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Double- and Triple-Hit Lymphomas Can Present with Features Suggestive of Immaturity, including TdT Expression, and Create Diagnostic Challenges

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Abstract

Double- and triple-hit lymphomas (DHL/THL) are aggressive B cell neoplasms characterized by translocation of *MYC* with concurrent *BCL2* and/or *BCL6* translocation. In this retrospective study from one institution, we report clinicopathologic features of 13 cases (9 DHL/4 THL). The median age was 59 years (range 30 to 74) and patients included 8 females and 5 males. Presentation included enlarging lymphadenopathy/masses (11 patients) and abnormal peripheral blood findings (2 patients). Features which raised the differential of an immature neoplasm included TdT positivity (4 cases, 2 THL/2 DHL); dim CD45 expression (7 cases), lack of CD20 (2 cases), or lack of surface immunoglobulin light chain (3 cases) by flow cytometry; and blastoid morphology (2 cases). We conclude that expression of TdT in a B cell lymphoma with mature features or expression of surface light chain in a case otherwise suggestive of B-lymphoblastic leukemia/lymphoma should prompt an expedited evaluation for DHL/THL.

Keywords

double hit lymphoma; triple hit lymphoma; B cell lymphoma unclassifiable; diffuse large B cell lymphoma; B lymphoblastic leukemia/lymphoma

INTRODUCTION

Translocation of the *MYC* proto-oncogene with a concurrent *BCL2* or *BCL6* translocation as detected by fluorescence in-situ hybridization (FISH) or standard cytogenetics (karyotype) defines double-hit lymphoma (DHL) ¹. Triple hit lymphoma (THL) is characterized by the presence of concurrent *MYC*, *BCL2*, and *BCL6* translocations. DHL and THL are morphologically heterogeneous and do not fall into a single WHO

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Classification of Tumours of Haematopoietic and Lymphoid Tissues (2008 WHO) category, with most cases being classified as either diffuse large B cell lymphoma (DLBCL) or B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma (BCLU). Less frequently, DHL have the morphology and immunophenotype of B-lymphoblastic leukemia/lymphoma (B-LBL) or follicular lymphoma²⁻¹⁰. The incidence of FISH-defined DHL (*MYC* and *BCL2*) in patients with DLBCL varies depending on the study, ranging from 3% to 13%¹¹. DHL with a concurrent *BCL6* translocation is less common¹²⁻¹⁴, and THL is rare¹⁴⁻²². Double and triple hit lymphomas are aggressive, with a poor prognosis likely due to a synergistic effect of *MYC*-driven proliferation signals and *BCL2* and/or *BCL6* deregulation^{11,23}.

In our practice, we have encountered cases of DHL/THL with features, including TdT expression, raising the differential of B-LBL versus DLBCL or BCLU. In this retrospective study from one institution, we report 13 cases of DHL/THL and describe clinical, morphologic, and immunophenotypic findings. Our series includes six cases which presented a diagnostic challenge due to features which raised the possibility of an immature neoplasm.

MATERIALS AND METHODS

The University of Minnesota Institutional Review Board approved this retrospective study. Our cohort included three cases of DHL and three cases of THL recently encountered in our clinical practice and notable for their difficulty in diagnosis as well as six additional cases of DHL and one additional case of THL identified through our electronic pathology database. The additional cases of DHL/THL were identified through multiple natural language searches, including for “double hit” or “triple hit”, in the diagnosis, diagnosis comment, or addendum portions of the pathology report. All cases were diagnosed between 2010 and 2015. The electronic medical record was used to extract clinical information including patient age, gender, medical history, staging, treatment, and follow-up.

Hematoxylin and eosin (H&E)-stained slides, immunohistochemically stained slides, and Wright-Giemsa stained aspirate smears and peripheral blood smears were examined by the pathologist at the time of diagnosis as well as retrospectively for this study by two authors (EC and LM). Only touch preparations from a lymph node were available for retrospective review in case 10. Based on review of slides, the morphology of the cases was retrospectively classified as centroblastic, Burkitt lymphoma-like, or blastoid based on the following criteria. Centroblastic cases had medium to large sized neoplastic cells with scant cytoplasm, round to irregular nuclear contours, vesicular chromatin, and multiple nucleoli. Burkitt lymphoma-like cases had monomorphic intermediate-sized neoplastic cells with scant cytoplasm, oval to irregular nuclear contours, finely stippled chromatin, and variable nucleoli. Blastoid cases were composed of intermediate sized cells with scant chromatin, round nuclear contours, and fine chromatin, reminiscent of lymphoblasts.

