CASE REPORT

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Primary diffuse large B-cell lymphoma of the testis

IONELA ROTARU[1)], JANINA GEORGIANA NACEA[2)], MARIA CAMELIA FOARFĂ[3)], DRAGOŞ VIOREL CIOVICĂ[4)], ANA-MARIA PĂTRAŞCU[3)]

[1)]*Department of Hematology, University of Medicine and Pharmacy of Craiova, Romania*
[2)]*Department of Obstetrics and Gynecology, University of Medicine and Pharmacy of Craiova, Romania*
[3)]*Department of Morphology, University of Medicine and Pharmacy of Craiova, Romania*
[4)]*Department of Urology, Emergency County Hospital, Craiova, Romania*

Abstract

Non-Hodgkin's lymphomas are chronic lymphoproliferative disorders, with nodal or extranodal onset, most of which being digestive tract lymphomas. Testicular primitive lymphoma generally affects men; it is rare but aggressive type of lymphoma. We present the case of the only patient diagnosed with testicular lymphoma in Hematology Clinic of Craiova, Romania, in the last 20 years. Histopathological and immunohistochemical exams confirmed the diagnosis of diffuse large B-cell lymphoma, and the stage was IIB. According to *International Prognostic Index* (IPI) score, the patient was classified as low risk. He received combined treatment consisting of surgery, chemotherapy, central nervous system prophylaxis and radiotherapy. The outcome was very good, the patient achieving complete remission. After 36 months, he is still in complete remission with clinical and biological evaluation performed every three month, and computerized tomography once a year.

Keywords: primary testicular lymphoma, diffuse large B-cell lymphoma, immunohistochemistry.

Introduction

Non-Hodgkin's lymphomas are chronic B-cell lymphoproliferative disorders, the majority of them present with nodal onset and 30% of cases with extranodal one [1]. The most frequent site involved in extranodal onset is the digestive one, and the majority are, on histopathological exam, diffuse large B-cell lymphoma (DLBCL). Generally, it could be *de novo*, but, there are situation of association with other neoplasia, for example chronic myeloid leukemia [2] or with immunodeficiency status, such as human immunodeficiency virus (HIV) infection or long-term immunosupresive treatment after transplantation.

Testicular primitive lymphoma represents 1–2% of malignant lymphomas and between 1–7% of primitive testicular tumors [3]. The particularities of this type of lymphoma are: low incidence, high clinical aggressiveness and complex therapeutic approach. Nearly all cases (80–90%) from histopathological point of view are DLBCL [4], up to 90% being diagnosed in stage I (60%) or II (30%) [5]. Type B symptoms generally occur in advanced stages, as cases diagnosed in stage IV are difficult to differentiate from a lymph node onset lymphoma and from secondary testicular damage. Despite the aggressive treatment approach, the majority of cases achieve complete remission, but, the most frequent relapses are the extranodal one [6].

We present the case of a 63-year-old male with DLBCL of testis treated in the Hematology Clinic of Craiova, Romania.

Case presentation

First hospitalization

A 63-year-old male observed the appearance of a left testicular tumor in March 2014. The tumor increased rapidly in size over the next three weeks. The patient was admitted to the Department of Urology, where a testicular left tumor with a diameter of 5.5 cm, with a high consistency, well defined, indolent, was detected. Left orchiectomy was performed with histopathological and immunohistochemical (IHC) examination.

Morphological assessment

Gross examination

A yellowish, firm mass, with rare hemorrhagic areas and necrosis that completely replaces testicular parenchyma, is observed.

Histopathological examination

The tissue sample was processed according to the classical histological technique (fixation in 10% buffered formalin and embedment in paraffin).

The usual staining was Hematoxylin–Eosin (HE). Microscopic examination revealed a diffuse, monomorphic, neoplastic proliferation in the testicular parenchyma (Figure 1a), consisting of medium and large round cells of lymphocyte type, marked with nuclear pleomorphism. The described cellular proliferation dissociates and compresses the seminiferous tubules (Figure 1b).

Immunohistochemistry technique has included an

antibody panel, shown in Table 1. The labeled Streptavidin Biotin–Horseradish peroxidase (LSAB–HRP) method was used.

