Accepted manuscripts are the articles in press that have been peer reviewed and accepted for publication by the Editorial Board of the *Vojnosanitetski Pregled*. They have not yet been copy edited and/or formatted in the publication house style, and the text could still be changed before final publication.

Although accepted manuscripts do not yet have all bibliographic details available, they can already be cited using the year of online publication and the DOI, as follows: article title, the author(s), publication (year), the DOI.

Please cite this article **SUCCESSFUL TREATMENT OF SYNCHRONOUS HAIRY CELL LEUKAEMIA AND DIFFUSE LARGE B CELL LYMPHOMA IN A PATIENT WITH SEVERE HYPERCALCEMIA AND EXTENSIVE OSTEOLYTIC LESIONS**

Authors Markovic O1,4, Gotic M2,4, Cemerikic V3, Divac A1, Marisavljevic D1,4, Vojnosanitetski pregled (2021); Online First July, 2021.

UDC:

DOI: https://doi.org/10.2298/VSP210118073M

When the final article is assigned to volumes/issues of the Journal, the Article in Press version will be removed and the final version appear in the associated published volumes/issues of the Journal. The date the article was made available online first will be carried over.
SUCCESSFUL TREATMENT OF SYNCHRONOUS HAIRY CELL LEUKAEMIA AND DIFFUSE LARGE B CELL LYMPHOMA IN A PATIENT WITH SEVERE HYPERCALCEMIA AND EXTENSIVE OSTEOLYTIC LESIONS

Markovic O¹, Gotic M², Cemerikic V³, Divac A¹, Marisavljevic D¹

Clinical-Hospital Center „Bezanijska Kosa“ Belgrade¹, Clinic for Hematology, Clinical Centar of Serbia², Belgrade, Beolab, Beograd³, School of Medicine, University of Belgrade⁴

Correspondence to:
Abstract

**Introduction:** Although secondary malignancies usually occur at different times after HCL treatment, (simultaneous) occurrence of HCL and other malignancies at the same time is very rare. Synchronous hairy cell leukemia (HCL) and diffuse large B cell lymphoma (DLBCL) has not been described so far.

**Case report:** We report a 62-years old female patient who presented with intense constitutional symptoms, hypercalcaemia, pancytopenia and osteolytic destruction of the left shoulder joint. Immunohistochemical analysis of the bone marrow revealed presence of two populations: a population of HCL cells and a population of DLBCL cells with the expression of c-myc and bcl-2 ("double expressor" DLBCL) and high proliferative activity (Ki-67+cells>90%). FISH analysis showed amplification of the bcl-2 gene. In addition, BRAF V600E mutation was detected. After intensive treatment with immunochemotherapy, radiotherapy and bisphosphonates patient achieved complete remission, lasting for more than two years.

**Conclusion:** As the association of hairy cell leukemia and lymphoma is a very rare, diagnosis of synchronous occurrence of two lymphoproliferative diseases is diagnostic and therapeutic challenge. It remains unclear whether DLBC and HCL are derived from two different malignant clones or DLBCL developed by transformation of HCL as the result of clonal evolution of B-cell clone.

**Key words:** hairy cell leukemia, diffuse large cell B-cell lymphoma, synchronous malignancies.
Introduction

Hairy cell leukemia (HCL) is uncommon type of hematological malignancy which constitutes 2% of all leukemias. Prognosis of HCL has considerable improved thanks to new effective chemotherapeutic agents. However, literature data showed that HCL patients have higher risk of developing second malignancies than the general population. In some studies second malignancies were the primary cause of death in patients with HCL. Although secondary malignancies usually occur at different times after HCL treatment, occurrence of HCL and other malignancies at the same time is very rare. It has been reported the simultaneous occurrence of HCL and several other lymphoproliferative diseases (follicular lymphoma, T cell lymphoma, B-cell chronic lymphocytic leukemia, multiple myeloma). However, no one described simultaneous HCL and diffuse large cell B-cell lymphoma (DLBCL) so far.