Terminal deoxynucleotidyl transferase (TdT) immunohistochemical stain was performed at the time of diagnosis in seven cases and performed retrospectively for the remaining cases. All other immunohistochemical stains were performed at the time of diagnosis using the

standard methods utilized at that time. Not all immunohistochemical stains were performed in all cases. Details of immunohistochemical stains currently used at the University of Minnesota are as follows: terminal deoxynucleotidyl transferase (TdT, concentrated 1:20, polyclonal, Supertechs, Bethesda, MD), CD10 (prediluted, clone 56C6, Cell Marque, Rocklin, CA), CD20 (prediluted, clone L26, Ventana, Tucson, AZ), CD22 (concentrated 1:50, clone FPC1, Biocare, Concord, CA), CD34 (prediluted, clone Qbend/10, Ventana), CD79a (prediluted, clone JCB117, Cell Marque), Ki-67 (prediluted, clone 30-9, Ventana), Pax5 (prediluted, clone SP34, Ventana), BCL2 (prediluted, clone Bcl-2/100/D5, Leica, Buffalo Grove, IL), BCL6 (prediluted, clone Gi191E/A8, Ventana), and MUM1 (concentrated 1:25, clone MUM1p, Dako, Carpinteria, CA). In situ hybridization for EBV-encoded RNA (EBER-ISH, prediluted, RNA probe, Ventana) was performed on select cases.

Flow cytometry was performed using routine protocols in place at the time of diagnosis. Flow cytometry was performed for all cases by 8-color analysis on a FACSCanto2 flow cytometer (Becton Dickinson, San Jose, CA) using the following fluorescently labeled antibodies obtained from Becton Dickinson (BD, San Jose, CA) and Dako (Carpinteria, CA): CD5 PE-Cy7 (BD), CD10 APC (BD), CD14 PerCP (BD), CD19 V450 (BD), CD20 APC-H7 (BD), λ FITC (Dako), and κ PE (Dako). A subset of the cases additionally had flow cytometry performed by 4-color analysis using the following fluorescently labeled antibodies: CD3 PE (BD), CD 19 APC (BD), CD45 PERCP-CY5.5 (BD), and terminal deoxynucleotidyl transferase (TdT) FITC (Supertechs, Bethesda, MD). Intracytoplasmic immunoglobulin light chains were evaluated in a subset of cases using antibodies conjugated to the following fluorochromes: λ PE (BD) and κ FITC (BD). Data was re-analyzed by authors (LM and EC) using Kaluza software (Beckman Coulter, Brea, CA). The intensity of CD 19 and CD20 was assessed relative to background polytypic B cells (if present), background T cells (if polytypic B cells absent), or the expected intensity for polytypic B cells (if only neoplastic lymphocytes present). The intensity of CD45 was assessed using the CD45 V500 fluorochrome-antibody combination with polytypic B cells, background T cells, or expected intensity for lymphocytes used as a reference, depending on the case. CD45 expression in cases considered dim had a level of intensity at or slightly above the usual level of intensity for blasts in our laboratory using this fluorochrome.

G-banded cytogenetic analysis was performed on unstimulated and B cell mitogen-stimulated samples after a 24- or 48-hour culture according to standard cytogenetic protocols at the University of Minnesota. Samples included peripheral blood, bone marrow aspirate, and tissue biopsy. Chromosome analysis was performed on 20 G-banded metaphase cells and the resulting karyotypes were described according to the International System for Human Cytogenetic Nomenclature (ISCN) ²⁴. FISH probes included a break-apart probe to the *BCL6* (3q27) locus, a break-apart probe to the *MYC* (8q24) locus, a break apart probe to the *BCL2* (18q21) locus, a dual-fusion probe set to the *IGH* (14q32) and *BCL2* (18q21) loci, and a dualfusion probe set to the *IGH* (14q32) and *MYC* (8q24) loci (Abbott Molecular, Abbott Park, IL). Not all FISH probes were used in all cases. Specimens included peripheral blood, aspirate smears/touch imprints from bone marrow biopsy, touch preparations from tissue biopsy, and formalin-fixed, paraffin-embedded tissue. Cytogenetic analysis for case 11 was performed on a cerebrospinal fluid sample at an outside hospital; the *MYC* translocation was confirmed by FISH at the University of Minnesota.