IHC analysis indicated CD20 and CD79a (membrane markers for B-lymphocytes) strongly positive and diffuse in tumor cells, CD45-RO (membrane marker for T-lymphocytes) positive with variable intensity in tumor cells, and CD5 (membrane marker for T-lymphocytes) positively intense and focal in tumor cells. Bcl-2 (cytoplasmic marker of interfollicular lymphocytes) was positively intense and diffuse in tumor cells.

Ki-67 (nuclear proliferation marker) was strongly positive in about 40% of tumor cells. Bcl-6 (nuclear marker of germinal center lymphocytes) and Cyclin D1 (proto-oncogenic nuclear marker) were negative in tumor cells (Figure 2). CD10 and multiple myeloma oncogene 1 (MUM1) were negative.

According to the *World Health Organization* (WHO) diagnosis criteria and using Hans algorithm, the diagnosis was primary DLBCL of testis with germinal center phenotype.

Second hospitalization

The patient was admitted in the Hematology Clinic of Craiova about two months after the testicular tumor was observed, causing physical asthenia, weight loss of about 8 kg over the last two months, intense night sweats. Clinical exam showed mobile, high consistency and painless left inguinal polyadenopathy of about 2 cm in diameter. Usual laboratory tests were performed. The following values were significantly high: erythrocyte sedimentation rate (ESR) 120/140 mm, uric acid 9.4 mg/dL, serum lactate dehydrogenase (LDH) 858 IU/L, beta2-microglobulin 5.4 mg/dL. There was no anemia, granulocytopenia or thrombocytopenia, and the leukocyte formula was normal. Hepatitis B surface (HBs) antigen and C-antiviral antibodies as well as HIV testing were all negative.

In order to establish the extension of the disease computed tomography (CT) scan, bone marrow biopsy and cerebrospinal fluid analysis were performed.

Neck, thorax, abdomen and pelvis CT scan (with Ultravist 370 contrast agent) indicated retroperitoneal and interaortocaval adenopathic block of 3/5/5.6 cm (Figure 3), but no supradiaphragmatic adenopathies and normal spleen size.

Bone marrow biopsies did not show disease involvement. Cerebrospinal fluid analysis indicated normal cytology, with no malignant cells. Cytogenetic and molecular analysis was not performed (it was not available).

According to the Ann-Arbor staging system for lymphomas, the patient stage was IIB (involvement of one part of the diaphragm).

International Prognostic Index (IPI) score was evaluated. Is a clinical tool to predicting the prognosis in aggressive lymphoma, according to risk factors. Negative prognosis factors are: age >60 years old, stage III/IV disease, elevated serum LDH, *Eastern Cooperative Oncology Group* (ECOG) performance status >1, and more than one extra-nodal site. Each one counts one prognosis point. Based on IPI scores, the patient can be classified into low-risk group (IPI 0–1), low intermediate risk group (IPI 2), high intermediate risk group (IPI 3) and high-risk group (IPI 4–5).

Using the IPI prognostic score, the patient was included in the low intermediate risk prognosis group (two prognostic points: age >60 years old and high serum LDH).

