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ARTICLE

MYC/BCL2/BCL6 triple hit lymphoma: a study of 40 patients with a comparison to *MYC/BCL2* and *MYC/BCL6* double hit lymphomas

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Abstract

High-grade B-cell lymphomas with *MYC*, *BCL2*, and *BCL6* rearrangements (triple hit lymphoma) are uncommon. We studied the clinicopathologic features of 40 patients with triple hit lymphoma and compared them to 157 patients with *MYC/BCL2* double hit lymphoma and 13 patients with *MYC/BCL6* double hit lymphoma. The triple hit lymphoma group included 25 men and 15 women with a median age of 61 years (range, 34−85). Nine patients had a history of B-cell lymphoma. Histologically, 23 (58%) cases were diffuse large B-cell lymphoma and 17 cases had features of B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma. Most cases of triple hit lymphoma were positive for CD10 (100%), BCL2 (95%), BCL6 (82%), MYC (74%), and 71% with MYC and BCL2 coexpression. P53 was overexpressed in 29% of triple hit lymphoma cases. The clinicopathological features of triple hit lymphoma patients were similar to patients with *MYC/BCL2* and *MYC/BCL6* double hit lymphoma, except that triple hit lymphoma cases were more often CD10 positive compared with *MYC/BCL6* double hit lymphoma ($p < 0.05$). Induction chemotherapy used was similar for patients with triple hit lymphoma and double hit lymphoma and overall survival in triple hit lymphoma patients was 17.6 months, similar to the overall survival of patients with double hit lymphoma ($p = 0.67$). Patients with triple hit lymphoma showing P53 overexpression had significantly worse overall survival compared with those without P53 overexpression ($p = 0.04$). On the other hand, double expressor status and prior history of B-cell lymphoma did not correlate with overall survival. In conclusion, most patients with triple hit lymphoma have an aggressive clinical course and poor prognosis and these tumors have a germinal center B-cell immunophenotype, similar to patients with double hit lymphomas. P53 expression is a poor prognostic factor in patients with triple hit lymphoma.

Introduction

It is well known that chromosomal translocations occur commonly in various types of B-cell non-Hodgkin lymphoma. *MYC*, located at chromosome 8q24, encodes an important transcription factor that is responsible for many cellular functions including proliferation, growth, apoptosis, and differentiation [1, 2]. *MYC* translocation is a hallmark of Burkitt lymphoma [3], but *MYC* translocation also can present less often in other types of B-cell lymphoma, including 5−14% of diffuse large B-cell lymphoma [4–7], and rarely in follicular lymphoma, mantle cell lymphoma, and chronic lymphocytic leukemia [8–10]. *BCL2*, located at chromosome 18q21, encodes an integral outer mitochondrial membrane protein that inhibits cell apoptosis. *BCL2* translocation, usually in the form of t(14;18)(q32;q21), is the hallmark of follicular lymphoma, but can be present in 20−30% of de novo diffuse large B-cell lymphoma [11].

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BCL6 encodes a transcriptional repressor within germinal center B cells mediating lymphomagenesis through aberrant proliferation, apoptosis, and differentiation blockade and is located at chromosome 3q27. *BCL6* translocation is the most common genetic abnormality in diffuse large B-cell lymphoma, in approximately 30−40% of cases [12, 13].

MYC translocation can be associated with *BCL2* or *BCL6* translocations in about 5−10% of cases of large B-cell lymphoma, also known as double hit lymphoma, of either *MYC/BCL2* or *MYC/BCL6* type. Large B-cell lymphomas with concurrent *MYC*, *BCL2*, and *BCL6* translocations are also designated as triple hit lymphoma [14]. *MYC/BCL2* double hit lymphoma is much more common than *MYC/BCL6* double hit lymphoma or triple hit lymphoma. Using 2008 World Health Organization (WHO) classification, most cases of double hit lymphoma and triple hit lymphoma were classified as either diffuse large B-cell lymphoma or as B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma. In the 2017 revision of the WHO classification, double hit lymphoma/triple hit lymphoma is placed in a new category designated as high-grade B-cell lymphoma with rearrangements of *MYC* and *BCL2* and/or *BCL6* [14].

Multiple retrospective studies have shown that patients with double hit lymphoma usually have an aggressive clinical course and very poor outcome [15–24]. Extranodal disease, a high serum lactate dehydrogenase level, bone marrow and central nervous system involvement and a higher international prognostic index score are frequent in double hit lymphoma patients. To date, many case series of double hit lymphoma, especially *MYC/BCL2* double hit lymphoma, have been reported in the literature, but relatively few cases of triple hit lymphoma have been reported. In this study, we report 40 cases of *MYC/BCL2/BCL6* triple hit lymphoma, the largest series to date, and systemically compared them with 157 cases of *MYC/BCL2* double hit lymphoma and 13 cases of *MYC/BCL6* double hit lymphoma to better characterize the clinicopathologic features and prognosis of triple hit lymphoma.

Materials and methods

Case selection

Forty cases of high-grade B-cell lymphoma with *MYC, BCL2*, and *BCL6* rearrangements diagnosed between January 1, 2011 and December 31, 2016 were included in this study. A small subset of cases has been reported previously [25]. All the cases were diagnosed as either diffuse large B-cell lymphoma or B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma based on their morphological features and all cases were classified as high-grade B-cell lymphoma with *MYC, BCL2*, and *BCL6* rearrangements based on the current WHO classification. The study group was further compared to 157 cases of *MYC/BCL2* double hit lymphoma and 13 cases of *MYC/BCL6* double hit lymphoma that have been reported previously [18, 19, 21] (Table S1). The overall survival (overall survival) of patients with triple hit lymphoma was also further compared with a group ($n = 467$) of patients with diffuse large B-cell lymphoma without *MYC* rearrangement.

