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# Hairy cell leukemia – a rare type of leukemia. A retrospective study on 39 patients

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## Abstract

Hairy cell leukemia (HCL) is a chronic B-cell lymphoid leukemia characterized by pancytopenia, splenomegaly, myelofibrosis and the presence in peripheral blood, bone marrow and spleen of atypical lymphoid cells with a hairy aspect. This is a retrospective analysis of 39 patients hospitalized in the Clinic of Hematology, "Filantropia" Municipal Hospital, Craiova, Romania, between 1997–2012, devised by age, sex, and HCL type. Characteristic features of diagnosis (including clinical features, laboratory data: complete blood cell count, differential count, peripheral blood and bone marrow infiltration with atypical lymphoid cells with cytoplasm fine prolongations, immunophenotyping of peripheral blood, bone marrow, spleen or lymph node biopsies with histopathological exams and immunohistochemistry), types of therapy (focused on IFN-α), complications (infections, hemorrhage, autoimmune, second malignancies) and survival rate were monitored. Conclusions of the study revealed the importance of histopathology and immunohistochemistry for diagnosis, of the therapeutic options in the absence of purine nucleoside analogues, the most frequent complications and the decrease of their incidence correlated with therapy and increased count of neutrophils.

***Keywords***: hairy cells, neutropenia, myelofibrosis, interferon-alpha.

## ⊡ Introduction

Hairy cell leukemia (HCL) is an uncommon B-cell lymphoproliferative disease characterized by pancytopenia, neutropenia, splenomegaly, myelofibrosis and the presence in peripheral blood, bone marrow and spleen of atypical lymphoid cells with irregular cytoplasm projections and unique immunophenotypic features. It is a rare disease (2% of leukemias in adults), more frequent in men than in women (4:1 ratio), appearing at around 50-year-old.

Hairy cells are transformed B-cells originating in the marginal zone of splenic white pulp and lymph node sinuses and release cytokines (IL6, TNF) that inhibit normal hematopoiesis and promote bone marrow fibrosis. TNF increases DNA synthesis in purified hairy cells *in vitro*, mediated by IL6 in an intracytoplasmatic mechanism [1]. Other studies reveal that TNF-α stimulates cell growth [2, 3]. Hairy cells express phosphorylated MEK and ERK, indicating a constitutive activation of the RAF-MEK-ERK mitogen-activated protein kinase pathway in HCL. Phosphorylated MEK and ERK are the downstream targets of the BRAF-kinase. The BRAFV600E mutation (oncogenic in many other neoplasms) has a high frequency in HCL constitutively activates the MEK-ERK pathway, involved in cell survival, differentiation and proliferation [4–6].

Clinical features are represented by splenomegaly ± hepatomegaly, pallor, fatigue, weight loss, infections, hemorrhage, rarely lymphadenopathy; in 10% of cases, the disease is asymptomatic.

Laboratory data show a moderate or severe pancytopenia. Peripheral blood smear reveals hairy cells with characteristic morphological features: large lymphoid cells, 10–15 μm

in diameter, with central or eccentric position of round, oval or indented nuclei, reticular or netlike chromatin pattern, with indistinct or absent nucleoli. The pale blue cytoplasm presents fine, hair-like projections or ruffled borders. Frequently, the hairy cells stain positively for tartrate resistant acid phosphatase (TRAP) [7].

Immunophenotyping in HCL shows characteristic surface markers: M-rosette+, SmIg++, CD5-, CD23-, FMC7++, B-ly-7++, CD25+, LeuM5+, HC2+, CD11c+, L30+.

Bone marrow trephine biopsy usually demonstrates a hypercellular bone marrow, diffuse or focal infiltration by hairy cells and a dense reticulin fiber pattern (promoted especially by direct fibronectin secretion of the hairy cells). Residual hematopoiesis is usually decreased, especially for granulocytes. Extravasated red cells and blood lakes may also be present. The spleen presents a distinctive pattern of diffuse red pulp infiltration by hairy cells with atrophy or replacement of white pulp. The liver reveals both sinusoidal and portal infiltration by hairy cells.

Two thirds of patients present clonal cytogenetic abnormalities, more frequently including chromosomes 1, 2, 5, 6, 11, 19, 20. In 40% of cases, chromosome 5 is involved as trisomy, interstitial deletions of band 5q13, pericentric inversions [8, 9].