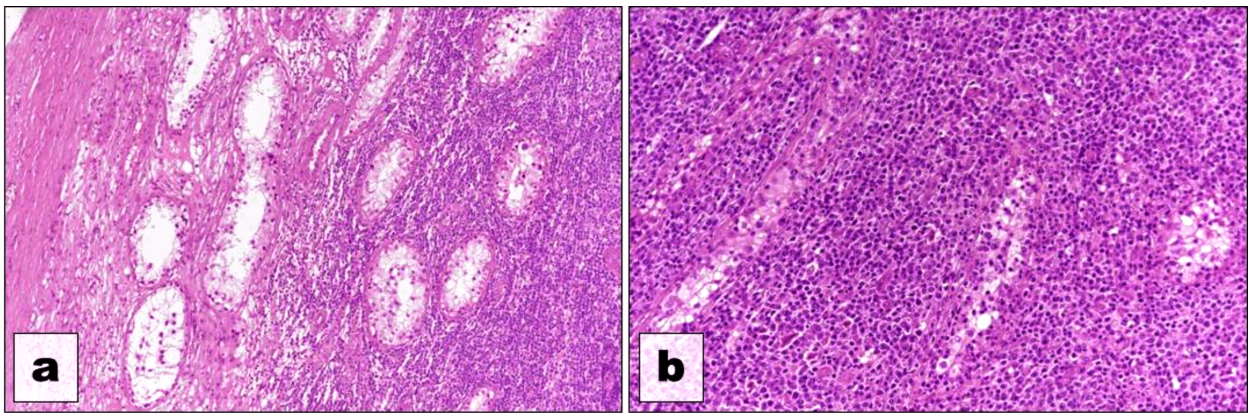

Figure 1 – ***Histopathological examination: (a) Testicular parenchyma invaded by malignant cellular proliferation; (b) Detail: Monomorphic, medium and large round cells with nuclear pleomorphism. HE staining: (a) ×100; (b) ×200.***

Table 1 – ***Antibodies used to identify the malignant proliferation***

Antibody	Clone	Source	Dilution	Specificity	Pretreatment
CD10	56C6	LEICA	1:100	Membrane	Five cycles citrate buffer
CD20	L26	DAKO	1:100	B Ly, membrane	Three cycles citrate buffer
CD79a	5P18	THERMO	1:300	B Ly, membrane	Seven cycles citrate buffer
CD5	SP19	THERMO	1:50	T Ly, membrane	Seven cycles citrate buffer
CD45RO	UCHL1	DAKO	1:200	T Ly, membrane	Seven cycles citrate buffer
Bcl-2	124	DAKO	1:50	Interfollicular Ly, cytoplasm	Seven cycles citrate TEDTA buffer
Bcl-6	LN22	LEICA	1:100	Germinal center Ly, nuclear	Five cycles TEDTA buffer
Cyclin D1	P2D11F11	LEICA	1:20	Proto-oncogene, nuclear	Five cycles citrate TEDTA buffer
Ki-67	MIB-1	DAKO	1:20	Nuclear	Seven cycles citrate buffer

CD: Cluster of differentiation; Bcl: B-cell lymphoma; Ly: Lymphocyte; TEDTA: Tetrasodium ethylenediaminetetraacetic acid.