RESULTS

Our series included four cases of THL and nine cases of DHL (8 *BCL2/MYC* and 1 *BCL6/MYC*) (see Table I). Cases 1- 3 and 10- 12 were encountered in our routine clinical practice, include both in-house and consult cases, and were notable at the time of diagnosis for features which raised the possibility of an immature neoplasm (B-LBL). The remaining are additional cases (all in-house) identified through search of our electronic pathology database. All cases were diagnosed between 2010 and 2015.

Clinical features of our cohort are outlined in Table II. Our series included eight females and five males, with a median age of 59 years (range 30 to 74 years). Eleven had no significant oncologic history while two had a history of follicular lymphoma. Case 2 had a history of low grade follicular lymphoma diagnosed 8 months prior at the same site and treated with rituximab and bendamustine. FISH analysis of the low-grade lymphoma showed 77% of interphase cells with a signal pattern indicative of an IGH/BCL2 fusion with no evidence found of rearrangement of BCL6 or MYC loci. Case 7 had a history of low-grade follicular lymphoma diagnosed 5 years prior at the same site and treated with rituximab and chlorambucil followed by R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone). Eleven patients presented with enlarging lymphadenopathy/masses and two presented with abnormal peripheral blood findings (moderate leukocytosis of $38.9 \times 10^9/L$ in case 1 and atypical circulating lymphoid cells seen on peripheral smear with a normal white blood cell count in case 12). By the Ann Arbor staging system, most patients (10, 77%) presented with extensive disease (stage IV). Three patients had cerebrospinal fluid (CSF) involvement. Treatment protocols varied and are outlined in Table II. At last follow-up (median follow-up of surviving patients 11 months, range 5 to 48 months), four patients had died of disease or treatment-related complications, two were alive with relapsed disease, and seven were alive with no evidence of disease.

Based on review of H&E and Wright-Giemsa stained slides by two of the authors (LM and EC), morphology of the cases was classified as centroblastic (7 cases), Burkitt-lymphoma like (4 cases), or blastoid (2 cases). Images representative of the spectrum of morphology encountered in our series are shown in Figure 1. Morphologic and immunophenotypic findings are detailed in Table III. All cases were positive for CD10 (by flow or immunohistochemistry). Ten of the 11 cases evaluated for BCL2 were positive. EBV by EBER-ISH was negative in all eight cases evaluated. The proliferation index, based on Ki-67 immunohistochemical stain, was 80-90% in eight cases, 95-100% in four cases, and 30-40% in one case. Terminal deoxynucleotidyl transferase (TdT) was positive by immunohistochemical stain in four cases, with the majority of neoplastic cells positive (see Figure 2); one case (Case 10) showed rare TdT positive cells, and the remaining cases were negative for TdT. Flow cytometric immunophenotyping confirmed TdT positivity in three of the four cases positive by immunohistochemistry. TdT was negative by flow cytometry in the one case showing rare TdT positive cells. All cases positive for TdT lacked CD34 expression by immunohistochemistry and/or flow cytometry.

CD45 was dim in seven cases; a single case (case 6) had a subset of neoplastic cells lacking CD45 entirely. CD19 was dim in two cases. CD20 was dim in three cases and absent in three

cases. Three cases lacked surface immunoglobulin light chain expression, at least in a subset of the neoplastic cells. The two cases evaluated for intra-cytoplasmic light chains lacked both surface and cytoplasmic light chain expression. A subset of the cells in case 3 had increased forward scatter suggesting increased size but otherwise had a similar immunophenotype. In case 10, a subset of the cells had increased forward scatter suggesting increased size; in addition, the cells with increased forward scatter lacked CD20 and light chain expression (both surface and cytoplasmic), had slightly dim CD45, and bright CD10. Figure 3 shows representative flow dot-plots.

