Original paper

Clinicopathological and fluorescence *in situ* hibridisation analysis of primary testicular diffuse large B-cell lymphoma: a single-centre case series

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Primary testicular diffuse large B-cell lymphoma (PT-DLBCL) represents a rare and aggressive extranodal non-Hodgkin's lymphoma (NHL) with some specific features that differ from other NHLs.

Formalin fixed, paraffin wax embedded (FFPE) samples of 21 PT-DLBCLs and 30 comparative patients with DLBCL were analysed. All PT-DLBCL patients were treated with rituximab-containing regimens, intrathecal prophylaxis (10 patients), and irradiation of the contralateral testis (9 patients). FFPE samples were additionally analysed by immunohistochemistry (Bcl-2, c-Myc protein expression) and fluorescence *in situ* hybridisation (FISH) (BCL2 and MYC).

The patients with PT-DLBCL (median age 48.5 years), had low frequency of B symptoms (28.6%) and were often diagnosed in I and II Ann Arbor clinical stage (66.0%). The majority of PT-DLBCL (80.9%) had a non-germinal centre B-cell-like immunophenotype. Immunohistochemical staining showed increased c-Myc protein expression in the PT-DLBCL group compared to the control group ($p = 0.016$). MYC rearrangement was detected in 1 of 14 (7.0%), and MYC amplification in 3 of 14 (21.0%) patients. One of the 14 cases (7.0%) in the PT DLBCL group showed BCL2 rearrangement, and four of 14 (28.05%) cases showed BCL2 amplification. Complete remission (CR) was achieved in 75.0% of PT-DLBCL patients who had superior survival compared to those who did not achieve CR (median 48 vs. 21 months, $p = 0.012$).

Patients with PT-DLBCL express some immunohistochemical, biological, and clinical features that might differentiate them from nodal and extranodal DLBCL patients, indicating the need for a more personalised treatment approach.

Key words: primary testicular lymphoma, diffuse large B cell lymphoma.

Introduction

Primary testicular lymphomas are rare, comprising 3.0% to 5.0% of all testicular tumours, with diffuse large B-cell lymphoma (DLBCL) as the dominant lymphoma subtype [1, 2]. Primary testicular diffuse large B-cell lymphoma (PT-DLBCL) has an aggressive clinical course and inferior response to current treatment therapy [3].

According to the cell of origin, PT-DLBCL are more often non-germinal centre B-cell-like (non-GCB) type, as are other primary extranodal lymphomas [4, 5, 6]. Most studies have shown that the clinical outcome of non-GC DLBCL is worse than germinal centre B-cell-like (GCB) subtype [5, 6, 7].

Numerous prognostic factors have been tested in DLBCL, including BCL2 and MYC translocations and protein expression [8]. In DLBCLs, a significant advance in understanding of MYC alterations has been made recently. MYC is rearranged in 5.0% to 15.0% of DLBCL, not otherwise specified (NOS), and is frequently associated with BCL2 or BCL6 translocation, in the so-called "double-hit" or "triple-hit" lymphoma [9, 10, 11, 12]. C-Myc protein expression is detected in a much higher proportion of DLBCL (30.0-50.0%) than MYC alterations (5.0-15.0%) and is associated with concomitant protein expression of Bcl-2 in 20.0% to 35.0% of cases [13, 14, 15]. Most of these tumours do not carry MYC/BCL2 alterations and have been named "double-expressor lymphoma". Several studies have shown that the double-expressor lymphomas have a worse outcome than other DLBCL, NOS [13, 14, 15]. However, due to its rarity and aggressiveness, and improvements on the molecular field, PT-DLBCLs are currently under investigation.

The aim of the current study was to analyse clinical, histological, immunohistochemical, and biological features of PT-DLBCL, and to compare them with nodal and extranodal DLBCLs.