Clinical data were collected from the electronic medical records, including a history of lymphoma, number and sites of involvement, Ann Arbor stage, international prognostic index score, treatment regimens, response to therapy, and overall survival. This is a retrospective study that was approved by the institutional review board of MD Anderson Cancer Center.

Immunophenotyping

Immunohistochemical analysis was performed using formalin-fixed, paraffin-embedded tissue sections, either at the time of diagnosis or retrospectively for the purpose of this study. The panel of monoclonal antibodies used was variable over time but included reagents specific for CD3 and CD20 (Ventana Medical Systems, Tucson, Arizona, USA); CD5, CD10, BCL2, BCL6, and MUM1/IRF4 (Leica Microsystems, Buffalo Grove, IL, USA); MYC (Epitomics, Burlingame, CA, USA); P53 (Leica Biosystems, Newcastle, UK); and Ki-67 (MIB-1) (DAKO, Carpinteria, CA, USA). The cutoffs for CD10, BCL6, and MUM1 positivity were 30% as used in the Hans algorithm [26]. The positive cutoffs for MYC, BCL2, and P53 expression were ≥40%, ≥50%, and ≥50% of cells, respectively, as have been reported previously [21, 27–31].

Conventional cytogenetics and fluorescence in situ hybridization

Conventional G band karyotype analysis was performed on nine cases of triple hit lymphoma, 46 cases of *MYC/BCL2* double hit lymphoma and one case of *MYC/BCL6* double hit lymphoma. The karyotypes were reported according to the 2016 International System for Human Cytogenetic Nomenclature [32].

Fluorescence in situ hybridization analysis for *MYC, BCL6*, and *BCL2* was performed in all cases using the LSI *MYC* or *BCL6* dual-color break-apart and LSI *IGH@BCL2* dual-color, dual fusion probes (Abbott Laboratories, Des Plaines, IL, USA). Triple hit lymphoma cases were identified if they had concurrent rearrangements of *MYC, BCL2*, and *BCL6*. For bone marrow aspirate specimens, fluorescence in situ hybridization was performed by using a freshly

dropped slide from a harvested bone marrow or a G-banded slide for metaphase mapping according to the manufacturer's instructions. For formalin-fixed, paraffin-embedded tissue samples, fluorescence in situ hybridization was performed on 4-μm-thick tissue sections and fixed onto slides according to the manufacturer's protocol. The signals from 200 nuclei were analyzed.

The cutoffs for considering a tumor sample positive for rearrangement of *MYC, BCL2*, and *BCL6* were different in bone marrow smears vs. formalin-fixed, paraffin-embedded tissue sections. However, these cutoffs were low (all <5% in bone marrow smears and <10% in formalin fixed, paraffin-embedded tissue sections), and all cases in this study with *MYC, BCL2*, and *BCL6* rearrangements had abnormal signals present in >20% of all nuclei assessed.

Statistical analysis

Overall survival was calculated from date of diagnosis to the date of death or last follow-up. Patient survival was analyzed using the Kaplan−Meier method and compared using the log-rank test (GraphPad Prism version 7 software). Fisher's exact test was used to compare differences between groups. A p value of less than 0.05 was considered statistically significant.

Results

Clinical characteristics

The study cohort included 25 men and 15 women with a median age of 61 years (range, 34−85). Thirty-one (77%) patients presented with de novo lymphoma and nine (23%) patients had a history of B-cell lymphoma, including eight follicular lymphoma and one diffuse large B-cell lymphoma without *MYC* abnormalities. Thirty-three (83%) patients had extranodal sites of disease at diagnosis, and 28 (70%) had two or more extranodal sites of involvement. Involved extranodal sites included: bone marrow, central nervous system, stomach, colon, kidneys, breasts, liver, spleen, skin, soft tissue, bones, and testes. Bone marrow involvement was observed in 20 of 36 (56%) patients assessed, 11 of the 20 cases were concordant (diffuse large B-cell lymphoma or HGBL) involvement while the other nine cases were involved by low grade B-cell lymphoma. The central nervous system was involved in 4 of 22 (18%) patients who underwent analysis of cerebrospinal fluid. The serum lactate dehydrogenase level was elevated in 24 of 31 (77%) patients tested. Thirty-four of 38 (89%) patients presented with high Ann Arbor stage (III/IV) disease, and 30 of 37 (81%) patients had high-intermediate to high international prognostic index score (Table 1).

Pathologic characteristics

Histologically, all tumors had a diffuse pattern (Fig. 1). A prominent starry sky pattern was present in 13 (33%) cases and 10 (25%) tumors showed a variable amount of coagulative necrosis. Based on morphology, 23 (58%) cases were classified as diffuse large B-cell lymphoma and 17 were B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma. Three cases had concurrent existence of another type of lymphoma: two follicular lymphoma and one mantle cell lymphoma.

Immunohistochemical analysis (Fig. 1) showed that all 39 (100%) tumors assessed were positive for CD10 and other markers were positive as follows: 35 of 37 (95%) for BCL2, 28 of 34 (82%) for BCL6, 23 of 31 (74%) for MYC, 8 of 19 (42%) for MUM1/IRF4, and 7 of 24 (29%) for P53. The median Ki-67 proliferation rate was 80% (range, 60−100%). Twenty-two of 31 (71%) tumors with available data showed coexpression of MYC and BCL2. Thirty-nine of 40 (98%) tumors had a germinal center B-cell immunophenotype according to the Hans algorithm.

