A case of adult T-cell leukemia and lymphoma in an Italian woman showing different malignant clones in tumor mass and in blood

Human T-lymphotropic virus (HTLV)-1 infections and their associated diseases are very rare in Italy, as indded they are in most parts of Europe, occurring prevalently in subjects in endemic areas. The HTLV-1-associated leukemia/lymphoma, ATLL, is a very aggressive T-cell non-Hodgkin's lymphoma which can be difficult to recognize in non-endemic areas. Here we describe the case of an elderly Italian woman, with no apparent risk factors, affected by a rapidly fatal ATLL who presented with an abdominal lymphomatous mass and circulating leukemic cells. The simultaneous presence of different T-cell clones in the tumor mass and in the blood was demonstrated by T-cell receptor gene rearrangement analysis and HTLV-1 integration pattern studies. After surgery, all the T-cell clones were present in the blood, indicating that tumor cells had spread from the mass. Phylogenetic analysis, using the complete LTR sequence, showed that the patient's HTLV-1 isolate belongs to the cosmopolitan subtype A.

Haematologica 2007; 88:(8)e101-e103

Adult T-cell leukemia/lymphoma (ATLL) is etiologically linked to human T lymphotropic virus type 1 (HTLV-1); less than 5% of infected individuals develop the disease after a latency period of several decades.[1,2] ATLL occurrence reflects the geographical distribution of the virus (i.e. Japan, the Caribbean basin, Melanesia, Africa, North/South America, and the Middle East), but sporadic cases have also been described in non-endemic areas.[3,4] These cases most often occur in immigrants from endemic areas or in individuals with relations to endemic areas, and only exceptionally in Caucasian patients with no apparent risk factors for HTLV-1 infection. ATLL can manifest as an acute form or lymphoma (highly aggressive and very difficult to treat), or as sub-acute/smoldering or chronic forms, but its diagnosis can be a difficult task in non-endemic areas. Here we present the case of an HTLV-1-positive ATLL occurring in an Italian woman without relation to endemic areas. An 85-year old, Caucasian woman complaining of abdominal pain was admitted to Padova City Hospital on February 15, 2001. Physical examination disclosed a large firm painful mass in the lower left abdominal quadrant. No lymphadenopathy, splenomegaly or hepatomegaly was recorded, and no skin lesions were present. A computerized tomography scan showed a 10x12 cm inhomogeneous mass in the left iliac cavity, as well as a few small masses in the para-aortic region that were probably enlarged lymph nodes. A chest X-ray was normal. Blood work-ups over the following two weeks showed a lymphocyte increase from 3.71 to 5.50x10^9/L, while flow cytometry analysis disclosed an increase in CD3+ lymphocytes, with a CD4/CD8 ratio of 2.95, and HLA-DR expression by 80% of the CD4+ cells. A blood smear showed atypical cells, characterized by a pleomorphic nucleus resembling the *flower cells* described in ATLL. *Bone marrow biopsy was not performed.* On March 15, the patient underwent surgery; the abdominal mass caused stenosis of the descending colon and also involved the retroperitoneum, aorta, and sigmoid colon. The omentum was entirely resected, and the tumor mass was biopsied. Histological examination of the tumor sample revealed a non-Hodgkin's lymphoma, with a CD2+/CD3+/CD4+ immunophenotype on immunohistochemistry. Neoplastic cells were also present in the ascitic fluid collected during surgery. A blood sample obtained on March 26 showed that serum Ca levels had increased from 9.5 mg/dL at admission to 10.9 mg/dL. On March 27, twelve days after surgery, the patient died of heart failure. The diagnosis of ATLL was confirmed by HTLV-1 analyses. A blood sample collected on March 7 exhibited strong Western blot reactivity against native (p19, gp21, p24, p26, p28, p32, p36, gp46, p53) and genetically engineered (GD21, rgp46-I) HTLV-1 proteins (HTLV BLOT 2.4; Genelabs). HTLV-1 sequences, amplified as previously reported,[4] in peripheral blood mononuclear cells were detected (PBMC) from the March 7 sample, in the tumor mass biopsied on March 15, and in the PBMC collected on March 26 (Figure 1). PCR findings indicated that three different T cell clones had developed independently. The rearranged T-cell receptor gamma (TCRγ) gene was amplified using consensus primers TVγ and TJγ under the conditions described by Benhattar et al.[5] One T-cell clone (indicated with * in Figure 1), likely characterized by rearrangement of both alleles (as suggested by the equimolar intensity of the two bands), was found in the March 7 PBMC sample, and two different distinct clones (one less represented than the other, and indicated with ** and ***, respectively, in Figure 1) were found in the tumor mass. Interestingly, TCR rearrangement analyses of the March 26 PBMC sample showed the presence of all the different clones, indicating that cells from the tumor mass had spread to the peripheral blood compartment (Figure 1). To analyze the integration pattern of HTLV-1, high-molecular weight DNA extracted from the March 26 PBMCs were digested by *Eco RI* restriction enzyme, which does not cut within the HTLV-1 provirus, and then hybridized with a probe representing the entire HTLV-1 genome (pMT2). This analysis disclosed two distinct bands greater than 9 kb (about 20 and 23 kb, Figure 2), thus suggesting the clonal integration of a single complete HTLV-1 genome in the two most represented T-cell clones (indicated with * and ** in Figure 1). A clonal change in patients with ATLL has been previously described at the time of crisis;[6] it is possible that our patient had been affected by a sub-acute/smoldering form for some time and came to clinical attention only at the time of the crisis. The precise mechanism of cell neoplastic transformation by HTLV-1 remains unclear, and it has been suggested that Epstein-Barr virus (EBV) might play a role in the *multi-hit* transformation process.[7,8] EBV