Material and methods

The pre-therapy formalin fixed, paraffin wax embedded (FFPE) samples of 21 PT-DLBCLs available for histological, immunohistochemical, and biological evaluation were analysed. Primary testicular lymphoma was defined as the presence of a testicular mass, with orchiectomy and no evidence of previously diagnosed lymphoma of other sites. As a comparative group, 30 newly diagnosed DLBCL patients (19 nodal and 11 extranodal, DLBCL, NOS), with available FFPE were additionally analysed. The control samples were randomly chosen by using a table of random numbers among the available FFPE archived samples of DLBCL, NOS. Subsequently, clinical data were collected. All patients were diagnosed in the Unit of Haematopathology, Department for Pathology, Clinical Centre of Serbia, Belgrade, between 2002–2016, and were treated at the Clinic for Haematology, Clinical Centre of Serbia, Belgrade, during the same period. All patients underwent standard staging procedures (physical examination, laboratory analyses, imaging techniques, and bone marrow biopsy). The patients with PT-DLBCL were regarded as advanced stage if there was evidence of enlarged lymph nodes elsewhere in the body according to Ann Arbor staging system. The International Prognostic Index was used for prediction of survival [16]. Twenty PT-DLBCL patients were treated with R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone) therapy, plus contralateral testicular radiotherapy applied in nine patients, and intrathecal therapy in 10 patients (Table I). All patients in the control group were treated with R-CHOP/R-CHOP-like regimens. Treatment response was evaluated according to the previously reported response criteria [17]. This work was done in accordance with the Declaration of Helsinki and was approved by the Ethics Board of the Faculty of Medicine, University of Belgrade.

Histologic review

Immunohistochemistry was performed on archived FFPE tissue samples of PT-DLBCL obtained after orchiectomy, and archived FFPE tumour tissue samples of DLBCL patients. For diagnostic purposes, immunohistochemical stains for CD20 (L26; Dako), CD3 (SP7; Thermoscientific), CD5 (4C7; Leica Biosystems), CD10 (clone 56C6; Dako), Ki-67 (MIB1; Dako), BCL6 (clone PG-B6p; Dako), MUM1 (clone MUM1p; Dako), BCL2 (clone 124; Dako) Cyclin D1 (SP4; Thermoscientific), CD23 (1B12; Leica Biosystems), and TdT (Clone EP266; Dako) were performed, according to the manufacturer's directions and current laboratory protocol applying an avidin-biotin complex method (LSAB2 kit peroxidase; Dako) using AEC as a chromogen. C-Myc was detected using Leica Bond Max Refine KIT method (Leica Bond Polymer Refine detection system – DS9800) and rabbit monoclonal anti c-Myc antibody (Abcam Rabbit monoclonal anti c-myc, Clone Y69) diluted 1 : 50. Sections were counterstained with Mayer haematoxylin.

Cases were considered positive when at least 50% of tumour cells expressed Bcl-2 protein and ≥ 40% of tumour cells expressed c-Myc, according to other studies [2, 4]. The patients were classified into germinal centre B-cell-like (GCB) and non-GCB subtypes according to the Hans algorithm [4].

Fluorescence *in situ* hybridisation

All cases with available additional paraffin-embedded material were studied using fluorescent *in situ* hybridisation (FISH) for MYC and BCL2 using

Table I. Clinical characteristics of 21 patients with primary testicular diffuse large B cell lymphoma (PT-DLBCL)