Six of the cases were notable in the course of our routine clinical practice for features which raised the differential of B-LBL including TdT expression (cases 2, 3, 11, and 12), blastoid morphology (cases 1 and 10), dim CD45 (cases 1, 2, 10, and 11), absence of CD20 (cases 2 and 10), and absence of light chains (cases 2 and 10). Of the seven additional cases identified via the electronic pathology database, features included dim CD45 (cases 5, 9, and 13), absence of CD20 (case 6), and absence of surface light chains (case 13).

Two of the cases in our series (cases 2 and 11) were classified at the time of diagnosis as most consistent with B-lymphoblastic lymphoma. The staging bone marrow biopsy in Case 2 did not show definitive morphologic evidence of marrow or blood involvement by neoplastic B cells; however, flow cytometry identified an abnormal B-cell population with a similar immunophenotype as seen in the diagnostic lymph node biopsy. Case 11 did not have blood or marrow involvement. Three (cases 3, 4, and 9) were diagnosed as B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma. Four (cases 5, 6, 7, and 13) were diagnosed as diffuse large B-cell lymphoma. The remaining four (cases 1, 8, 10, and 12) were given a descriptive diagnosis of “high grade B-cell lymphoma” with the translocations noted in the diagnostic line.

DISCUSSION

Double-hit lymphoma (DHL) and triple-hit lymphoma (THL), united by cytogenetic/FISH findings, are morphologically heterogeneous and do not fall into a single 2008 WHO diagnostic category. In this retrospective study from one institution, we report 13 cases of DHL or THL seen recently at our institution including six cases notable for features raising the differential of an immature neoplasm (B-LBL). Our series supports the literature on DHL/THL and contributes additional examples of rare presentations.

Many of the cases had one or more of the flow cytometric features of DHL that have been described previously²⁵⁻²⁷, including dim CD45, dim or absent CD20, and absent light chain expression. Our patients had similar clinical features as previously reported in DHL/THL^{28,29}, including occurrence in predominantly older adults, most with extensive (stage IV) disease, and not uncommon CSF involvement. Our series includes one case of DHL with *MYC* and *BCL6* translocations, a less common entity than DHL with *MYC* and *BCL2*^{13,30}. Mature DHL presenting in leukemic phase and mimicking B-LBL has been reported³¹, and this series provides an additional two patients presenting with extensive peripheral blood and/or bone marrow involvement (cases 1 and 12). The morphology of the neoplastic cells in case 1, particularly in the peripheral blood, was suggestive of blasts, mimicking B-

ALL at presentation, although subsequent immunophenotyping was not supportive of B-ALL. Case 12 was positive for TdT but expressed monotypic surface light chain, bright CD45 and CD20, and had a centroblastic morphology, favoring a mature neoplasm.

Our series includes two patients with a prior history of follicular lymphoma (FL). In case 2, the subsequent DHL was diagnosed as most consistent with B-LBL. Case 7 was diagnosed as DLBCL with the diagnostic specimen lacking features of immaturity. Transformation of FL to a high grade lymphoma occurs in approximately 30% of patients, and the high grade lymphoma is usually DLBCL. Transformation of FL to B-LBL is rare but well documented in the literature, with most cases showing a *MYC* rearrangement in addition to *IGH/BCL2* fusion^{4-8,32-36}. Geyer, et al⁵ recently published a series of 7 patients with TdT+ B-lymphoblastic transformation of FL. In that series, the authors raise the possibility that patients presenting with de novo double-hit B-LBL may have a pre-existing low-grade FL masked by the higher-grade process. The flow cytometry findings in case 10 in our series, occurring in a patient with no oncologic history, raise the possibility of a high-grade lymphoma with some features of immaturity, along with a synchronous FL or FL in transformation.