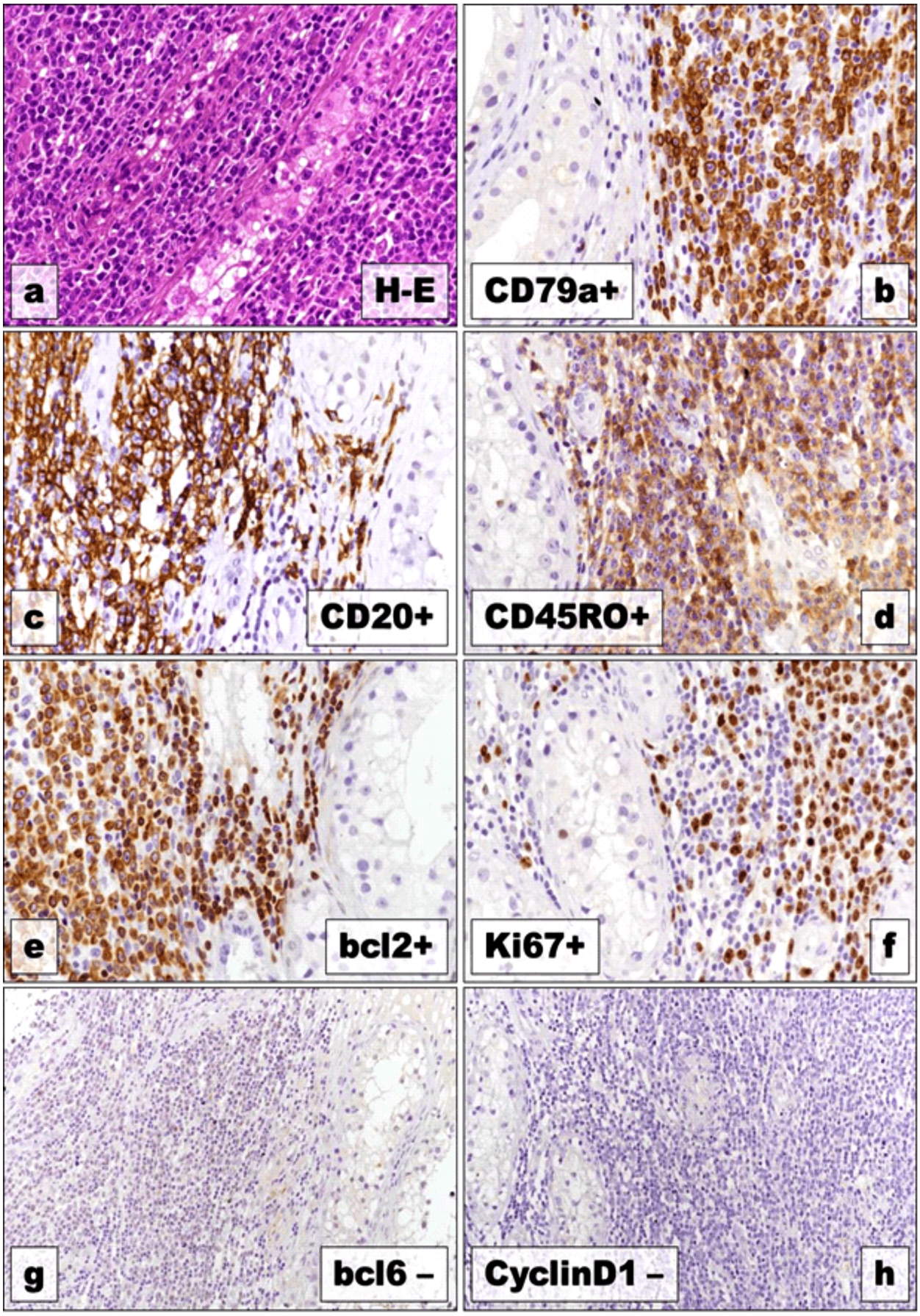

Figure 2 – ***Primary diffuse large B-cell lymphoma of the testis. HE staining: (a) ×400. Immunohistochemical panel: (b–f) ×400; (g and h) ×200.***

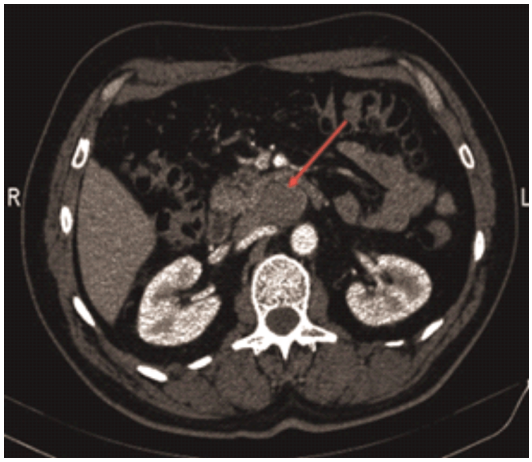

Figure 3 – ***CT examination: retroperitoneal, inter-aortocaval adenopathic block, 5.6 cm maximum diameter (red arrow).***

Poly-chemotherapeutic treatment was initiated [eight cycles R-CHOP – Rituximab, Cyclophosphamide, Hydroxy-daunomycin (Doxorubicin), Oncovin® (Vincristine), Prednisolone], four intrathecal administrations with Methotrexate, Cytosar and Dexamethasone, and 30 Gy radiotherapy has been applied on the right testis, according to the current recommendations of the *International Extra-nodal Lymphoma Study Group* (IELSG).

Outcome

The applied treatment led to complete remission of the disease, confirmed by positron-emission tomography (PET)-CT. After the end of the therapeutic protocol in December 2014, complete remission confirmed by PET-CT was obtained.

Clinical-biological follow-up was performed every three months in the first two years of complete remission, then at six months, followed by annual monitoring, up to five years if complete remission is maintained. Currently, the patient is in complete remission of the disease, two years and six months after termination of the therapeutic protocol.

Discussions

The case report describes a patient with DLBCL, with activated phenotype and extranodal onset, with particular localization at testicular level. Characteristic of this localization is the increased aggressiveness and high risk of relapse, even after 10–15 years of onset, especially in the contralateral testicle (5–35%) [7], and in patients who have not receiving radiotherapy on the contralateral testicle [8], with a risk of central nervous system (CNS) relapse, at five years 20% and at 10 years 35% [7], but also in other extranodal sites. The patient was staged IIB at diagnosis; approximately 20% of patients with primitive testicular lymphoma were stadialized in stage II at diagnosis [9].