No	Age	Ann Arbor Stage	IPI	Therapy	Rtx	IT	CR	Relapse	OS (months)	Vital status
1	74	III-IV	High	R-CHOP	No	Yes	Yes	No	28	Alive
2	67	I-II	Low	R-CHOP	No	No	Yes	No	24	Alive
3	61	III-IV	High intermediate	R-CHOP	No	Yes	Yes	Yes	10	Alive
4	62	I-II	High intermediate	R-CHOP	Yes	Yes	No	Resistant disease	24	Dead
5	67	III-IV	High intermediate	R-CHOP	Yes	No	Yes	Yes	16	Dead
6	62	I-II	High	R-CHOP	Yes	No	Yes	No	39	Alive
7	64	I-II	Low intermediate	R-CHOP	Yes	No	Yes	No	72	Alive
8	55	III-IV	Low	R-CHOP	Yes	No	Yes	No	20	Alive
9	66	I-II	Low	R-CHOP	Yes	Yes	No	No	16	Alive
10	78	I-II	Low	N/A	N/A	N/A	N/A	N/A	N/A	N/S
11	77	I-II	Low	R-CHOP	N/A	Yes	Yes	No	8	Alive
12	69	III-IV	High intermediate	R-CHOP	No	Yes	No	Resistant disease	7	Dead
13	72	I-II	Low	R-CHOP	No	No	No	Resistant disease	9	Alive
14	70	I-II	Low	R-CHOP	No	No	Yes	No	44	Dead
15	54	I-II	Low	R-CHOP	Yes	Yes	Yes	Yes	18	Dead
16	66	I-II	Low	R-CHOP	No	Yes	Yes	No	46	Alive
17	59	III-IV	High intermediate	R-CHOP	No	Yes	No	Resistant disease	21	Dead
18	76	I-II	High intermediate	R-CHOP	Yes	No	Yes	No	40	Alive
19	32	I-II	Low	R-CHOP	Yes	Yes	Yes	No	12	Alive
20	58	III-IV	Low intermediate	R-CHOP	No	No	Yes	Yes	48	Dead
21	64	I-II	Low intermediate	R-CHOP	No	No	Yes	No	119	Dead

IPI – International Prognostic Index; Rtx – radiotherapy; IT – intrathecal therapy; CR – complete remission; OS – overall survival; R-CHOP – rituximab, cyclophosphamide, vincristine, prednisone; N/A – not applicable

commercial, directly labelled split probes, BCL-2 and C-MYC so as with centromeric probes for chromosomes 8 and 18 (Abbott Vysis, USA) [18].

Results

Clinical evaluation

Clinical characteristics of patients with PT-DLBCL are summarised in Table I. The median age at presentation was 64 years (range, 32 to 78 years), with the majority of patients (14 patients, 66.0%) diagnosed in early disease stage (I and II) according to the Ann Arbor classification, and seven patients (33.0%) in advanced stage (III and IV) of disease. Six patients (28.6%) complained of B symptoms. High and high-intermediate IPI was present in 10 (47.6%) PT-DLBCL patients. The median age in the control group of DLBCL patients (15 men/15 females) was 49 years (range 19 to 81 years). They were more often diagnosed in advanced Ann Arbor clinical stage compared to PT-DLBCL (22 patients, 73.3%, $p = 0.011$), more often complained of B symptoms (23 patients, 76.7%, $p = 0.002$), and had bulky disease (seven patients, 23.3%, $p = 0.033$). Low and low-intermediate IPI was seen in 18 patients (60.0%), which did not differ from that of PT-DLBCL ($p > 0.05$).

Table II. Immunohistochemical and FISH analysis of 21 patients with primary testicular diffuse large B-cell lymphoma (PT-DLBCL)

No	Cell of origin	CD10	Bcl-6	MUM-1	Bcl-2	c-Myc (%)	Double expressors	C-myc FISH	bcl-2 FISH	Double HIT FISH
1	Non GCB	No	No	Yes	No	40	No	AMP	AMP	No
2	Non GCB	No	Yes	Yes	Yes	20	No	NEG	AMP	No
3	Non GCB	No	No	Yes	Yes	60	Yes	NEG	NEG	No
4	Non GCB	No	No	Yes	Yes	10	No	NEG	NEG	No
5	Non GCB	No	Yes	Yes	No	40	No	NEG	NEG	No
6	GCB	Yes	Yes	No	Yes	40	No	REA	NEG	No
7	GGB	Yes	Yes	Yes	No	80	No	NEG	NEG	No
8	Non GCB	No	No	Yes	Yes	40	No	NEG	NEG	No
9	Non GCB	No	No	Yes	Yes	10	No	NEG	NEG	No
10	Non GCB	No	Yes	Yes	Yes	20	No	NEG	REA	No
11	GCB	No	Yes	No	Yes	1	No	AMP	NEG	No
12	Non GCB	No	Yes	Yes	Yes	80	Yes	NEG	NEG	No
13	GCB	Yes	Yes	Yes	Yes	60	Yes	NEG	AMP	No
14	Non GCB	No	Yes	Yes	Yes	10	No	AMP	AMP	No
15	Non GCB	No	Yes	Yes	Yes	N/A	N/A	N/A	N/A	N/A
16	Non GCB	No	Yes	Yes	Yes	N/A	N/A	N/A	N/A	N/A
17	Non GCB	No	No	N/A	Yes	N/A	N/A	N/A	N/A	N/A
18	Non GCB	No	Yes	Yes	Yes	N/A	N/A	N/A	N/A	N/A
19	Non GCB	No	Yes	Yes	Yes	N/A	N/A	N/A	N/A	N/A
20	Non GCB	No	No	N/A	No	N/A	N/A	N/A	N/A	N/A
21	Non GCB	No	No	N/A	no	N/A	N/A	N/A	N/A	N/A