Rare cases of DHL with TdT positivity have been reported^{2,4-6,10,33,37,38}, most with the morphology and immunophenotype of B-LBL, arising from or presenting concurrently with follicular lymphoma. In our study, four cases were TdT positive, approaching one-third of our case series. Two of our four cases of THL were TdT positive, a finding reported previously in only a single case³⁸. The high proportion of TdT positive cases in our series is not representative of our patient population because we enriched our series with memorable cases (including outside cases and consult cases) that created a diagnostic challenge. Of the additional routine cases identified through our electronic pathology database, none were positive for TdT. Of the four cases with TdT positivity, one (case 2) had multiple additional features supportive of immaturity (dim CD45, lack of CD20, lack of surface or cytoplasmic light chains). The remaining three expressed monotypic surface light chain and had CD20 expression by flow cytometry; one of the three had dim CD45 expression. Classification of TdT positive DHL/THL cases as B-LBL is problematic, particularly when additional features are not supportive of immaturity. Two of the TdT positive cases in this series were classified as B-LBL at the time of diagnosis. On review of the cases for this study, we feel only case 2 qualifies for the diagnosis of B-LBL, and, as it arose in a patient with a history of follicular lymphoma, the case is perhaps better classified as TdT+ B-lymphoblastic transformation of FL, as suggested by Geyer, et al and Loghavi, et al^{5,38}.

In summary, features seen in the 13 DHL/THL cases presented in this paper included TdT positivity, dim CD45 expression, absence of CD20, lack of immunoglobulin light chains, leukemic presentation, and/or blastoid morphology. We conclude that incongruous features, such as dim CD45, absence of CD20 or surface light chain, or expression of TdT in a B cell lymphoma with mature morphology and immunophenotype or expression of surface light chain in a case suggestive of B-lymphoblastic leukemia/lymphoma, should prompt an expedited evaluation for DHL/THL. Further investigation into cases of DHL/THL with immature features, particularly TdT positivity, is warranted, both to determine the

appropriate classification of these neoplasms, as well as to gain further insight into prognosis.

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Figure 1:

Representative images showing morphologic spectrum. Case 1 (A) blastoid morphology presenting in bone marrow, and peripheral blood (inset); case 8 (B) Burkitt-like morphology in a lymph node; and case 9 (C) centroblastic morphology in a small intestinal mass; all H&E stained sections except Wright-Giemsa stained peripheral blood, 100X objective.

Figure 2:

Representative images from TdT positive cases. Case 3 (A and B), case 12 (C and D), case 11 (E and F), and case 2 (G and H). A, C, E, and G: H&E stained sections, 50X objective. B, D, F, and G: TdT immunohistochemical stain, 20X objective.

Figure 3: Representative flow cytometry plots. Case 2 (A), case 12 (B), case 10 (C), and case 13 (D). For all plots: events include all viable leukocytes and are color-gated as follows: purple= T cells; orange/light blue = kappa/lambda polytypic B cells; blue = neoplastic population; black = second neoplastic population.

Table 1:

Cytogenetic Data

Case	Karyotype	FISH analysis (% interphase cells)		
		BCL12	BCL6	MYC
1	48,XX,+X,del(6)(q23q25),-8,del(11)(q23q25),del(13)(q12q14),t(14;18)(q32;q21),+18,+t[11]/46,XX[9]	-Positive for <i>IGH-BCL2</i> gene fusion (76.5%)	-Negative for <i>BCL6</i> rearrangement	-Positive for <i>MYC</i> rearrangement (38.5% with loss of the 3' portion of the <i>MYC</i> locus and 26.5% with gains of one or more copies of the 5' portion) -Negative for <i>IGH-MYC</i> gene fusion
2	47,XX,+del(1)(p10),der(1;16)(q10;p10),t(8;22)(q24.1;q11.2),t(14;18)(q32;q21),-20,+mar[cp8]/46,XX[2]	-Positive for <i>IGH-BCL2</i> gene fusion (92.5%)	-Negative for <i>BCL6</i> rearrangement	-Positive for <i>MYC</i> rearrangement (94%)
3	47,XX,t(8;14)(q24.1;q32),der(13)(1;13)(q12;p13),t(14;18)(q32;q21),-der(18)t(14;18)[4]/47, idem,der(21)(1;21)(q12;p13)[5]/48,idem,+Y,dup(1)(q21q32),-der(13),-18,+mar[3]	-Positive for <i>IGH-BCL2</i> gene fusion (83%)	-Negative for <i>BCL6</i> rearrangement	-Positive for <i>MYC</i> rearrangement (72.5%)
4	Not performed	-Positive for <i>IGH-BCL2</i> gene fusion (46%)		-Positive for <i>MYC</i> rearrangement (44%)
5	Not performed	-Positive for <i>IGH-BCL2</i> gene fusion (50%)	-Negative for <i>BCL6</i> rearrangement	-Positive for <i>MYC</i> rearrangement (76%) -Negative for <i>IGH-MYC</i> gene fusion
6	Not performed	-Positive for <i>IGH-BCL2</i> gene fusion (96.3%)		-Positive for <i>MYC</i> rearrangement (99.5%)
7	Not performed	-Positive for <i>BCL2</i> rearrangement (74%)	-Negative for <i>BCL6</i> rearrangement	-Positive for <i>MYC</i> rearrangement (81.5%)

