

LARGE GRANULAR LYMPHOCYTE LEUKEMIA ASSOCIATED WITH HEPATOCELLULAR CARCINOMA: A CASE REPORT

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ABSTRACT

The association of large granular lymphocyte leukemia (LGL-L) with hepatocellular carcinoma in a 55-year-old patient is described. An increased number of LGL was seen on peripheral blood smears. The immunophenotype was CD3⁺, CD4⁺, CD8⁺, and a study of the TCR gene rearrangement indicated the monoclonal nature of the proliferation. A liver mass was detected on CT scan and an ultrasound-guided fine needle biopsy revealed the presence of hepatocholangiocellular elements. A right hepatectomy was performed. Major neutropenia persisted despite corticosteroids and granulocyte colony-stimulating growth factor (G-CSF) therapy. Methotrexate at 20 mg/week failed to control lymphocytosis after three months of treatment. A new nodule of hepatocarcinoma reappeared twelve months after surgery and a liver resection was performed.

Key words: large granular lymphocyte, leukemia, T-lymphocytes, neutropenia, hepatoma, hepatectomy.

Large granular lymphocyte leukemia (LGL-L) is a rare hematologic disease. The majority of the 150 patients described in a recent review¹ exhibited a clonally expanded LGL population with a CD3⁺, CD8⁺ phenotype. The association of LGL-L with rheumatoid arthritis is well established; it occurs in 20-30% of patients in Western countries.¹ Furthermore, this form of leukemia has been found to be associated with other diseases such as pure red blood cell aplasia, idiopathic thrombocytopenic purpura, chronic infections, autoimmune diseases, and immunodeficiencies.²⁻⁴ The association of LGL-L with solid neoplasms is rare^{2,5-7} and its concomitant occurrence in primary hepatic neoplasms has, to our knowledge, never been described in the English language literature.

Case report

PN, a 55-year-old Caucasian woman, was admitted for suspected cholecystitis. She had been complaining since March 1994 of weight

loss (about 8 kg) and, since October 1994, of general fatigue and intermittent-remittent fever, apparently related to dental granulomas.

Physical examination revealed a 5 cm painless mass in the right hypochondrium. Peripheral blood smear, stained with May Grünwald-Giemsa, showed an increased number of LGL with pale cytoplasm and fine prominent azurophilic granules. A bone marrow biopsy was stained using the hematoxylin-eosin, Giemsa, Gomori, Pearls, Pas methods. An immunohistochemical study was performed with CD3 and CD45 (leukocyte common antigen) monoclonal antibodies. A moderate interstitial bone marrow infiltration by CD3⁺ and CD 45⁺ lymphoid cells and a reduction of myeloid precursors were revealed. Results of laboratory investigations and immunophenotypic analysis are reported in Table 1. A diagnosis of LGL-L was suspected on the basis of the results of morphologic and immunophenotype studies. A study of TCR gene rearrangement was performed by PCR amplification of the

Table 1. Immunophenotypic features of peripheral blood mononuclear cells in the patient studied (WBC $3.9 \times 10^9/L$, neutrophils $0.4 \times 10^9/L$, lymphocytes $3.0 \times 10^9/L$, LGL $2.0 \times 10^9/L$).

CD3 = 82%	(n.v. 70-80%)
CD4 = 16%	(n.v. 32-55%)
CD8 = 67%	(n.v. 20-37%)
CD19 = 4%	(n.v. 5-15%)
CD16 = 42%	(n.v. 5-22%)

junctional region (VJ) of the genes encoding the γ -chain. A monoclonal TCR rearrangement was detected, indicating the clonal nature of the LGL proliferation. Further laboratory investigations excluded rheumatoid factor, cryoglobulins and antinuclear antibodies. Antibodies against neutrophils were detected by a cytofluorographic method. Antibodies against hepatitis A, B and C viruses, human immunodeficiency virus (HIV), Epstein Barr virus and HTLV-1 were absent. Abdominal ultrasonography showed a solid nodule occupying segments 5 and 6 of the liver and splenomegaly. Total body CT scan confirmed the presence of a low-density mass occupying segments 5 and 6 of the liver. No lymph node enlargement was detected. Ultrasound-guided fine needle biopsy of the liver revealed the presence of cells with PAS-positive cytoplasm, a finding compatible with a diagnosis of biliary carcinoma. Chest X-ray, bone scintigraphy, upper and lower gastrointestinal endoscopy showed no abnormalities.