Case report

We present a 62-year-old female patient who admitted to our hospital in August 2018 with fatigue, fever, weight loss (15kg/two months) and left shoulder pain. Physical examination showed poor general condition (ECOG 3), fever 38°C, pallor of the skin and weakness of the left hand. Skeletal radiography showed osteolytic lesions in glenoid region of scapula, whilst chest X-ray showed consolidation in the area of the left lower lobe of the lung. CT
of thorax showed non-homogeneous consolidation in S3 zone of the left lung with a negative bronchogram and hilar lymphadenopathy. Blood counts showed pancytopenia (Hb 74g/L, Plt 84x10^9/L, WBC 3.8x10^9/L with 48% neutrophiles, 44% lymphocytes, 6% monocytes, 2% eosinophiles and 3 erythroblasts/100 leukocytes in differential count). Biochemistry analysis showed hypercalcemia (3.3mmol/L), elevated potassium (5.42mmol/L), CRP (147.2mg/L), LDH (4008U/L) and β2-microgloblin (4.48mg/L). ESR was significantly accelerated (140mm/1st hour). Needle biopsy of lung and shoulder was unsuccessful several times. However, morphological and immunohistochemical analysis of the bone marrow revealed presence of two populations: a population of hairy-cell leukemia cells (CD103+, TRAP+, annexin+) and a population of diffuse large B-cell lymphoma cells with the expression of c-myc and bcl-2 ("double expressor" DLBCL), expression of MUM-1 and BCL-6 antigens, and high proliferative activity (Ki-67 cells >90%) (Figure 1). Flow cytometric immunophenotyping of bone marrow cells revealed a clonal population of atypical mature B cells (16% of cells) with Ig-kappa light chain expression in combination with heavy IgD or IgM chains and high expression of CD19, CD103, CD11c, CD305 and FMC7 antigen. The same population was detected by flow cytometry in the peripheral blood (6% of cells). FISH analysis showed amplification of the bcl-2 gene, whereas c-myc gene amplification was not detected. In addition, BRAF V600E mutation was detected. NMR of left shoulder showed complete destruction of left shoulder joint, with osteolytic lesion of left scapula in glenoid and coracoid region and focal infiltration of surrounding soft tissue (Figure 2). PET-CT scan showed hypermetabolic lesions on left axillary and retroperitoneal lymphadenopathy and multiple bone lesions (highest metabolic activity of SUV 25.52 noted in left scapula). We concluded that patient has synchronous HCL and "double expressor" DLBCL in IVB clinical stage with high IPI and high NCCN-IPI score.
Treatment consisted of immunochemotherapy and bisphosphonates (zolendronic acid). Although lymphoid cells were not detected in cerebrospinal fluid using flow cytometry, because of high CNS-IPI score CNS prophylaxis with intrathecal metotrexate injections was applied. After eight cycles of R-CHOP chemotherapy, local irradiation of residual area with PET positivity (left shoulder soft tissue) was applied. Therapy resulted in a complete hematological remission, lasting for almost two years after completion of the treatment (last follow-up, December 2020).

**Discussion**

Presented patient is the first reported case of synchronous hairy cell leukemia and diffuse large B cell lymphoma. The patient also had very uncommon clinical presentation since the association of osteolytic lesions and hypercalcaemia are extremely rare initial symptoms of lymphoma (approximately 2%)\(^6\). Hypercalcemia in malignancies can be mediated by a number of different mechanisms. PTH-like substances (parathyroid hormone-related protein) secreted by malignant cells, secreting other cytokines that activate osteoclasts, ectopic activity of 1-alpha-hydroxylase and the production of 1,25-dihydroxycholecalciferol as well as the excessive production of PTH are some of well characterized mechanisms of hypercalcemia\(^7\). Hypercalcemia of malignancy is the most often related with PTHrP\(^8\), but in some cases is associated with extensive bone metastases which was the case in our patient. It was previously considered that hypercalcemia is a result of direct destruction of bone by metastases. However, it was found that hypercalcemia is consequence of the release of local cytokines from the tumor cells, which activate osteoclasts and stimulate bone resorption, usually through RANK/RANKL\(^9\). Patients with hypercalcemia of malignancy have limited survival of several months, thus it is considered a marker of poor prognosis\(^7\). It is not clear whether this poor prognosis is
related to the advanced stage of malignancy associated hypercalcemia or it is just a simply marker of underlying cancer. Our patient has our patient had numerous osteolytic lesions and advanced disease, but despite this, owing to optimal therapy, a complete therapeutic response was achieved.

Nevertheless, it remains unclear whether DLBC and HCL are derived from two different malignant clones or DLBCL developed by transformation of HCL as the result of clonal evolution of B-cell clone. Based on phenotypic and molecular studies, it is now well established that HCL derives from germinal or post-germinal center cells. Based on phenotype and molecular features of HCL cells malignant transformation occurs at the level germinal or post-germinal center cells. This is confirmed by the presence somatic mutations of variable region of the immunoglobulin genes, which are the hallmark of a germinal center origin. Lymphoma cells of DLBCL in our patient originate from transformed germinal center B-cells (i.e. GBC type). This all together supports a common origin of synchronous HCL and DLBCL in this case. On the other hand, hypothesis on the existence of two different malignant clones can be supported by common finding of amplification of the bcl-2 gene in DLBCL patients and BRAF V600E mutation, a genetic alteration invariably associated with hairy cell leukemia.