FISH – fluorescence in situ hybridisation; GCB – germinal centre B cell; N/A – not applicable

Morphological, immunohistochemical, and FISH analysis

A centroblastic morphology was shown in all cases. All cases were CD20 positive. GC marker, CD 10 was expressed by three of 21 (14.0%), Bcl-6 by 13 of 21 (61.0%), and MUM-1 by 16 of 18 (88.0%) PT-DLBCL cases (Table II). Most cases of PT-DLBCL (17 of 21, 80.95%) had a non GCB-like immunophenotype. Four of 21 (19.0%) cases were classified as GCB-like subtype. Two of four GCB cases had all three markers positive (CD10+, Bcl-6+, MUM-1+). In the control group, 23 of 30 cases (76.0%) were non-GCB subtype ($p > 0.05$).

Bcl-2 protein expression was detected in 16 of 21 (76.0%) PT-DLBCL patients and in 67.0% of patients in the DLBCL NOS control group ($p > 0.05$).

C-Myc protein was overexpressed in eight of 14 cases (57.0%), compared to 23.0% in the control group ($p < 0.05$; Fig. 1). There was an observed difference in c-Myc protein expression in the PT-DLBCL group (median 40.0%, $n = 14$) and control group (median 10.0%, $n = 29$) (Mann Whitney test, $U = 111.50$, $z = –2.415$, $p = 0.016$).

Concurrent protein expression of c-Myc and Bcl-2 was present in 5 of 14 PT-DLBCL patients (36.0%), and classified as "double expressors". In the control group, there was a 13.0% rate of "double expressors" ($p > 0.05$).

In total, four of 14 (28.5%) patients showed *MYC* alterations by FISH in the PT-DLBCL group. MYC rearrangement was detected in one of 14 (7.0%), and MYC amplification in three of 14 (21.0%) patients (Fig. 2). One case with MYC rearrangement lacked BCL2 rearrangement, but showed c-Myc and Bcl-2 protein overexpression, and GCB immunophenotype. Two PT-DLBCL cases (two of four, 50.0%) with MYC alterations showed c-Myc staining in ≥ 40.0% of tumour cells (range 40.0-80.0%). Six cases with positive c-Myc protein expression did not have MYC alterations. Two cases with MYC amplification also had BCL2 amplification. In the control DLBCL group, three patients (10.0%) had MYC rearrangement, and three patients had amplifications (10.0%).

One of the 14 cases (7.0%) in the PT-DLBCL group showed BCL2 rearrangement, four of 14 (28.05%) cases

showed *BCL2* amplification. Four of five cases with *BCL2* alterations showed Bcl-2 positivity by immunohistochemistry. One patient had BCL2 translocation associated with the non-GCB subtype (Table II).

In the mentioned cases with MYC amplification signals, at least five of them were seen in each of the analysed nuclei.

Therapy response and survival

Complete remission (CR) was achieved in 15 of 20 PT-DLBCL patients (75.0%), while four patients had primary resistant disease. Three patients out of four with resistant disease died within two years following the diagnosis. Among the patients who achieved remission, four out of 16 PT-DLBCL patients (25.0%) relapsed. During the follow-up period, eight patients (40.0%) with PT-DLBCL succumbed to the disease. Regarding control group, CR was achieved in 24 patients (80.0%).

