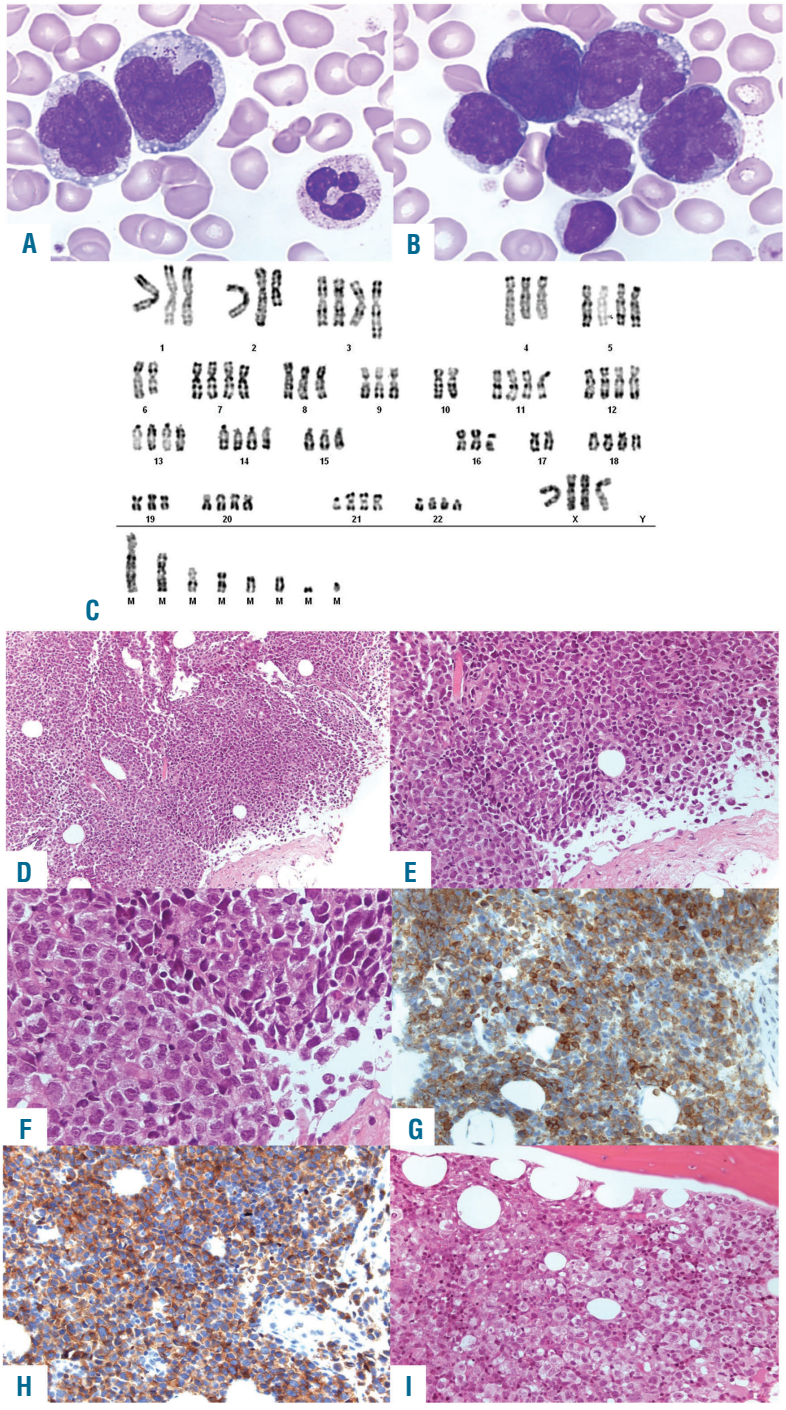

Figure 1. Cytologic, cytogenetic and pathological analysis of patient 1. (A-B) Blood smear revealing a lymphocyte population made of large cells with abundant, moderately basophilic cytoplasm containing granules, vacuoles and irregular nuclei (MGG, 100x). (C) Complex «near-tetraploid» karyotype (peripheral blood): 79~89,XXXX,-1,-2,add(2)(q23),-3,der(3)t(1;3)(q31;q27),-4,-6,-6,del(6)(q?q?),-8,-9,-10,-10,-15,-16,-17,-17,-19,i(21)(q10)x3,+7~14mar[cp16]. Ish 17p13.1(TP53,17qtel)x2,mar(TP53-,17qtel++)x3[8]. (D-E-F) Lymph node biopsy showing massive infiltration by medium- to large-sized cells with irregular pleomorphic nuclei, and abundant cytoplasm (H&E-safran 10x, 20x 40x). (G-H) By immunohistochemistry in the lymph node, tumor cells are CD3$^+$ and CD4$^+$ (SABC, 20x). I: The same tumor cell population was also found in the bone marrow (H&E-safran, 20x).