Conventional cytogenetic analysis was performed on nine cases, and a complex karyotype was detected in all nine cases (Table 2). The t(14;18)(q32;q21) was present in all nine cases that suggest BCL2 was always rearranged with *IGH*. The rearrangement partner of 3q27/*BCL6* was an immunoglobulin (*IG*) locus in three cases and a non-*IG* locus in six cases (including three with 8q24/*MYC*). 8q24/*MYC* was translocated with an *IG* locus in four cases and a *non-IG* gene locus in five cases. Fluorescence in situ hybridization analysis was performed and rearrangements for *MYC, BCL2*, and *BCL6* were detected in all 40 cases (Fig. 1).

Treatment and response

Detailed treatment information was available for 36 patients, all of whom received immune-chemotherapy induction. Twelve patients were treated initially with rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP). Twenty-three patients received more aggressive regimens, including 18 with rituximab, etoposide, prednisone, vincristine, and doxorubicin (R-EPOCH) and three with rituximab hyperfractionated, cyclophosphamide, vincristine, adriamycin, dexamethasone (R-HyperCVAD). The remaining two patients had a history of follicular lymphoma and received other regimens: one with rituximab, ifosfamide, carboplatin, and etoposide (R-ICE) and one with rituximab, dexamethasone, cytarabine, cisplatin (R-DHAP). After the initial treatment, 14 of 32 (44%) patients with complete follow-up achieved a complete remission; 12 (38%)

Table 1 Comparison of clinicopathologic features of THL to MYC/BCL6 DHL to MYC/BCL2 DHL

Features	MYC/BCL2/BCL6 THL (n=40) % (Positive/Evaluated)	MYC/BCL2 DHL (n=157) % (Positive/Evaluated)	MYC/BCL6 DHL (n=13) % (Positive/Evaluated)
Age (yrs), Median (range)	61 (34−85)	61 (18−87)	63 (34−86)
Age > 60 (yrs)	50 (20/40)	57 (89/157)	54 (7/13)
Male:Female	25:15	103:54	8:5
Previous FL/B-NHL	23 (9/40)	31 (49/157)	8 (1/13)
BM positive	56 (20/36)	50 (68/136)	36 (4/11)
CNS positive	18 (4/22)	18 (14/78)	50 (3/6)
Extranodal sites ≥ 2	70 (28/40)	54 (85/157)	82 (9/11)
Elevated serum LDH	77 (24/31)	84 (107/128)	67 (6/9)
Stage III or IV	89 (34/38)	87 (125/144)	82 (9/11)
High-Intermediate/ High IPI	81 (30/37)	82 (111/135)	80 (8/10)
Morphology			
DLBCL	58 (23/40)	58.5 (96/157)	85 (11/13)
HGBCL	43 (17/40)	39 (61/157)	15 (2/13)
GCB subtype	98 (39/40)	99 (151/152)	90 (9/10)
Immunophenotype			
CD10	100 (39/39)	98 (148/151)	75 (9/12)*
BCL6	82 (28/34)	92 (90/98)	100 (11/11)
BCL2	95 (35/37)	91 (129/141)	80 (8/10)
MYC	74 (23/31)	80 (48/60)	67 (2/3)
MYC/BCL2 Coexpress	71 (22/31)	71 (42/59)	33 (1/3)
P53	29 (7/24)	30 (14/47)	50 (2/4)
Ki67, Median (range)	80 (60-100)	85 (20-100)	80 (60-100)
Complex karyotype	100 (9/9)	100 (46/46)	100 (1/1)
Initial chemotherapy			
R-CHOP	33 (12/36)	38 (54/144)	55 (6/11)
Aggressive regimens	64 (23/36)	48 (68/144)	45 (5/11)
With SCT	31 (11/36)	27 (39/144)	18 (2/11)
CR after initial chemo	44 (14/32)	44 (63/144)	27 (3/11)

*$p < 0.05$ compared to THL

patients showed a partial response; and 6 (19%) patients had persistence or progression of disease (primary refractory). Three of six primary refractory patients had a history of follicular lymphoma. Eleven of 36 (31%) patients underwent stem cell transplant (stem cell transplant), including ten autologous and one allogeneic. With a median follow-up of 13 months (range, 1−47 months), 13 (33%) patients died with 11 (85%) of them died within 12 months of diagnosis. Four patients with initial complete remission died after disease relapse. The median overall survival was 18 months and the 1-year overall survival rate was 61%.

Prognostic factors in triple hit lymphoma

Univariate analysis showed that among the ten clinicopathologic factors analyzed, older age (≥60 years) and P53 overexpression were associated with a worse prognosis (Table 3). Although patients with tumors overexpressed P53 had a significantly worse overall survival than those without P53 overexpression (Fig. 2a, $p = 0.04$), there was no significant difference in overall survival between triple hit lymphoma with MYC and BCL2 double expression (double expressor) and those without double expressor (Fig. 3a, $p = 0.46$), and