The median follow-up of PT-DLBCL patients was 22.5 months (range 7-119 months). The median overall survival (OS) for the PT-DLBCL group was 48 months (95% CI: 21.084-74.916) (Fig. 3). PT-DLBCL patients with low and low-intermediate IPI had median OS of 48 months vs. 24 months for high-intermediate and high IPI (95% CI: 21.084-74.916, log-rank = 3.192, p = 0.074). The PT-DLBCL patients who achieved CR had significantly superior OS compared to those who did not achieve CR (median 48 months vs. 21 months, log-rank = 6.240, p = 0.012). When other clinical, morphological, immunohistochemical, and biological parameters were analysed by Kaplan-Meyer method and log-rank test, none of them showed any influence on survival. Regarding the control DLBCL group, OS was influenced by the presence of B symptoms (log-rank = 7.777, p = 0.027), IPI (log-rank = 10.932, p = 0.001), achievement of CR (log-rank = 37.884, p < 0.0001), and detected amplification of CMYC by FISH (log-rank = 8.977, p = 0.011).

Discussion

Primary testicular diffuse large B-cell lymphoma is the most common malignant testicular tumour in men over the age of 60 years [3, 6]. In our study, age, clinical stage, and B symptoms were in accordance with literature data [3, 6].

Despite significant progress in treatment of DLBCL patients, with the addition of rituximab to standard chemotherapy, radiotherapy, and central nervous system (CNS) prophylaxis, the outcome is fatal in almost half of patients with DLBCL [6, 19, 20, 21]. The role of CNS prophylaxis and radiotherapy is still unknown [20, 21]. The outcome of PT-DLBCL has been inferior compared to nodal DLBCL, according to the specificity of testicular anatomic and

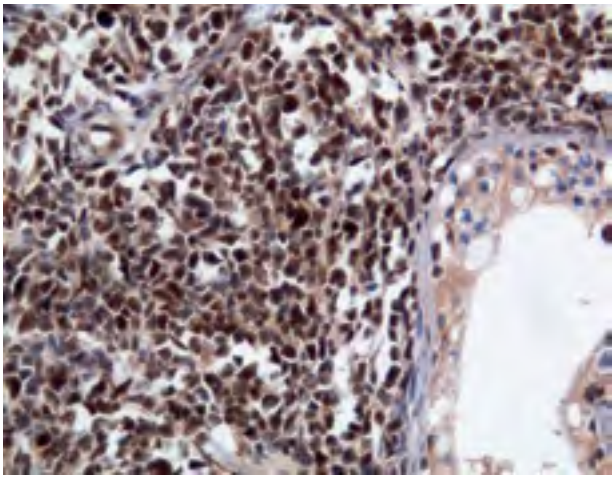

Fig. 1. c-Myc protein expression in PT-DLBCL (immunohistochemistry c-Myc, original magnification 200×)