Diagnosis of HCL is based on the presence of typical hairy cells associated with splenomegaly, neutropenia, myelofibrosis. Differential diagnosis should be made with splenic lymphomas, chronic lymphocytic leukemia, aplastic anemia, idiopathic myelofibrosis. HCL is a slowly progressing disease with a median survival of over five years. The most frequent complications are infections, autoimmune complications, hemorrhage, second malignancy. Treatment

includes purine nucleoside analogues, anti-CD20 monoclonal antibody, interferon-alpha, splenectomy.

### Aim of study

The study aims to evaluate a group of 39 patients with hairy cell leukemia, hospitalized in the Clinic of Hematology, "Filantropia" Municipal Hospital, Craiova, Romania, on a period of 15 years, correlated with particular features of diagnosis, types of therapy, evolution, complications and survival rate.

### ⊟ Patients and Methods

This is a retrospective analysis of 39 patients hospitalized in the Clinic of Hematology, "Filantropia" Municipal Hospital, Craiova, between 1997–2012, devised by age, sex, type of HCL. Clinical features at diagnosis (splenomegaly ± hepatomegaly, pallor, fatigue, weight loss, infections, hemorrhage), complete blood cell count, differential count, peripheral blood (determined with a Cobas C311 analyzer) and bone marrow infiltration with atypical lymphoid cells with cytoplasmic fine prolongations (Hematoxylin–Eosin stain sections, IHC stains for CD20+ and for DBA44), immunophenotyping of peripheral blood or bone marrow aspirate, hepatic and renal tests, abdominal CT, spleen or lymph node biopsy (Hematoxylin–Eosin stain sections) in some cases, types of therapy, complications and survival rate were monitored.

### ⊟ Results

The median age of patients with HCL was of 52 years with a high frequency in men (M:F=3:1). Approximately 70% of them had splenomegaly at diagnosis (moderate splenomegaly in 23 cases and tumoral splenomegaly in four cases), hepatomegaly in 15.4% of cases; most patients presented symptoms related to neutropenia, anemia, thrombocytopenia, data corresponding to literature [7]. Pancytopenia was present in 61.5% of patients at diagnosis, more than in literature data (50%). Median hemoglobin value was of 10.3 g/dL, leukocytes $2.4 \times 10^9$/L, absolute neutrophil count $0.82 \times 10^9$/L, platelets $83 \times 10^9$/L; abnormal hepatic transaminases (15.4%), azotemia (19.3%), hyper-gammaglobulinemia (11.5%). On the peripheral blood smear, the number of circulating hairy cells was variable, usually low (Figure 1). Immunophenotyping showed characteristic surface markers: B-cell markers (CD19, CD20, CD22, CD79a) and activated cell markers (CD11c, CD25, CD38). The bone marrow was hypercellular in 32 (82%) patients (Figure 2) and hypocellular in seven (18%) patients (Figure 3). Infiltration with hairy cell was focal or diffuse, with characteristic halo of cytoplasm confirmed by immuno-histochemistry with anti-CD20/DBA44 (Figures 4 and 5). Lymph node biopsy was made in two cases (Figure 6) and revealed parafollicular infiltration with hairy cells; spleen biopsy was made in five cases and showed diffuse infiltration of red pulp with hairy cells (Figure 7).
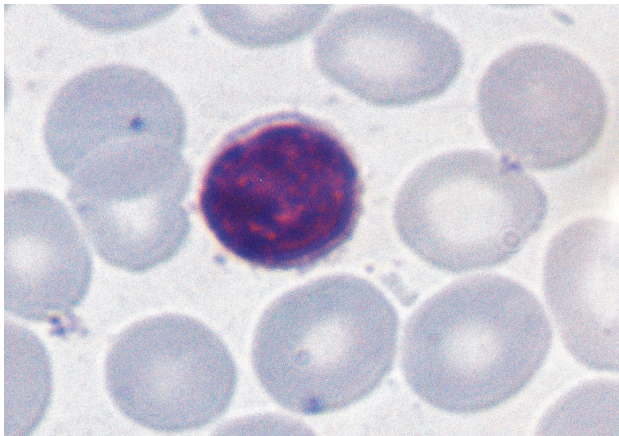