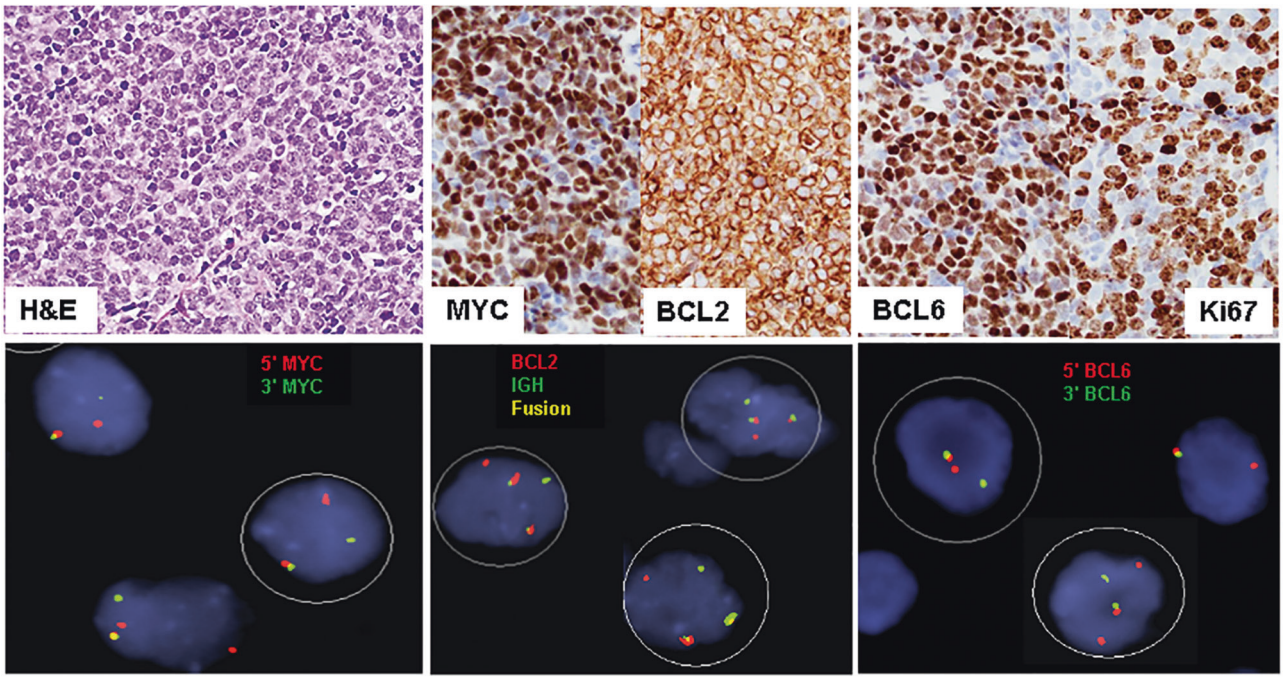

Fig. 1 A representative case of high grade B-cell lymphoma with *MYC, BCL2*, and *BCL6* rearrangements (triple hit lymphoma) **a** H&E, with high MYC (**b**), BCL2 (**c**), BCL6 (**d**), and Ki67 (**e**) expression (**a**–**e**, ×400), and rearrangement of *MYC, BCL2*, and *BCL6* by fluorescence in situ hybridization (**f**–**h**)

Table 2 Conventional cytogenetic findings in MYC/BCL2/BCL6 THL

Case	Cytogenetics
1	44,XX,+1,del(1)(q32q44),der(1;7)(q10;p10),t(3;11)(q26.2;q21),−4,del(4)(p14),+7,+7,der(8)t(8;14)(q24.1;q32)t(14;18)(q32;q21.3),der(14)t(8;14),der(18)t(14;18),der(15;22)(q10;q10),−17,−20[1]/46, XX[19]
3	73~82<3n>,XXY,+X,+3,t(3;4)(q27;q22)x2,+4,+5,-6,-9,del(9)(p13),del(10)(q24q26)x2,+11,+12,add(12)(p13)x2,−13,der(13)ins(13;8)(q32;q24.1q24.1)t(8;?)(q24.1;?),−14,t(14;18)(q32;q21.1),del(16)(p11.2)x2,+17,add(17)(p12)x2,−18,−19,add(20)(q13.3),+21,+22,+7~10mar[cp13]/46,XY[7]
6	84~87,XXYY,+X,add(1)(q21),-2,+3,+3,+3,der(3)t(3;8)(q27;q24),del(3)(q11.2)x2,del(3)(q21)x2,−4,−6,add(7)(q36)x2,−8,ider(8)(q10)t(3;8),–10,−10,−11,t(14;18)(q32;q21)x2,−15,−17,−17,+18,+18,-19,−19,−19,−22,−22,+2~4mar[cp4]/46,XY[16]
9	49,X,i(X)(q10),add(2)(p23),der(3)t(3;8)(q27;q24.1),+7,add(7)(q22),add(8)(q13),der(10)t(10;?)(q22:?)ins(?;8)(?;q24.1)x2,−13,+14,add(14)(p11.2),t(14;18)(q32;q21.3),+2mar[3]/47~51,X,i(X)(q10),add(2)(p23),der(3)t(3;8)(q27;q24.1),del(6)(q13q23),+7,add(7)(q22),add(8)(q13),add(10)(q22),der(10)t(10;?)(q22:?)ins(?;8)(?;q24.1)x2,−13,+14,add(14)(p11.2), t(14;18)(q32;q21.3),+22,+2mar[cp12]/46,XX,t(11;14)(p15;q21)[1]/46,XX[4]
18	47,XY,t(3;22)(q27;q11.2),+i(6)(p10),t(8;14)(q24.2;q32.3),add(10)(p13),t(14;18)(q32.3;q21.3)[cp15]/46,XY[5]
29	39-47,XY,−2,del(3)(q21q27)der(4)t(1;4)(q21;q35),t(8;18)(q24;q32),−9,t(14;18)(q32;q21.3),add(16)(p13.3)−17,+1−4mar[cp11]/46,XY[1]
34	49~50,Y,add(x)(p22.1),+5,+8,+12,del(13)(q12q22),t(14;18)(q32;q21),+mar[cp11]/46,XY[9]
35	50,XY,t(3;8)(q27;q24.2),+der(8)t(3;8),add(13)(q34),+der(18)t(18;22)(q21.1;q11.2),t(18;22),+2mar[11]/46,XY[9]
37	49,XY,XY,?t(2;14)(p13;q32),+?add(5)(q12),+7,?add(11)(q23),del(13)(q12q14),t(14;18)(q32;q21),add(22)(q11.2);+mar[cp18]/46,XY[2]

between patients who had a history of B-cell lymphoma and those with de novo lymphoma (Fig. 3b, $p = 0.34$). Similarly, the median overall survival in those patients who received a stem cell transplant was not significantly different from those who did not receive stem cell transplant ($p = 0.24$).