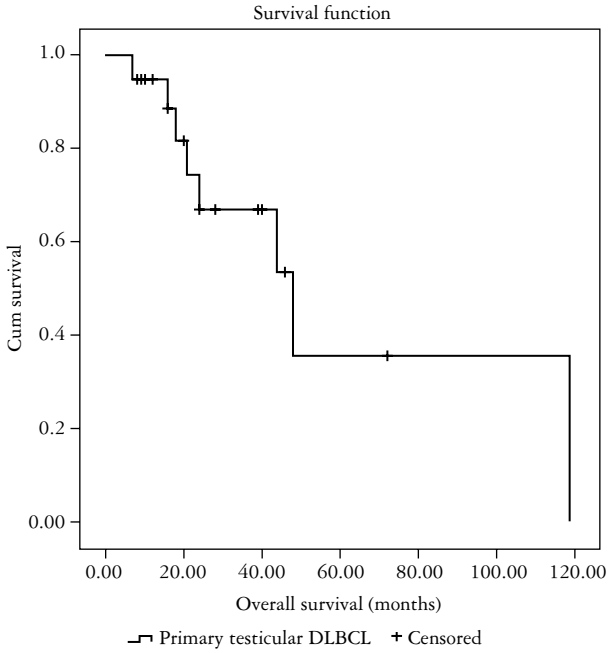

Fig. 2. Overall survival of PT-DLBCL

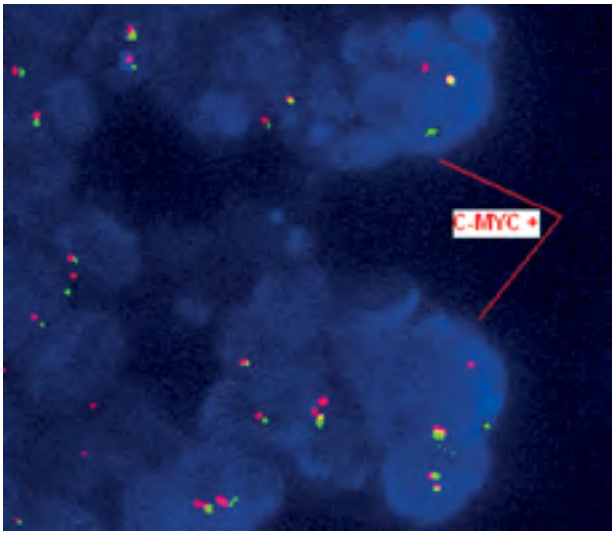

Fig. 3. MYC rearrangement in PT-DLBCL (FISH)

functional properties [1, 2]. Regarding OS, in the current study, our data suggest inferior survival of PT-DLBCL, potentially due to disease aggressiveness. Furthermore, many reports have indicated that IPI has an important role as a prognostic index on the survival of DLBCL patients [22]. This was confirmed in our control DLBCL group, where IPI retained its prognostic value. Regarding PT-DLBCL, our data confirm that the majority of PT-DLBCL patients present with limited stage disease, and have typically low and low-intermediate IPI risk, which has, however, limited prognostic utility.

Although clinical factors may contribute to the outcome after rituximab-based therapy, there is a need for evaluation of molecular and genetic markers associated with survival [23]. Booman *et al.* [7], demonstrated that PT-DLBCL has an almost exclusive activated B cell (ABC) type of gene expression, while one-third of the same lymphomas show an ambiguous protein expression pattern with strong co-expression of both germinal centre and activated B-cell markers. Most cases of PT-DLBCL in the study of Deng *et al*. [23] had a non-GCB-like (84.0%) immunophenotype and an ABC-like (86.0%) gene expression profile (GEP) subtype. In our study, most of the PT-DLBCL cases (81.0%) showed non-GCB immunophenotype (compared to 76.0% of control DLBCL patients); two were clearly GCB, and two cases were ambiguous with CD10+, Bcl-6+, and MUM-1+. Some authors reclassified ambiguous cases as ABC type by gene expression analysis [7]. This predominance of non-GCB-like type may partially account for the historically poor outcomes from PT-DLBCL [1].

The prognostic significance of Bcl-2 protein expression in DLBCL is controversial, but some recent studies have shown it to be associated with aggressive behaviour and shortened overall survival [13, 14, 15, 24]. However, it is observed that Bcl-2 overexpression has prognostic value in the GCB subtype only, related to the concomitant presence of BCL2 translocations, which are more frequently found in the GCB subtype [24]. In our study, Bcl-2 protein expression was seen in 76.0% of non-GCB-like PT-DLBCL cases. BCL2 translocation was observed in only one patient and was associated with a non-GCB immunophenotype. The incidence of the BCL2 genetic changes and Bcl-2 protein expression in our study is similar to that found in other series [12, 13, 14, 15].