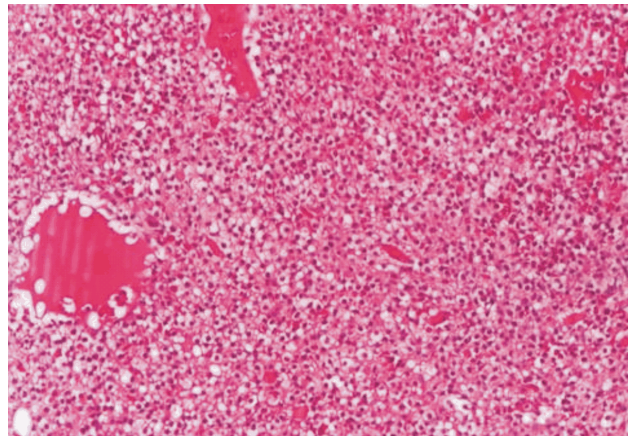Figure 1 – *Peripheral blood smear: circulating hairy cells (HE staining, ×1000).*



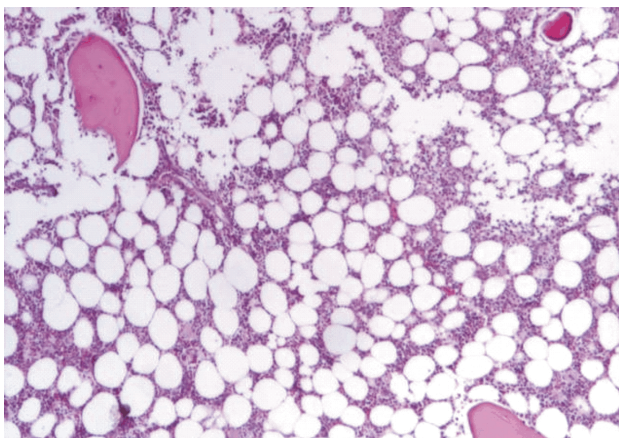Figure 2 – *Hypercellular bone marrow: diffuse infiltration with hairy cells (HE staining, ×40).*



Figure 3 – *Hypocellular bone marrow: diffuse infiltration with hairy cells (HE staining, ×200).*


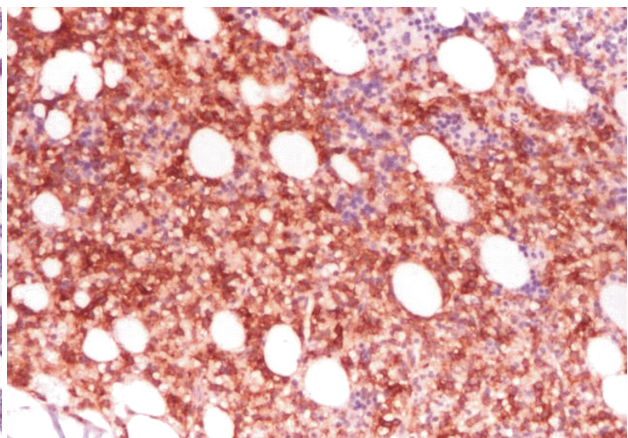
Figure 4 – *Bone marrow biopsy: diffuse CD20+ (CD20 immunohistochemistry, ×200).*
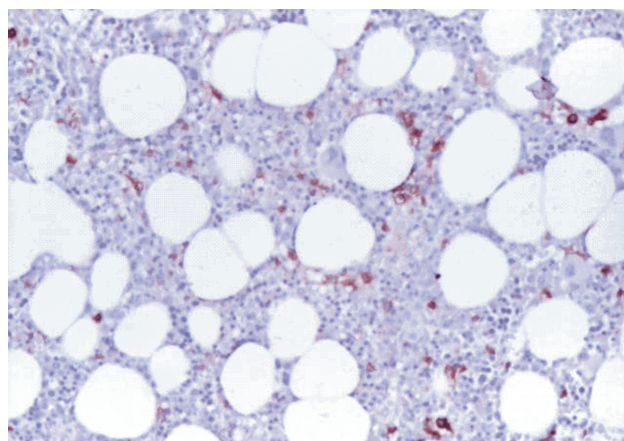
**Figure 5 –** *Bone marrow biopsy: diffuse DBA44 (DBA44 immunohistochemistry, ×200).*
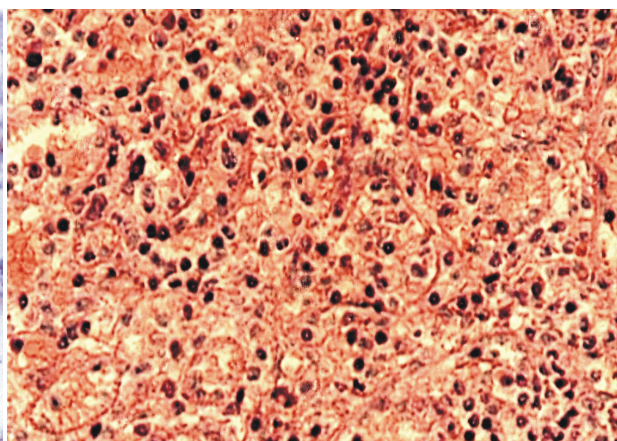