A right hepatectomy was performed on December 5, 1994. A margin of more than 2 cm of normal liver surrounded the mass. The neoplasm was classified as expansive uninodular according to Nakashima.⁸ No satellite nodules were present. Histologic examination revealed the presence of a hepatocarcinoma showing mixed hepatocholangiocellular elements with a large sclerotic component. The carcinoma tended to invade the intrahepatic bile duct wall with the formation of intraluminal neoplastic emboli up to the segmental ducts. Neoplastic emboli were also present in portal vessels in proximity

to the mass. The liver tissue around the tumor was normal. The postoperative course was complicated by hemoperitoneum, which required re-laparotomy. No active bleeding sources were found. Three days after re-laparotomy a continuous fever appeared. Multiple cultures of blood, urine and sputum were negative. Fever disappeared three weeks later following broad spectrum antibiotic therapy. Corticosteroids and granulocyte colony-stimulating factor (G-CSF) at 300 IU daily did not improve neutropenia. The patient recovered completely and resumed her habitual activities. The patient took methotrexate, 20 mg a week for three months, in an attempt to control the abnormal LGL proliferation, without apparent success. At 12 months after surgery, total WBC were $2.16 \times 10^9/L$, neutrophils $0.15 \times 10^9/L$ and lymphocytes $1.52 \times 10^9/L$. RBC were $4.55 \times 10^{12}/L$, Hb 13.6 g/dL and platelets were $157 \times 10^9/L$. LGL were still present in peripheral blood smears. Liver laboratory tests were normal. Ultrasonography and CT scan showed a regenerated liver with a single nodule in segment 2. Fine-needle biopsy confirmed the presence of hepatocellular carcinoma. The patient was re-hepatectomized 13 months after the first procedure without post-operative complications.

Discussion

This study reports on the first patient showing the association of a monoclonal proliferation of LGL expressing the CD3 and CD8 immunophenotype with hepatocellular carcinoma. A similar association with other neoplasms is known, the incidence of which ranges from 0 to 7%.^{5-7,9} Most of these tumors are of hematologic origin (non-Hodgkin's lymphomas, acute myeloid leukemia, hairy cell leukemia), while a minority of them are epithelial neoplasms (lung, gastrointestinal tract, prostate).

The prevalence of LGL-L and hepatocellular carcinoma in its mixed cholangiocellular variant are quite rare in Western populations.

Two hypotheses might explain this association: 1) LGL proliferation might occur as a reaction to the presence of the hepatic tumor;

2) LGL proliferation and hepatocarcinoma might be two independent entities. The latter hypothesis seems more likely since LGL lymphocytosis did not subside following removal of hepatocarcinoma, as had been observed by others,¹⁰ and the resected tumor showed no LGL infiltration. Moreover, the LGL proliferation in the present case was monoclonal, whereas polyclonality is usually found in reactive proliferations.

As a consequence of neutropenia, recurrent bacterial infections are frequent and are the main cause of death in patients with LGL-L. As previously described by others,¹¹ neutropenia can be caused by the production of specific antibodies against neutrophils. Both G-CSF and corticosteroids failed to ameliorate this neutropenia. As recently proposed,¹² low-dose methotrexate was administered to our patient. This therapy, reported to be effective in nearly 50% of patients, failed to reduce lymphocytosis after three months of treatment in our patient.

Despite major neutropenia, no overt clinical infections developed in our patient apart from fever of unknown origin subsequent to reoperation. Although neutropenia persists after discharge, the patient is well, without fever. This seems to suggest that neutropenia *per se* should not be considered an absolute contraindication to surgery, provided that broad spectrum antibiotic therapy is administered in the perioperative period. Unfortunately, few details are available on the outcome of other patients with

LGL-L treated surgically for associated secondary neoplasms.

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