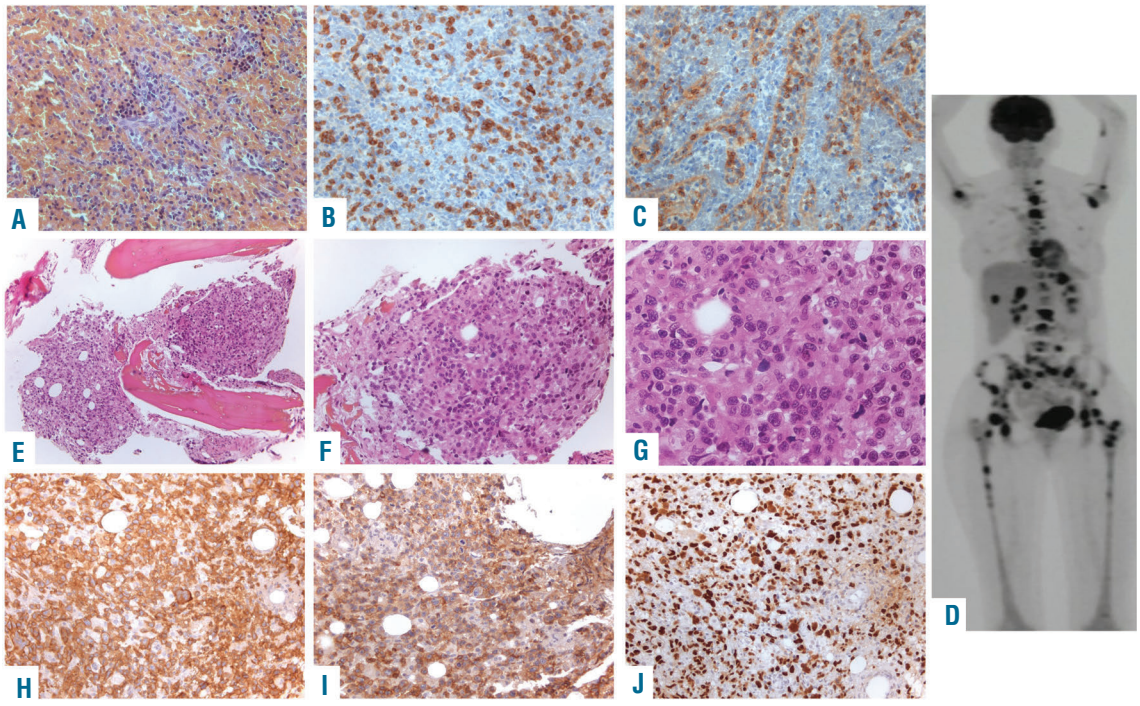

Figure 2. Cytologic, cytogenetic and pathological analysis of patient 2. (A) Splenectomy showed preserved architecture, with small- to medium-sized atypical lymphocytes infiltrating both cords and sinusoids (H&E-safran, 20x). (B) CD3 immunostaining highlights cells within splenic sinusoids (SABC, 20x). (C) Tumor cells in the spleen, as well as approximately 60% of T-LGL, are CD8-positive (SABC, 20x). (D) Multifocal hypermetabolic bone lesions on F-18 FDG PET/CT. (E-F-G) Spinal bone (L3) biopsy showing extensive infiltration by large cells with irregular nuclei, abundant, slightly basophilic cytoplasm, along with large anaplastic cells (H&E-safran 10x, 20x, 40x). (H-I-J) By immunohistochemistry, tumor cells in bone biopsy are $CD3^+$ (G) and $CD8^+$ (H) with a diffuse and intense nuclear expression of p53 (I) (SABC, 20x).