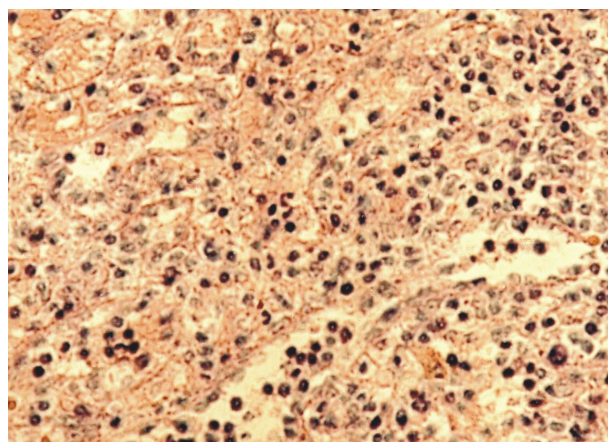**Figure 6 –** *Lymph node biopsy: parafollicular infiltration with hairy cells (HE staining, ×100).*



**Figure 7 –** *Spleen biopsy: diffuse infiltration of red pulp with hairy cells (HE staining, ×100).*

## Discussion

Diagnosis was based on the presence of hairy cells in peripheral blood and bone marrow and was associated with bi-/pancytopenia, splenomegaly, myelofibrosis. HCL is divided into three phenotypes: (a) typical HCL, (b) Japanese type of HCL (HCLJV), and (c) HCL variant (HCLV). The hairy cells of the Japanese type of HCL have less marked hairy projections, a rather round nucleus and weaker positivity for TRAP [10, 11]. HCL variant presents dimorphic features, both hairy cells and of prolymphocytes, splenomegaly and a leukocytosis more than $50×10^9$/L; the hairy cells have a prominent nucleolus and are usually CD25 negative and TRAP negative. A complex karyotype is observed, including translocation t(14;18)(q32;q21) present in follicular lymphoma and t(2;8)(p12;q24) observed in variant Burkitt's lymphoma, and a bad prognosis with a shorter median survival than typical HCL [12, 13].

In this study, 38 patients presented typical HCL and one patient presented HCL variant (splenomegaly, leukocytosis $56×10^9$/L; the hairy cells had a prominent nucleolus like prolymphocytes and were CD25 negative).

Differential diagnosis was made with: splenic marginal zone lymphoma (characteristic villous lymphocytes CD103 negative, TRAP negative, the bone marrow infiltrates sharply demarcated), prolymphocytic leukemia (marked elevation of the white blood count, morphology of the prolymphocytes), chronic lymphocytic leukemia (lymphoid cells with mature aspect, CD5 positive), aplastic anemia (hypoplasia/aplasia of bone marrow, absence of hairy cells), myelodysplastic syndromes (hypercellular bone marrow without hairy cells, dysplasia, the absence of splenomegaly), idiopathic myelofibrosis (the tear-drop red cells, the presence of erythroblasts and left-shift on the peripheral smear, the absence of hairy cells).

The most frequent complications were infectious (51.3% patients): pulmonary infections – 11 cases (three cases of mycobacterial infections), urinary infections – five cases, cutaneous infections – one case, severe sepsis after splenectomy – three cases. Infectious complications were related to granulocytopenia and cellular immune deficiency. In three cases associated type 2 diabetes mellitus. Hemorrhage occurred in 33% of patients, related of severe thrombocytopenia: digestive hemorrhage – five cases, epistaxis – four cases, genital hemorrhage – four cases. Autoimmune complications were present in four (10.2%) cases, second malignancies in three cases (skin, lungs). In two cases, HCL evolved to aplastic anemia.