Case	Karyotype	FISH analysis (% interphase cells)		
		BCL12	BCL6	MYC
8 ^A	48,X,Y,der(8)(pter->8q24.1::14q32->18q21->18qter),+12,+13,der(14)t(8;14)(q24.1;q32), der(18)t(14;18)(q32;q21)[7]/46,XY[13]	-Positive for <i>IGH-BCL2</i> gene fusion (84%)		-Positive for <i>IGH-MYC</i> gene fusion (82%)
9	Not performed	-Negative for <i>BCL2</i> rearrangement	-Positive for <i>BCL6</i> rearrangement (64%)	-Positive for <i>MYC</i> rearrangement (54%)
10	48,XX,del(2)(p11.2),+mar1,+mar2,inc[cp5]	-Positive for <i>BCL2</i> rearrangement (64%)	-Positive for <i>BCL6</i> rearrangement (68%),	-Positive for <i>MYC</i> rearrangement (56.5%)
11	51 – 55, XX, add(3)(q27),+6,der(8)t(8;22)(q24.2),add(9)(9p12),+11,+12,der(12;17)(q10;q10),+13,+13,+14,t(14;18)(q32;q21), -15,add(15)(p12),der(18)t(14;18)(q32;q21),+ider(18)(q10)(14;18)(q32;q21),+20,+21,-22,+1-2mat[cp14]	-Positive for <i>IGH/BCL2</i> gene fusion	-Positive for <i>BCL6</i> rearrangement	-Positive for <i>MYC</i> rearrangement
12	47,XX,t(14;18)(q32;q21),-der(18)t(14;18)[1]/54,idem,+add(1)(p13),t(1;10)(q21;q22),-2,-2,add(3)(q27),+5,+del(6)(q13q27),+add(7)(p22),add(8)(q24)+12,ins(12;?)(q13;?)x2,+t,+3-4mar[cp18]/46,XX[1].	-Positive for <i>IGH/BCL2</i> gene fusion (52.5%)	-Positive for <i>BCL6</i> rearrangement (41%)	-Positive for <i>MYC</i> rearrangement (32.5%)
13	Not performed	-Positive for <i>BCL2</i> rearrangement (82%),	-Positive for <i>BCL6</i> rearrangement (91%)	-Positive for <i>MYC</i> rearrangement (78.5%)

Shaded cases are THL; the remaining cases are DHL. Bold highlights abnormalities in the karyotype associated with *MYC*, *BCL2*, or *BCL6* loci. *BCL6* rearrangement was not evaluated by FISH in cases 7, 10, and 12

^AKaryotype from bone marrow involved by lymphoma

Clinical Features

Table II:

Case	Age/ Gender	Diagnostic tissue	Stage	CSF involvement	Treatment	Follow-up (length)
1	74/F	Bone marrow and peripheral blood	IV	Yes	R-CHOP	Deceased (3 months)
2	74/F*	Groin mass	IV	No	R-HyperCVAD → modified POMP	Alive with relapsed disease on supportive care (14 months)
3	59/M	Supraclavicular lymph node	IV	Unknown	Rituximab + dexamethasone	Deceased (2 weeks)
4	46/F	Abdominal mass	II	Unknown	R-CHOP → autologous transplant	Alive no evidence of disease (32 months)
5	62/F	Femur mass	IV	No	DA-EPOCH-R	Alive with no evidence of disease (11 months)
6	67/M	Signoid mass	IV	No	R-CHOP	Alive with no evidence of disease (11 months)
7	60/M*	Small intestine	IV	No	R-CHOP → ICE + ofatumumab → DHAP → FCR	Deceased (7 months)
8	71/M	Mediastinal lymph node	IV	No	R-CV → R-EPOCH → R-ICE → autologous transplant	Deceased (14 months)
9	30/M	Adrenal mass	II	No	HyperCVAD	Alive with no evidence of disease (48 months)
10	48/F	Supraclavicular lymph node	IV	No	R-EPOCH	Alive with relapsed disease (5 months)
11	39/F	Subareolar breast mass	IV	Yes	R-HyperCVAD → myeloablative allogeneic SCT	Alive with no evidence of disease (22 months)
12	55/F	Bone marrow and peripheral blood	IV	Yes	R-HyperCVAD → autologous bone marrow transplant with BEAM conditioning	Alive with no evidence of disease (10 months)
13	52/F	Peripancreatic lymph node and celiac trunk lymph node	I	No	DA-EPOCH-R	Alive undergoing workup for autologous transplant (6 months)