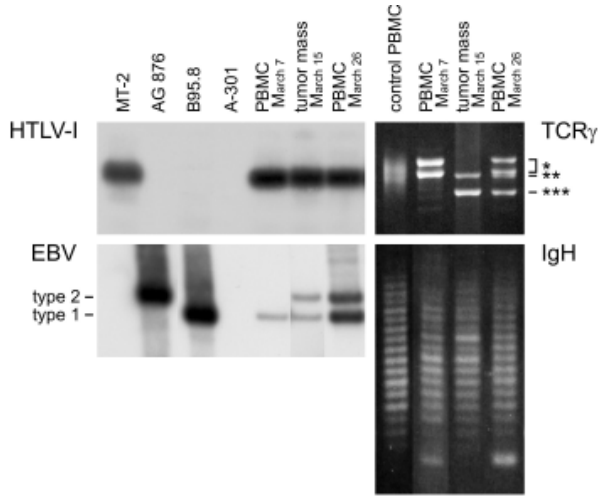

Figure 1. Molecular analysis of HTLV-1 DNA, EBV DNA, TCR? and IgH rearrangements.HTLV-1 detection was performed by PCR using DNA extracted from fresh peripheral blood mononuclear cells (PBMC) and from paraffin-embedded tumor mass, and primers specific for the Tax/Rex region, which anneal to positions 7344-7364 and 7455-7474 of the HTLV-1 ATK isolate. The amplification product was electrophoresed on agarose gel, transferred to filter, and hybridized with an oligonucleotide probe specific for the HTLV-1 sequence 7399-7419. DNA from MT-2 cells was used as the positive control. EBV DNA was investigated and typed using primers common to EBNA2 sequences of types 1 and 2 viruses, which amplify a type 1 fragment of 168 bp from B95.8 prototype virus and a type 2 fragment of 184 bp from the AG876 isolate. The PCR product was electrophoresed on a polyacrylamide gel, electrotransferred onto nylon filters, and hybridized with an internal probe common to both viral types. DNA from A-301 cells was used as the negative control for HTLV-1 and EBV detection. The analysis of TCR_ gene was performed using consensus primers which amplify almost all TCR_ gene rearrangements found in lymphoblastoid cells and generate a PCR product of ~160-190 bp5; the PCR product was electrophoresed on a polyacrylamide denaturing gel, and stained with ethidium bromide (*, **, *** indicate the three different T-cell clones detected). IgH gene rearrangements were analyzed by semi-nested PCR using the consensus primers Fr3A (outer/inner, forward), LJH (outer, reverse), and VLJH (inner, reverse), as previously described9. PBMC from a healthy donor were used as control of polyclonal pattern for TCR_ and IgH gene rearrangements.