erately basophilic cytoplasm containing granules and vacuoles, and irregular nuclei (Figures 1A-B). Their phenotype was identical to T-cell LGL leukemia except for a weak CD3 expression. Blood karyotype at evolution was hyperploid and complex, and FISH analysis detected 17p deletion (Figure 1C). Lymph node biopsy showed massive and diffuse infiltration by medium to large-sized atypical cells with irregular and pleomorphic nuclei without nucleoli and abundant cytoplasm (Figures 1D-E-F). Tumor cells were $CD5^+$, $CD4^+$, $CD3^{+/-}$, $CD30^+$, granzyme B^+ and $TiA1^+$ (Figures 1G-H). An identical proliferation was found in the bone marrow (Figure 1I). The same clonal TCR rearrangement was detected, confirming that $CD4^+$ T-LGL leukemia had transformed into aggressive T-cell lymphoma. Two mutations were found in the *TP53* gene: the first (R282W in exon 8) was detected in a blood sample from 2010 with a variant allelic frequency (VAF) of 6% and was deleterious according to IARC TP53 database.[2] At evolution, R282W was only 1% whereas the second mutation (V157I in exon 5) was detected in blood samples in 2010 and at transformation, but its germline origin was confirmed by a skin biopsy. No mutation of *STAT3* or *STAT5B* genes was detected.

The patient received 2 cycles of CHOP, 3 cycles of CHOEP and one cycle of DHAX without success. She then achieved complete remission after one cycle of high-dose Methotrexate and L-Asparaginase which made it possible to perform an autologous stem cell transplant (ASCT) with BEAM conditioning, but disease progressed rapidly after ASCT and the patient died.

A 30-year-old Caucasian woman presented with isolated neutropenia in 2011 (neutrophils $0.76x10^9$/l and lymphocytes $2.8x10^9$/l). LGL were detected on blood smear, representing 61% ($1.7x10^9$/l) of total lymphocytes, with a $CD3^+$, $CD8^+$, $CD4^-$, weak CD5, partial CD56 and CD57 phenotype and clonal TCR-γ gene rearrangement which confirmed the diagnosis of $CD8^+$ T-LGL leukemia. There was no TP53 gene mutation, but a heterozygous *STAT3* mutation (Y640F in exon 21) was detected. Physical examination and blood biochemistry were normal.

In 2013, she presented with fatigue, fever, hepatosplenomegaly and pericarditis. Blood cell count showed: leucocytes $1.9x10^9$/l, neutrophils $0.34x10^9$/l, lymphocytes $0.78x10^9$/l, platelets $426x10^9$/l, hemoglobin 6.4g/dl. Intramedullary hemolytic anemia related to hemophagocytosis and disseminated intravascular coagulation (DIC) were present. A liver biopsy revealed lobular hepatitis without lymphoid infiltration. No viral infection was found to explain hemophagocytosis. She received two cycles of etoposide with corticosteroids and improved quickly but relapsed within a month. She then received etoposide and cyclophosphamide with intravenous gamma-globulin, allowing her condition to improve for a month before relapsing. Splenectomy, liver and bone marrow biopsies were performed. Splenic infiltrates accumulating in the sinusoids and cords with medium-sized T-lymphocytes infiltrating the red pulp were detected with a $CD3^+$, $CD5^+$, $CD8^+$, $CD7^-$, $TIA\text{-}1^+$, $CD57^+$ phenotype (Figures 2A-B-C). Bone marrow biopsy showed weak interstitial and intra-vascular infiltration by the same clonal LGL population. This third episode was treated with etoposide, cyclophosphamide and corticosteroids, but the patient relapsed three months later. F-18 FDG PET/CT imaging showed hypermetabolic lesions on

L3 vertebra, left rib, sternum and iliac crest (Figure 2D). L3 vertebra biopsy showed extensive infiltration by large cells with irregular nuclei, abundant slightly basophilic cytoplasm and large anaplastic cells (Figures 2E-F-G). Their phenotype was CD3$^+$, CD7$^+$, CD8$^+$, CD30$^+$, TiA-1$^+$, Granzyme B$^+$, P53$^+$, CD2$^{+/-}$, CD56$^+$, CD57$^+$, CD4$^-$, CD20$^-$, CD79a$^-$, ALK$^-$ (Figure 2H-I-G). The same clonal TCR rearrangement was confirmed. *TP53* gene analysis detected a deleterious *P151T* mutation in exon 5 (VAF 85%) which was not detected at diagnosis. *STAT3* gene analysis revealed that the heterozygous mutation detected in 2011 had turned into a homozygous one at transformation. An additional mutation in BCOR was found (p.G599fs, VAF 57%).

The patient received one cure of Brentuximab-Vedotin (BV)-CHEP and one cure of BV-CHOEP, but disease progressed. Treatment was changed to BV-DHAX with etoposide and infliximab because of recurrent episodes of DIC and hemophagocytosis, but the patient died of disease progression.