Comparison of *MYC/BCL2/BCL6* triple hit lymphoma to double hit lymphoma

The clinicopathologic features of the triple hit lymphoma cohort were compared to those of 157 patients with *MYC/*

Table 3 Univariate analysis for OS in triple hit lymphoma (THL)

Factors	HR	95% CI	*p*
Age ≥60 (yrs)	2.67	1.04−7.68	**0.04**
Male:Female	0.93	0.31−2.76	0.89
Previous FL/B-NHL	1.76	0.45−6.84	0.33
BM positive	1.21	0.41−3.64	0.73
Extranodal sites ≥ 2	1.31	0.25−2.37	0.61
Elevated serum LDH	2.97	0.74−11.83	0.27
Stage III/IV	3.09	0.57−16.63	0.19
High-intermediate/High IPI	2.46	0.58−10.46	0.37
MYC/BCL2 double expression	1.76	0.46−6.77	0.46
P53 ≥ 50%	3.17	1.05−11.93	**0.04**

Bold values indicate *P* value was < 0.05

BCL2 double hit lymphoma and 13 with *MYC/BCL6* double hit lymphoma (Table 1). Compared to *MYC/BCL6* double hit lymphoma, cases with triple hit lymphoma more often expressed CD10 ($p < 0.05$). Both the range and mean expression level of p53 in triple hit lymphoma was not significantly different from *MYC/BCL2* double hit lymphoma (Fig. 2b, $p = 0.96$). Only four cases in *MYC/BCL6* double hit lymphoma group had a P53 results and therefore they were not included in this comparison. All other clinicopathologic features of triple hit lymphoma including induction chemotherapy were similar to the *MYC/BCL2* double hit lymphoma and *MYC/BCL6* double hit lymphoma groups ($p > 0.05$; Table 1). Patients with triple hit lymphoma had a very poor prognosis, with an overall survival of 18 months, similar to the overall survival of patients with *MYC/BCL6* double hit lymphoma (17 months) and those with *MYC/BCL2* double hit lymphoma (20 months) (Fig. 3c, $p = 0.67$), but much worse than patients with diffuse large B-cell lymphoma without *MYC* rearrangement (diffuse large B-cell lymphoma−overall survival, Fig. 3d, $p < 0.0001$). Since P53 expression is a prognostic factor in triple hit lymphoma as well as in double hit lymphoma [30], further survival analyses were performed to compare the survival of triple hit lymphoma patients to double hit lymphoma patients when P53 < 50%, and still showed no survival difference between them (Fig. 3e). Similarly, the patients with triple hit lymphoma without P53 overexpression still showed significant worse overall survival than patients with diffuse large B-cell lymphoma, not otherwise specified (Fig. 3f).

Discussion

Triple hit lymphoma is a rare and aggressive large B-cell lymphoma that carries translocations involving *MYC*, *BCL2,* and *BCL6*. Because of its low frequency, a relatively large case series of triple hit lymphomas to better characterize this tumor is not yet available in the literature. In this study, we retrospectively reviewed 40 patients with triple hit lymphoma and compared their clinicopathologic features and prognosis to 157 patients with *MYC/BCL2* double hit lymphoma and 13 patients with *MYC/BCL6* double hit lymphoma.

Numerous previous studies have shown that *MYC/BCL2* double hit lymphoma occurs most often in older patients with an aggressive clinical course and poor prognosis. Relatively limited data are available for patients with *MYC/BCL6* double hit lymphoma, but it appears that the clinical features and the prognosis of this group are similarly poor [18, 24, 33]. This study of 40 cases of triple hit lymphoma showed that most patients are elderly (median age 61 years) with a male predominance, similar to double hit lymphomas presented in this study and to those reported previously [25, 34, 35]. Most patients with triple hit lymphoma presented with advanced stage disease, a high serum lactate dyhydrogenase level, extranodal sites of disease and high international prognostic index score, similar to the double hit lymphomas. About one quarter of patients with triple hit lymphoma had a history of B-cell lymphoma, most often follicular lymphoma (8 of 9, 89%), also similar to patients with double hit lymphoma [21].

Morphologically and immunophenotypically, cases of triple hit lymphoma were more similar to cases of double hit lymphoma than *MYC/BCL6* double hit lymphoma. Cases of triple hit lymphoma either were high-grade with a starry sky appearance resembling B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma or they resembled cases of diffuse large B-cell lymphoma, very similar to that observed in *MYC/BCL2* double hit lymphoma. In contrast, triple hit lymphoma appear to differ from *MYC/BCL6* double hit lymphoma in that the latter group more often showed diffuse large B-cell lymphoma morphology, although the difference was not statistically significant, presumably due to the small number of cases. Immunophenotypically, most cases of triple hit lymphoma (98%) had a germinal center B-cell (germinal center B cell) immunophenotype, similar to *MYC/BCL2* double hit lymphoma (99%). The frequency of germinal center B-cell type appeared higher than that in *MYC/BCL6* double hit lymphoma (90%); however, it was not statistically significant ($p = 0.10$). It is well known that the double expressor immunophenotype has prognostic significance in patients with diffuse large B-cell lymphoma treated with RCHOP [27, 36]. In an earlier study the double expressor phenotype was not associated with prognosis in patients with *MYC/BCL2* double hit lymphoma [21]. In this study, approximately 70% of triple hit lymphoma cases had a double