Our data support the results of previous studies that have reported c-Myc protein to be overexpressed in a higher percentage of PT-DLBCL patients compared to other DLBCL cases [2, 12, 13, 14, 15]. Regarding FISH analysis, in our study, MYC rearrangement was observed in 7.0% of PT-DLBCL cases, which did not significantly differ from DLBCL patients in the control group, and was in accordance with previously reported data [11, 12, 24, 25].

We did not detect MYC/BCL2 double-hit cases by FISH, but "double-expressor" DLBCL cases were present in 36.0% of PT-DLBCL patients. In the control group, there were 13.0% of "double-expressor" cases. Patients with c-MYC/Bcl-2-positive tumours had significantly inferior outcomes compared with patients with tumours that did not display both c-MYC and Bcl-2 positivity [11, 12, 13, 14, 25, 26, 27].

Our results confirm previously published data showing more frequent occurrence of non-GCB subtype among PT-DLBCL. Despite the evident pathologic overlap between PT-DLBCL and systemic or nodal DLBCL, the introduction of high-throughput sequencing procedures revealed numerous mutated genes that contribute to sustained signalling pathways in PT-DLBCL [28]. BCR, NF-κB, and PI3K signalling are specifically deregulated in PT-DLBCL, which represent potential drug targets. Ibrutinib, lenalidomide, checkpoint inhibitors, and other agents are currently under investigation in not only systemic and nodal DLBCL patients, but also in PT-DLBCL [28]. They might contribute to improved outcome of PT-DLBCL. However, future studies are needed to reveal weather PT-DLBCL truly has the mutational underpinnings and phenotypic profile that will classify this entity separately from nodal non-GCB DLBCL and provide specific therapeutic strategies for PT-DLBCL.

Conclusions

Data from our study demonstrate that PT-DLBCL has immunohistochemical and biological features that differ from its nodal and other extranodal counterparts. Regarding clinical data that might support such a conclusion, the IPI lost its prognostic significance in PT-DLBCL compared to the control group. Despite the limitations of the current study, it emphasises the variability of PT-DLBCL, potential inferior survival, and disease aggressiveness. Because of the rarity of PT-DLBCL, prospective multinational studies are needed to advance our understanding of the biology of this tumour and revision of the current treatment approach.

The authors declare no conflict of interest.

References

1. Cheah CY, Wirth A, Seymour JF. Primary testicular lymphoma. Blood 2014; 123: 486-493.
2. Menter T, Ernst M, Drachneris J, et al. Phenotype profiling of primary testicular diffuse large B-cell lymphomas. Hematol Oncol 2014; 32: 72-81.
3. Zucca E, Conconi A, Mughal TI, et al.; International Extranodal Lymphoma Study Group. Patterns of outcome and