As the association of hairy cell leukemia and lymphoma is a very rare, diagnosis of synchronous occurrence of two lymphoproliferative diseases is diagnostic and therapeutic challenge. Establishing adequate diagnosis in cases of double malignancies is particularly important because it allows the implementation of optimal therapeutic approach which is usually directed to more aggressive disease. Such an approach resulted in complete remission of disease in our patient. It is important to emphasize that our patient achieved complete clinical remission after treatment with immune-chemotherapy (i.e. rituximab-
CHOP protocol), which can be explained by responsiveness of malignant B-cell clone of 
HCL to monoclonal anti-CD20 antibody\textsuperscript{13}. 

Conflict of interest

The authors declare they have no conflict of interest.

LITERATURE

1. Hisada M, Chen BE, Jaffe ES, Travis LB. Second cancer incidence and cause-specific 
mortality among 3104 patients with hairy cell leukemia: a population-based study. J Natl Cancer Inst 2007; 99:215-22. https://doi.org/10.1093/jnci/djk030

2. McDonald L, Fadalla K. Synchronous follicular non-Hodgkin’s lymphoma and hairy cell leukaemia: a case report. Annals of Hematology 2019, 98(6):1517–18. https://doi.org/10.1007/s00277-018-3573-5

3. Gasljevic G1, Kloboves-Prevodnik V, Gazic B, Vovk M. Coexistent hairy cell leukaemia and hepatosplenic T-cell lymphoma: a case report. Diagn Pathol 2014; 9:58. http://www.diagnosticpathology.org/content/9/1/58

4. Rastogi P, Jeyaraman P, Sachdeva MU et al. Synchronous hairy cell leukemia and chronic lymphocytic leukemia: a case report with a brief review of literature. Blood Res 2018; 53(2):160-163. https://doi.org/10.5045/br.2018.53.2.160

5. Zhao X, Maric I. Chance identification of synchronous hairy cell leukemia and plasma cell myeloma in a potential HSC donor. Blood 2014; 123(24): 3694. https://doi.org/10.1182/blood-2014-02-558189

6. Matsuhashi Y, Tasaka T, Uehara E, et al. Diffuse large B-cell lymphoma presenting with hypercalcemia and multiple osteolysis. Leuk Lymphoma 2004; 45:397-400. https://doi.org/10.1080/10428190310001593139
7. Goldner W. Cancer-Related Hypercalcemia. J Oncol Practice 2016;12(5):426-432. DOI: 10.1200/JOP.2016.011155

8. Stewart AF: Clinical practice. Hypercalcemia associated with cancer. N Engl J Med 2005;352:373-9. DOI: 10.1056/NEJMcp042806

9. Sternlicht H, Glezerman IG: Hypercalcemia of malignancy and new treatment options. Ther Clin Risk Manag 2015;11:1779-1788. https://doi.org/10.2147/TCRM.S83681

10. Burthem J, Zuzel M, Cawley JC. What is the nature of the hairy cell and why should we be interested? Br J Haematol 1997;97:511–4. https://doi.org/10.1046/j.1365-2141.1997.00087.x

11. Küppers R, Klein U, Hansmann ML, Rajewsky K. Cellular origin of human B-cell lymphomas. N Engl J Med 1999;11:1520–1529. doi: 10.1056/NEJM199911113412007.

12. Tiacci E, Trifonov V, Schiavoni G, Holmes A, Kern W, Martelli MP, et al. BRAF mutations in hairy-cell leukemia. N Engl J Med. 2011; 364(24):2305-15. Doi: 10.1056/NEJMoa1014209.

13. Ravandi F. Chemo-immunotherapy for hairy cell leukemia. Leuk Lymphoma. 2011;52:72-74. DOI: 10.3109/10428194.2011.565096
Figure 1. Presence of two populations of cells in bone marrow: a population of hairy-cell leukemia cells and a population of diffuse large B-cell lymphoma cells a) Hematoxilin Eosin (H&E) 100x. Immunohistochemical stains of formalin-fixed, paraffin-embedded sections for: b) CD20, c) CD103, d) TRAP, e) anexin, f) c-myc (x100)
Figure 2. NMR of left shoulder showed complete destruction of left shoulder joint, with osteolytic lesion of left scapula.