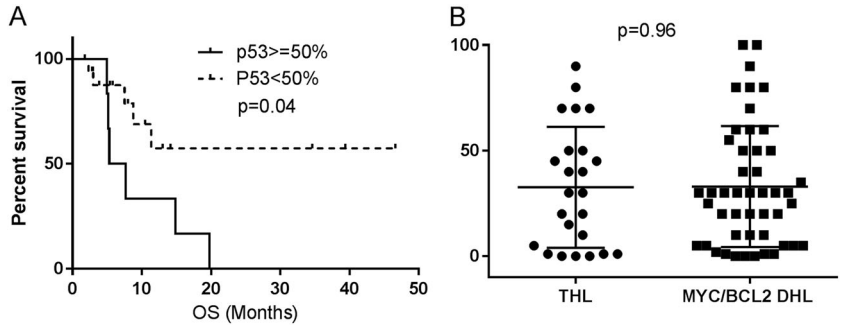

Fig. 2 Comparison of overall survival (overall survival) between P53-positive triple hit lymphoma and P53-negative ones (**a**); and P53 expression in triple hit lymphoma vs. *MYC/BCL2* double hit lymphoma (**b**)

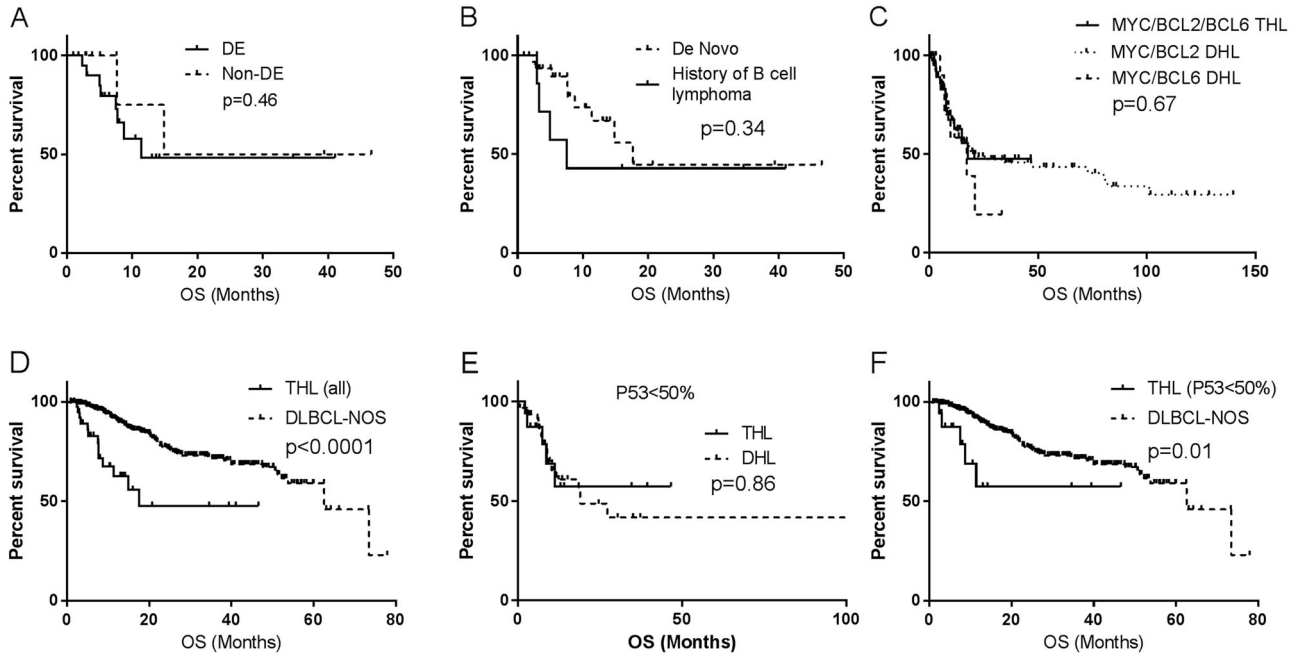

Fig. 3 Comparison of overall survival (overall survival) between triple hit lymphoma with or without MYC and BCL2 dual expression (**a**), de novo triple hit lymphoma and those with a history of B-cell lymphoma (**b**), triple hit lymphoma vs. double hit lymphomas (**c**), triple hit lymphoma vs. diffuse large B-cell lymphoma–overall survival (**d**), triple hit lymphoma vs. double hit lymphomas when P53 < 50% (**e**), and triple hit lymphoma with P53 < 50% vs. diffuse large B cell lymphoma−overall survival (**f**)

expressor immunophenotype, positive for both MYC and BCL2. Similarly, MYC and BCL2 dual expression was not predictive of prognosis in triple hit lymphoma patients presented here.

TP53 is an important tumor suppressor gene that plays a critical role in the pathogenesis of multiple tumors including diffuse large B-cell lymphoma. Studies have shown that *TP53* mutation is associated with P53 overexpression and both are associated with an inferior prognosis in patients with diffuse large B-cell lymphoma [37–39]. Previous studies have shown that P53 expression can enhance the negative effect of *MYC* translocation and expression [40, 41]. In earlier studies, P53 overexpression was shown to augment the negative prognostic effect of *MYC/BCL2* double hit lymphoma and MYC/BCL2 double expression lymphoma [28, 30]; in other words, P53 expression is a poor prognostic factor in *MYC/BCL2* double hit lymphoma. In this study, P53 expression was observed in 29% of triple hit lymphoma cases evaluated and was associated with an inferior overall survival compared to cases without P53 overexpression ($p = 0.04$), similar to the negative impact of P53 reported in *MYC/BCL2* double hit lymphoma. However, the negative prognostic effect of double hit lymphoma and triple hit lymphoma is independent of P53, as patients of triple hit lymphoma and double hit lymphoma with tumors showing <50% of P53 expression still showed similar overall survival, and these triple hit lymphoma patients still demonstrated a worse overall survival than patients with diffuse large B-cell lymphoma—not otherwise specified.