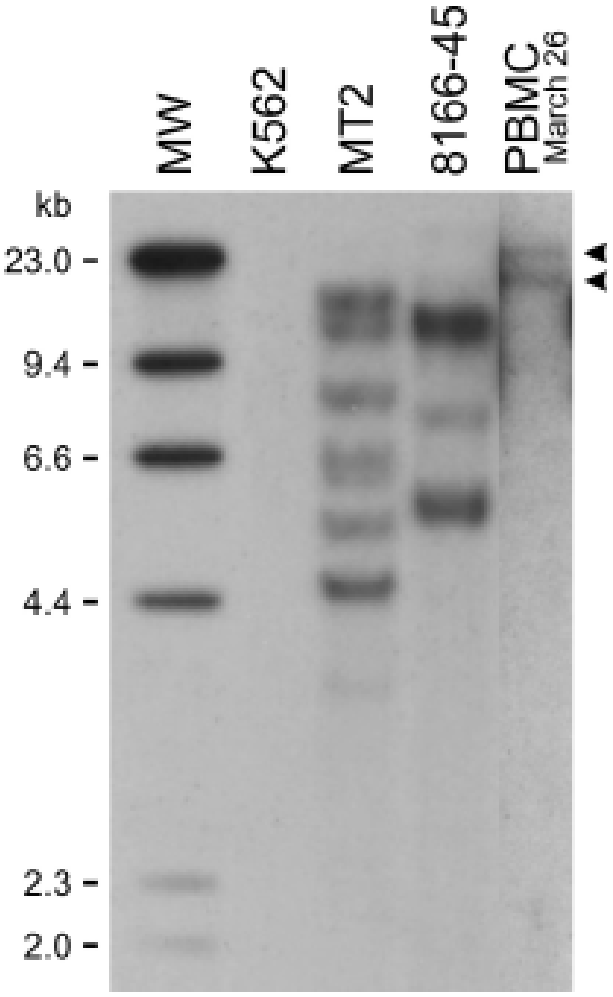

Figure 2. Detection of proviral HTLV-1 integration by Southern blot analysis. DNA was digested by Eco RI, subjected to agarose gel electrophoresis, transferred to nylon membrane, and hybridized with the entire HTLV-1 genome probe (pMT2). DNA from K562 cell line was used as negative control. The positive controls were DNA from MT-2 and 8166-45 cell lines. Two bands larger than 9 kb (about 20 and 23 Kb, arrowheads) were detected in PBMC from the patient.

sequences have been detected in 22% (21/96) of ATLL cases in Japan.[8] Molecular analysis for EBV sequences, performed as previously reported,[9] showed a small amount of EBV type 1 DNA in the March 7 PBMCs, and the presence of EBV types 1 and 2 in the tumor mass and in the March 26 PBMC sample (Figure 1). The increased level of EBV in the March 26 blood sample appeared not to be sustained by a clonal proliferation of B cells, since the semi-nested PCR for immunoglobulin heavy chain gene (IgH), performed as previously described,[9] disclosed a normal B polyclonal pattern in all the samples analyzed (Figure 1). Since an increase in EBV DNA in PBMC has been demonstrated in HTLV-1-infected patients under 70 years of age, as well as in infected and non-infected older patients,[10] the EBV infection and/or reactivation and the simultaneous presence of both types in our patient might have resulted from an impaired cell-mediated immunity due to HTLV-1 infection and old age, and might have contributed to the rapid progression. Since HTLV-1 infection is almost absent in Italy,[3] we were interested in understanding the origin of the HTLV-1 infection in our patient. She was widowed and lived alone, and was unfortunately, very deaf and illiterate, so it was impossible to interview her or other close relatives. She was born in Puglia (Southeast Italy) but had lived most of her life in Padova (Northeast Italy). No close family members were alive and no other relatives could be traced to screen for HTLV-1 infection. Her birthplace is indeed intriguing, since serological HTLV reactivity, whose significance is still uncertain, has been observed in about 2% of blood donors from Puglia,[3] and a familial clustering of HTLV-1 infection has been described.[11] The molecular epidemiology of HTLV-1 proviruses has shown that little variation exists among strains and that the few nucleotide changes observed are specific for the geographical origins of the patients; six major geographical subtypes, A-F, have been defined thus far.[12,13] To characterize the HTLV-1 subtype,