T-cell LGL leukemia is an indolent disease with survival rates around 70% at 10 years.[1] Very rare cases of aggressive forms of T-cell LGL leukemia have been reported.[3,4]

Whilst the median age at diagnosis of T-LGL leukemia is 60,[1] our patients were 55 and 30 years old, respectively. In the transformed cases published, age at diagnosis of aggressive forms is around 41 years,[5] which is concordant with our experience. The sex ratio, which is 1:1 in T-cell LGL leukemia, may be 2:1 in favor of women in aggressive forms, and our patients were female.

In our cases, demonstration of clonal evolution of T-LGL leukemia in aggressive lymphoma came from the phenotypic conservation of the cell of origin: CD4$^+$, CD8$^+$ in patient 1, which is an uncommon phenotype, and CD8$^+$ in patient 2 expressing the cytotoxic markers granzyme B and TiA1, but essentially from a common clonal TCR-gamma rearrangement. Previously reported cases generally describe: appearance of acute B signs, rapidly enlarging lymph-nodes, infiltration of amygdales and subcutaneous adipose tissues, cytopenia due to bone marrow infiltration, hepatosplenomegaly in which the architecture of the spleen and the liver with persistent infiltration by small T cells of the LGL clone is usually conserved.

In most reported cases, the morphologic aspect does not differ from that observed in peripheral T-cell lymphomas.[6] In NOS T-cell lymphoma,[7] malignant cells are large pleomorphic cells with irregular nuclei, immature chromatin and often a well-defined nucleolus associated with abundant and clear cytoplasm with detection of cells in mitosis. Similar morphologic aspects were present in our cases.

Concerning the biological characteristics associated with transformation, only cytogenetic analysis was previously reported in one case with complex cytogenetics.[8] In patient 1, complex karyotype, near tetraploid, with del 17p deletion was detected. No molecular study has previously been reported. It is suggested that T-cell LGL leukemia arises from antigenic stimulation of effector memory cytotoxic T-cells leading to up-regulation of the JAK-STAT3 signaling pathway that drives sub-clone selection with acquired *STAT3* mutations, which are reported in 28-75% of LGL leukemia.[1] In 2% of cases, *STAT5b* mutations are found.[9] In patient 1, no mutation in the *STAT3* or *STAT5b* genes was detected, whereas patient 2 had a heterozygous *STAT3* mutation which turned into a homozygous mutation at transformation. The biological consequence of this evolution is unknown. We also detected acquired mutations in the *TP53* gene that caused protein inactivation: R282W[10] in exon 8 since diagnosis in patient 1, and P151T in exon 5 in patient 2 only at transformation. In patient 1, complete loss of the p53 pathway was present at the stage of aggressive T-cell lymphoma. The role of the mutations in the process of transformation is unknown, but they probably participated in chemo-resistance and chromosomal instability. For patient 2, a *BCOR* mutation was present at transformation. It is known that 50% of mice missing BCOR (exon 4 deletion) develop T-cell acute lymphoblastic leukemia (T-ALL) providing evidence of a tumor suppressor role for BCOR in T-cell malignancies, and cooperation between BCOR mutation and inactive *TP53* allele in the development of T-ALL was documented.[11] Furthermore, in a cohort of extra-nodal NK/T-cell lymphoma, nasal type, *BCOR* was the second most frequent mutated gene after *STAT3*, suggesting a role of BCOR mutation in lymphoma pathogenesis together with alteration of the JAK/STAT cascade.[12] The T-cell lymphoma was refractory to almost all chemotherapy regimens in our 2 patients. In patient 1, complete remission was only obtained after one course of Methotrexate and L-Asparaginase, suggesting that tumor survival was dependent on asparagine and/or glutamine metabolism. In patient 2, all treatments were ineffective, including combination of chemotherapy to BV currently used in peripheral CD30$^+$ T-cell lymphomas with encouraging results.[13] From these 2 cases and the few already published, we cannot propose a strategy of first-line treatment. Even high dose chemotherapy and auto-grafting in patient 1 was unsuccessful. Alternatively, chemotherapy induction regimens used in T-cell ALL were recommended to propose allografting in younger patients.[14] Modern immunotherapy with CAR-T cells may be more efficient, but the antigens to target need to be identified. Alternatively, the rationale for testing compounds such as APR-246, which can restore function and transcriptional activity of p53 mutated proteins, may be high.[15]

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