About two thirds of triple hit lymphoma patients in this cohort received intensive induction immunochemotherapy regimens, mainly R-EPOCH. Despite the use of aggressive therapy, including radiotherapy and stem cell transplant in a subset of patients, the outcome of triple hit lymphoma was

still very poor, similar to patients with double hit lymphoma. About 45% of patients achieved complete remission after therapy and a third of patients had died at time of last follow-up. The median overall survival of the triple hit lymphoma patients in this study was 18 months, which is longer than the median overall survival of 4 months reported in two previous studies that included seven and eight triple hit lymphoma patients, respectively [34, 35]. Several factors may have contributed to this difference: (1) treatment regimens; (2) underlying patient characteristics in different cohorts; (3) and most likely the small sample sizes in previous reports. Recent large series studies of double hit lymphoma patients have shown a possible superior effect of intensive induction regimen including R-EPOCH and R-Hyper-CVAD, but mainly the former, on the prognosis of double hit lymphoma patients [22, 23] and two thirds of the triple hit lymphoma patients in this study received such induction regimen. Similar to findings in patients with *MYC/BCL2* double hit lymphoma, a history of B-cell lymphoma or stem cell transplant was not associated with survival in patients with triple hit lymphoma.

In summary, *MYC/BCL2/BCL6* triple hit lymphoma is an uncommon subset of high-grade B-cell lymphoma with aggressive clinical behavior. The clinicopathologic features are similar to double hit lymphoma, especially *MYC/BCL2* double hit lymphoma. P53 overexpression is a poor prognostic factor in triple hit lymphoma. Despite of a germinal center B-cell immunophenotype and the use of intensive induction chemotherapy regimens in most of the triple hit lymphoma patients, the prognosis was poor, similar to that of patients with *MYC/BCL2* double hit lymphoma and *MYC/BCL6* double hit lymphoma. Novel therapies are needed for patients with triple hit lymphoma.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Dang CV, O'Donnell KA, Zeller KI, Nguyen T, Osthus RC, Li F. The c-Myc target gene network. Semin Cancer Biol. 2006;16:253–64.
2. Nie Z, Hu G, Wei G, et al. c-Myc is a universal amplifier of expressed genes in lymphocytes and embryonic stem cells. Cell. 2012;151:68–79.
3. Dalla-Favera R, Bregni M, Erikson J, Patterson D, Gallo RC, Croce CM. Human c-myc onc gene is located on the region of chromosome 8 that is translocated in Burkitt lymphoma cells. Proc Natl Acad Sci USA. 1982;79:7824–7.
4. Barrans S, Crouch S, Smith A, Turner K, Owen R, Patmore R, Roman E, Jack A. Rearrangement of MYC is associated with poor prognosis in patients with diffuse large B-cell lymphoma treated in the era of rituximab. J Clin Oncol. 2010;28:3360–5.
5. Valera A, Lopez-Guillermo A, Cardesa-Salzmann T, et al. MYC protein expression and genetic alterations have prognostic impact in patients with diffuse large B-cell lymphoma treated with immunochemotherapy. Haematologica. 2013;98:1554–62.
6. Savage KJ, Johnson NA, Ben-Neriah S, Connors JM, Sehn LH, Farinha P. Horsman double expressor, Gascoyne RD. MYC gene rearrangements are associated with a poor prognosis in diffuse large B-cell lymphoma patients treated with R-CHOP chemotherapy. Blood. 2009;114:3533–7.
7. Quesada AE, Medeiros LJ, Desai PA, et al. Increased MYC copy number is an independent prognostic factor in patients with diffuse large B-cell lymphoma. Mod Pathol. 2017;30:1688–97.
8. Hu Z, Medeiros LJ, Chen Z, et al. Mantle cell lymphoma with MYC rearrangement: a report of 17 patients. Am J Surg Pathol. 2016;41:216–24.
9. Li Y, Hu S, Wang SA, Li S, Huh YO, Tang Z, Medeiros LJ, Tang G. The clinical significance of 8q24/MYC rearrangement in chronic lymphocytic leukemia. Mod Pathol. 2016;29:444–51.
10. Miao Y, Hu S, Lu X, Li S, Wang W, Medeiros LJ, Lin P. Double-hit follicular lymphoma with MYC and BCL2 translocations: a study of 7 cases with a review of literature. Hum Pathol. 2016;58:72–77.
11. Jacobson JO, Wilkes BM, Kwaiatkowski DJ, Medeiros LJ, Aisenberg AC, Harris NL. bcl-2 rearrangements in de novo diffuse large cell lymphoma. Assoc Distinctive Clin Features Cancer. 1993;72:231–6.
12. Basso K, Saito M, Sumazin P, et al. Integrated biochemical and computational approach identifies BCL6 direct target genes controlling multiple pathways in normal germinal center B cells. Blood. 2010;115:975–84.
13. Parekh S, Polo JM, Shaknovich R, et al. BCL6 programs lymphoma cells for survival and differentiation through distinct biochemical mechanisms. Blood. 2007;110:2067–74.
14. Kluin PM, Harris NL, Jaffe ES, Stein H, Gascoyne RD, Leoncini L, Swerdlow EH. High grade B-cell lymphoma. In: Swerdlow EH, Harris NL, Jaffe ES et al., editors. WHO classification of tumours of haematopoietic and lymphoid tissues. Revised 4th ed. Lyon, France: IARC; 2017.
15. Aukema SM, Kreuz M, Kohler CW, et al. Biological characterization of adult MYC-translocation-positive mature B-cell lymphomas other than molecular Burkitt lymphoma. Haematologica. 2014;99:726–35.
16. Aukema SM, Siebert R, Schuuring E, van Imhoff GW, Kluin-Nelemans HC, Boerma EJ, Kluin PM. Double-hit B-cell lymphomas. Blood. 2011;117:2319–31.
17. Johnson NA, Savage KJ, Ludkovski O, et al. Lymphomas with concurrent BCL2 and MYC translocations: the critical factors associated with survival. Blood. 2009;114:2273–9.
18. Li S, Desai P, Lin P, Yin CC, Tang G, Wang XJ, Konoplev SN, Khoury JD, Bueso-Ramos CE, Medeiros LJ. MYC/BCL6 double-hit lymphoma (double hit lymphoma): a tumour associated with an aggressive clinical course and poor prognosis. Histopathology. 2015;68:1090–8.
19. Li S, Lin P, Fayad LE, Lennon PA, Miranda RN, Yin CC, Lin E, Medeiros LJ. B-cell lymphomas with MYC/8q24 rearrangements and IGH@BCL2/t(14;18)(q32; q21): an aggressive disease with heterogeneous histology, germinal center B-cell immunophenotype and poor outcome. Mod Pathol. 2012;25:145–56.
20. Li S, Lin P, Young KH, Kanagal-Shamanna R, Yin CC, Medeiros LJ. MYC/BCL2 double-hit high-grade B-Cell lymphoma. Adv Anat Pathol. 2013;20:315–26.
21. Li S, Saksena A, Desai P, et al. Prognostic impact of history of follicular lymphoma, induction regimen and stem cell transplant in patients with MYC/BCL2 double hit lymphoma. Oncotarget. 2016;7:38122–32.
22. Oki Y, Noorani M, Lin P, et al. Double hit lymphoma: the MD Anderson Cancer Center clinical experience. Br J Haematol. 2014;166:891–901.