prognostic factors in primary large-cell lymphoma of the testis in a survey by the international extranodal lymphoma study group. J Clin Oncol 2003; 21: 20-27.
4. Hans CP, Weisenburger DD, Greiner TC, et al. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood 2004; 103: 275-282.
5. Fonseca R, Habermann TM, Colgan JP, et al. Testicular lymphoma is associated with a high incidence of extranodal recurrence. Cancer 2000; 88: 154-161.
6. Gundrum JD, Mathiason MA, Moore DB, et al. Primary testicular diffuse large B-cell lymphoma: a population-based study on the incidence, natural history, and survival comparison with primary nodal counterpart before and after the introduction of rituximab. J Clin Oncol 2009; 27: 5227-5232.
7. Booman M, Douwes J, Glas AM, et al. Primary testicular diffuse large B-cell lymphomas have activated B-cell-like subtype characteristics. J Pathol 2006; 210: 163-171.
8. Stein H, Warnke RA, Chan WC, et al. Diffuse large B-cell lymphoma, not otherwise specified. In: Swerdlow SH, Campo E, Harris NL, et al. (eds.). WHO classification of tumours of haematopoietic and lymphoid tissues. World Health Organization Classification of Tumours. IARC, Lyon 2008.
9. Scott DW, Wright GW, Williams PM, et al. Determining cell-of-origin subtypes of diffuse large B-cell lymphoma using gene expression in formalin-fixed paraffin-embedded tissue. Blood 2014; 123: 1214-1217.
10. Swerdlow SH, Campo E, Pileri SA, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Blood 2016; 127: 2375-2390.
11. Karube K, Campo E. MYC alterations in diffuse large B-cell lymphomas. Semin Hematol 2015; 52: 97-106.
12. Swerdlow SH. Diagnosis of 'double hit' diffuse large B-cell lymphoma and B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma: when and how, FISH versus IHC. Hematology Am Soc Hematol Educ Program 2014; 2014: 90-99.
13. Horn H, Ziepert M, Becher C, et al. German High-Grade Non-Hodgkin Lymphoma Study Group. MYC status in concert with BCL2 and BCL6 expression predicts outcome in diffuse large B-cell lymphoma. Blood 2013; 121: 2253-2263.
14. Green TM, Young KH, Visco C, et al. Immunohistochemical double-hit score is a strong predictor of outcome in patients with diffuse large B-cell lymphoma treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone. J Clin Oncol 2012; 30: 3460-3467.
15. Johnson NA, Slack GW, Savage KJ, et al. Concurrent expression of MYC and BCL2 in diffuse large B-cell lymphoma treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone. J Clin Oncol 2012; 30: 3452-3459.
16. The International Non-Hodgkin's Lymphoma Prognostic Factors Project. A Predictive Model for Aggressive Non-Hodgkin's Lymphoma. N Engl J Med 1993; 329: 987-994.
17. Cheson BD, Horning SJ, Coiffier B, et al. Report of an international workshop to standardize response criteria for non-Hodgkin's lymphomas, NCI Sponsored International Working Group. J Clin Oncol 1999; 17: 1244.
18. Chin SF, Daigo Y, Huang HE, et al. A simple and reliable pretreatment protocol facilitates fluorescent in situ hybridisation on tissue microarrays of paraffin wax embedded tumour samples. Mol Pathol 2003; 56: 275-279.
19. Chapuy B, Roemer MG, Stewart C, et al. Targetable genetic features of primary testicular and primary central nervous system lymphomas. Blood 2016; 127: 869-881.
20. Schmitz N, Nickelsen M, Savage KJ. Central nervous system prophylaxis for aggressive b-cell lymphoma: who, what, and when? Hematol Oncol Clin North Am 2016; 30: 1277-1291.
21. Kridel R, Telio D, Villa D, et al. Diffuse large B-cell lymphoma with testicular involvement: outcome and risk of CNS relapse in the rituximab era. Br J Haematol 2017; 176: 210-221.
22. Vaidya R, Witzig TE. Prognostic factors for diffuse large B-cell lymphoma in the R(X)CHOP era. Ann Oncol 2014; 25: 2124-2133.
23. Deng L, Xu-Monette ZY, Loghavi S, et al. Primary testicular diffuse large B-cell lymphoma displays distinct clinical and biological features for treatment failure in rituximab era: a report from the International PTL Consortium. Leukemia 2016; 30: 361-372.
24. Visco C, Tzankov A, Xu-Monette ZY, et al. Patients with diffuse large B-cell lymphoma of germinal center origin with BCL2 translocations have poor outcome, irrespective of MYC status: a report from an International DLBCL rituximab-CHOP Consortium Program Study. Haematologica 2013; 98: 255-263.
25. Bernasconi B, Uccella S, Martin V, et al. Gene translocations in testicular lymphomas. Leuk Lymphoma 2014; 55: 1410-1412.
26. Twa DD, Mottok A, Chan FC, et al. Recurrent genomic rearrangements in primary testicular lymphoma. J Pathol 2015; 236: 136-141.
27. Wang XJ, Medeiros LJ, Lin P, et al. MYC cytogenetic status correlates with expression and has prognostic significance in patients with MYC/BCL2 protein double-positive diffuse large B-cell lymphoma. Am J Surg Pathol 2015; 39: 1250-1258.
28. Twa DDW, Mottok A, Savage KJ, et al. The pathobiology of primary testicular diffuse large B-cell lymphoma: Implications for novel therapies. Blood Rev 2018; 32: 249-255.

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