According to IHC expression of CD10, Bcl-6 and MUM1, DLBCL is classified as a germinal center B-cell and non-germinal center B-cell like [3, 10]. The majority of DLBCL cases belong to the non-germinal center B-cell like subgroup [3, 10]. There are also aggressive histological subtypes, described such as high-grade DLBCL, Burkitt and Burkitt's-like types, which represent a minority, only 10–20% and are more frequent in patients carrying the HIV. Our case was negative for CD10, Bcl-6 and MUM1, so is a germinal center B-cell proliferation. The positivity of Bcl-2 in DLBCL was identified in 50% of cases in 97 patients newly discovered cases with nodal and extranodal DLBCL, between January 2007 and December 2016, in the Hematology Clinic of Craiova [11]. Our case was positive for Bcl-2, but negative for Bcl-6. Unfortunately, we could not determine c-myc protein expression on IHC exam. If c-myc is positive in IHC exam, the lymphoma is double expressor, with poor outcome [12]. DLBCL with activated phenotype, such as the current case, is associated with a lower rate of complete remissions using poly-chemotherapy regimens containing anthracyclines compared to the DLBCL of the germinal center. Combination of anti-CD20 monoclonal antibodies to chemotherapy did not increase the percentage of patients achieving complete remission in testicular primitive DLBCL [13]. In addition to the IPI score, other prognostic value factors have been looked for. Thus, a testicular tumor diameter greater than 7.5 cm fits into negative prognosis [14, 15]. In a multi-variate analysis of 24 patients, the IPI score <1 proved to be a statistically significant prediction factor for superior survival compared to patients with an IPI score >1 [13].

In the case presented, the tumor had a maximum diameter of 5.5 cm and the prognostic risk group was low-intermediate risk. Despite the recent advanced and aggressive treatment, prognosis is often poor [3]. The therapeutic approach to primitive testicular lymphoma remains a challenge, due to the risk of late relapses. Although there is not yet a current standard of care in testicular primitive lymphoma, the recommendations of the IELSG are to use the combination therapy: surgical resection, chemotherapy associated with CNS prophylaxis and radiotherapy on the contralateral testicle. The recommendations are based on the results of retrospective and prospective studies conducted by this group [7, 8]. Because of the risk of relapse in the CNS, a Phase II prospective study is underway, in which 54 patients with testicular primitive non-Hodgkin's malignant lymphoma were enrolled. All patients followed combined therapy: chemoimmunotherapy, CNS prophylaxis (intrathecal liposomal Cytarabine and systemic with intermediate-dose Methotrexate), and locoregional radiotherapy [6]. The study of the gene expression profile in primitive testicular lymphoma revealed the increase in the expression of oncogenes that interfere with the signaling pathways of the B-lymphocyte antigen receptor as well as the decrease in the expression of some antioncogenes. These recurrent rearrangements result in the appearance of proteins involved in cell proliferation and survival and may be therapeutic targets for new molecules [15]. According to current recommendations of the IELSG, the patient underwent surgical treatment (left orchiectomy) followed by chemo-immunotherapy (eight R-CHOP cycles), CNS prophylaxis (four intrathecal administration of Methotrexate in combination with Cytosar and Dexamethasone) and radiotherapy on the right testicle at a total dose of 30 Gy. Evolution was favorable, the patient being in complete remission two and a half years after the end of the therapeutic protocol.

Conclusions

This paper presents the only patient diagnosed with primary testicular lymphoma in the Hematology Clinic of Craiova in the last 20 years, with favorable evolution after combined therapy (surgical resection, chemoimmunotherapy, CNS prophylaxis and contralateral testicular irradiation). The patient is in complete remission of the disease two years and a half at the end of the therapeutic protocol. Despite the complex therapeutic protocol, the prognosis in these locations remains reserved. Future prospective studies, some of them already underway, will provide additional information on the most effective therapeutic approach with significant impact on survival.

Conflict of interests

The authors declare that they have no conflict of interests.

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Corresponding author
Janina Georgiana Nacea, MD, PhD Student, Department of Obstetrics and Gynecology, Faculty of Medicine, University of Medicine and Pharmacy of Craiova, 2 Petru Rareş Street, 200349 Craiova, Dolj County, Romania; Phone +40764–197 444, e-mail: janinanacea@yahoo.com

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