23. Petrich AM, Gandhi M, Jovanovic B, et al. Impact of induction regimen and stem cell transplantation on outcomes in double-hit lymphoma: a multicenter retrospective analysis. Blood. 2014;124:2354–61.
24. Pillai RK, Sathanoori M, Van Oss SB, Swerdlow SH. Double-hit B-cell lymphomas with BCL6 and MYC translocations are aggressive, frequently extranodal lymphomas distinct from BCL2 double-hit B-cell lymphomas. Am J Surg Pathol. 2013;37:323–32.
25. Wang W, Hu S, Lu X, Young KH, Medeiros LJ. Triple-hit B-cell lymphoma with MYC, BCL2, and BCL6 translocations/rearrangements: clinicopathologic features of 11 cases. Am J Surg Pathol. 2015;39:1132–9.
26. 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;103:275–82.
27. 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;30:3452–9.
28. Li S, Weiss VL, Wang XJ, Desai PA, Hu S, Yin CC, Tang G, Reddy NM, Medeiros LJ, Lin P. High-grade B-cell lymphoma with MYC rearrangement and without BCL2 and BCL6 rearrangements is associated with high P53 expression and a poor prognosis. Am J Surg Pathol. 2016;40:253–61.
29. 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 diffuse large B cell lymphoma rituximab-CHOP Consortium Program Study. Haematologica. 2013;98:255–63.
30. Wang XJ, Jeffrey Medeiros L, Bueso-Ramos CE, et al. P53 expression correlates with poorer survival and augments the negative prognostic effect of MYC rearrangement, expression or concurrent MYC/BCL2 expression in diffuse large B-cell lymphoma. Mod Pathol. 2016;30:194–203.
31. Wang XJ, Medeiros LJ, Lin P, Yin CC, Hu S, Thompson MA, Li S. 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;39:1250–8.
32. McGowan JSA Schmid M, editors. An international system for human cytogenetic nomenclature (2016). First ed. Switzerland: Karger, Basel; 2016.
33. Turakhia SK, Hill BT, Dufresne SD, Nakashima MO, Cotta CV. Aggressive B-cell lymphomas with translocations involving BCL6 and MYC have distinct clinical-pathologic characteristics. Am J Clin Pathol. 2014;142:339–46.
34. Bacher U, Haferlach T, Alpermann T, Kern W, Schnittger S, Haferlach C. Several lymphoma-specific genetic events in parallel can be found in mature B-cell neoplasms. Genes Chromosomes Cancer. 2011;50:43–50.
35. Tomita N, Tokunaka M, Nakamura N, et al. Clinicopathological features of lymphoma/leukemia patients carrying both BCL2 and MYC translocations. Haematologica. 2009;94:935–43.
36. 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;30:3460–7.
37. Chang CC, Liu YC, Cleveland RP, Perkins SL. Expression of c-Myc and p53 correlates with clinical outcome in diffuse large B-cell lymphomas. Am J Clin Pathol. 2000;113:512–8.
38. Koduru partial remission, Raju K, Vadmal V, Menezes G, Shah S, Susin M, Kolitz J, Broome JD. Correlation between mutation in P53, p53 expression, cytogenetics, histologic type, and survival in patients with B-cell non-Hodgkin's lymphoma. Blood. 1997;90:4078–91.
39. Xu-Monette ZY, Wu L, Visco C, et al. Mutational profile and prognostic significance of TP53 in diffuse large B-cell lymphoma patients treated with R-CHOP: report from an International diffuse large B cell lymphoma Rituximab-CHOP Consortium Program Study. Blood. 2012;120:3986–96.
40. Xie Y, Bulbul MA, Ji L, Inouye CM, Groshen SG, Tulpule A, O'Malley DP, Wang E, Siddiqi IN. p53 expression is a strong marker of inferior survival in de novo diffuse large B-cell lymphoma and may have enhanced negative effect with MYC coexpression: a single institutional clinicopathologic study. Am J Clin Pathol. 2014;141:593–604.
41. Clipson A, Barrans S, Zeng N, et al. The prognosis of MYC translocation positive diffuse large B-cell lymphoma depends on the second hit. J Pathol Clin Res. 2015;1:125–33.