About 10% of patients (asymptomatic without severe cytopenias) do not require treatment. Treatment is necessary in patients with neutrophils <1000/μL, platelets <$100×10^9$/L, Hb<10 g/dL, symptomatic splenomegaly, recurrent infections, autoimmune complications, progressive disease. The treatment of hairy cell leukemia had a multi-step evolution. The purine nucleoside analogues (Deoxycoformycin, 2-Chlorodeoxyadenosine) represent the first-line therapy, often associated with immunosuppression and secondary malignancies [14, 15]. In cases resistant to purine analogues or in refractory relapses anti-CD20 monoclonal antibody, 375 mg/m²/week i.v. ×8–12 represent an alternative, as well as immunotoxins, TNF-α inhibitors, angiogenesis inhibitors [16–18]. IFN-α, 3 MU/m², three times weekly, may induce complete remission in 10–20% of cases and partial remission in the rest of cases [20]. Splenectomy is indicated for patients refractory to therapy or with bleeding due to severe thrombocytopenia [21]. Supportive care (antibiotics, corticosteroids for autoimmune complications) may be necessary.

In this study, all patients received treatment: one patient substitution and corticotherapy, one patient Chlorambucil 4 mg/day for six months (increased in blood counts, but significant risk of infection related-neutropenia), three chemotherapy (two CVP – Cyclo-

phosphamide, Vincristine, Prednisone and one CHOP – Cyclophosphamide, Doxorubicin, Vincristine, Prednisone) with good response but significant toxicities. Five patients were splenectomised (four open splenectomy and one laparoscopic splenectomy). The indications for splenectomy were: severe thrombocytopenia – two patients, pancytopenia – two patients, compressive and painful splenomegaly – one patient. In all cases splenectomy corrected cytopenias, troublesome to the tumoral splenomegaly. The early post-splenectomy complications (<3 months) were: hyper-thrombocytosis (four patients), thrombosis (one patient), infections (one patient). The late post-splenectomy compli-cations (>3 months) were represented by lung micro-embolies with secondary pulmonary hypertension syndrome (one case) and tumoral hepatomegaly (one case). The median survival post-splenectomy rate was of 35 months. Twenty-nine patients were treated with IFN-α as first-line therapy 3 MU/m$^2$ three times weekly for 12–24 months. Four (14%) patients achieved complete response with negative bone marrow and eighteen (62%) patients had a partial response. Fourteen (49%) patients received maintenance therapy with IFN-α. Eight (28%) patients never needed a second therapy and seven (24.5%) patients were retreated with IFN-α. The median follow-up was of 52 months and the disease-free survival in patients with maintenance therapy was of 28 months. One patient developed a secondary neoplasia (lung cancer) [22]. Some studies reported a significantly increased incidence of second malignancies in HCL patients treated with IFN; others reported no excess of second neoplasms after IFN or purine nucleoside analogues [23, 24]. In two cases, IFN-α was badly tolerated (peripheral neuropathies, flu-like symptoms). The failure free survival after discontinuation of treatment was seven, respectively 11 months. Purine nucleoside analogues and anti-CD20 monoclonal antibody were not available in our hospital for the treatment of HCL.

HCL is a slowly progressing disease with a median survival of over five years with classic therapy. New agents (Deoxycoformycin, 2-Chlorodeoxyadenosin) increased the median survival rate to 10 years. The median survival in this study was of eight years.

## ⚗ Conclusions

HCL is a rare type of leukemia, sometimes difficult to diagnose, which may be considered in the presence of pancytopenia associated with neutropenia, splenomegaly, myelofibrosis, presence in peripheral blood, bone marrow and spleen of atypical lymphoid cells with irregular cytoplasm projections and unique immunophenotypic features. Histopathological examinations of bone marrow, spleen, lymph node associated with immunohistochemistry are absolutely necessary to positive and differential diagnosis of HCL. IFN-α remained the best option of therapy in the absence of purine nucleoside analogues, having a significant efficacy in patients with HCL. The maintenance therapy seems to increase disease free survival rate, usually short after discontinuation of treatment. The most frequent complications are infections related to granulocytopenia and an immune deficiency; their incidence was significantly decreased after three months of therapy with IFN-α and statistically correlated ($p<0.05$) with increased count of neutrophils.

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