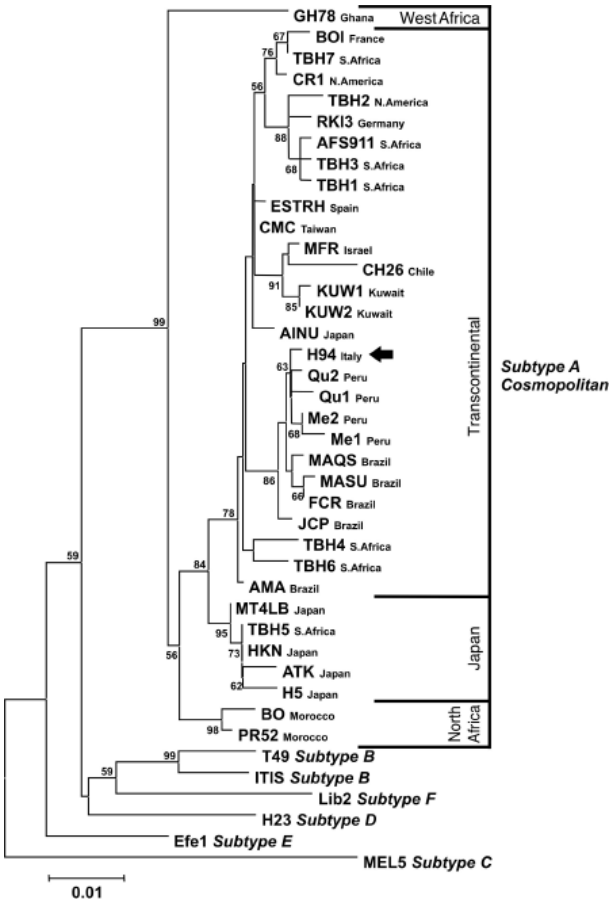

Figure 3. HTLV-1 phylogenetic tree.Analysis of the complete LTR region was performed by two semi-nested PCRs, as described by Mahieux et al12; the purified PCR products were sequenced in an ABI PRISM 377 according to manufacturer's instructions, using primers 8255, 420LTR, TATAbox and 5PLTR. The tree was constructed by the neighbor-joining method using the MEGA program and 1000 replicates (bootstrap) in order to test the reliability of the final tree topology. *H94 Italy* indicates the HTLV-1 isolate from the tumor mass of the patient (Gene Bank accession number AY223517).

sequencing of the complete LTR was carried out as described by Mahieux *et al.,*[12] and nucleotide sequences were aligned with reference nucleotide sequences of HTLV-1 subtypes (A-F). On the basis of the complete sequence of the HTLV-1 LTR region, the neighbor joining tree identified the isolate of our patient as HTLV-1 cosmopolitan subtype A with a 99% bootstrap value (Figure 3). Within this subtype, our case is included with 78% bootstrap value in the transcontinental subgroup, which comprises isolates from patients originating from different geographical areas (Latin America, Japan, the United States, the Middle East); however, when and how HTLV-1 infection occured in our patient remains an unresolved question. In conclusion, although ATLL is very rare in non-endemic areas - to our knowledge, only two other ATLL cases have been described in Italian Caucasians[11,14] it should be considered when a T-cell non Hodgkin's lymphoma presents features such as *flower cells* in the peripheral blood or hypercalcemia, even if the patient is not linked to known risk factors, and HTLV-1 infection analyses should therefore be included in the differential diagnosis.

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Key Words: HTLV-1, ATLL, Italy

Acknowledgments: This work was supported in part by AIRC (Associazione Italiana Ricerca sul Cancro) and FIRC (Federazione Italiana Ricerca sul Cancro).

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