Shaded cases are THL; the remaining cases are DHL

* Patient had a history of follicular lymphoma

Abbreviations: BEAM (carmustin, etoposide, cytarabine, melphalan); CSF (cerebrospinal fluid); DA-EPOCH-R (dose-adjusted etoposide, doxorubicin, and cyclophosphamide with vincristine, prednisone and rituximab); DHAP (dexamethasone, cytarabine, cisplatin); FCR (fludarabine, mitoxantrone, rituximab); R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone); R-hyperCVAD (rituximab, cyclophosphamide, vincristine, doxorubicin, dexamethasone, methotrexate, and cytarabine); POMP (prednisone, vincristine, methotrexate, and 6-mercaptopurine maintenance); R-bendamustine (rituximab, bendamustine); R-CVP (rituximab, cyclophosphamide, vincristine, prednisone); R-EPOCH (Etoposide-Prednisone-Oncovin-Cyclophosphamide-Hydroxydaunorubicin; R-ICE (rituximab, ifosfamide, carboplatin, etoposide); CEP (cytotoxic/etoposide/prednisone)

Table III

Morphologic and Immunophenotypic Features

Case	Original Diagnosis	Morphology	Immunohistochemistry		Flow cytometric immunophenotyping		
			Positive	Negative	Positive	Negative	Light chains
1	High grade BCL	Blastoid	BCL2, BCL6, Ki67 (>95%)	EBER, TdT	CD10, CD19(very dim), CD20, CD45(dim)	CD5, CD34, TdT	Monotypic κ
2	B-LBL	Burkitt-like	CD10, CD79a, BCL2, Pax5, Ki67 (~100%), TdT	CD5, CD20, CD22, CD34, BCL6, EBER	CD10, CD19(dim), CD45(dim), TdT	CD5, CD20, CD34	Negative for s/cy light chains; rare neoplastic cells (0.7%) show λ
3 ^{AA}	BCLU	Centroblastic	CD10, CD20, BCL2, Ki67 (30-40%), TdT	CD34	CD10, CD19, CD20, CD45	CD5, TdT	Monotypic λ
4	BCLU	Burkitt like	CD20, BCL2, Ki67 (90%)	TdT	CD10, CD19, CD20, CD45	CD5	Monotypic κ
5	DLBCL	Centroblastic	CD10, CD20, BCL6, Ki67 (80-90%)	CD5, BCL2, MUM1, EBER, TdT	CD10, CD19, CD20, CD45(very dim)	CD5	Monotypic λ
6	DLBCL	Burkitt like	CD10, CD20, Pax5, BCL6, Ki67 (90%)	CD5, MUM1, EBER, TdT	CD10, CD19, CD45(negative on a subset)	CD5, CD20	Monotypic κ
7	DLBCL	Centroblastic	CD10, CD20, Pax5, BCL2, BCL6, Ki67 (80-90%)	TdT	CD10, CD19, CD20, CD45	CD5	Monotypic λ
8	High grade BCL	Burkitt like	CD10, CD20, BCL2, Ki67 (80%)	EBER, TdT	CD10, CD19, CD20(dim), CD45	CD5	Monotypic κ
9	BCLU	Centroblastic	CD10, CD20, BCL2, BCL6, Ki67 (80-90%)	MUM1, EBER, TdT	CD10, CD19, CD20, CD45(dim)	CD5	Monotypic κ
10	High grade BCL	Blastoid	CD10, CD79a, BCL2, BCL6, Pax5, MUM1, Ki67 (80-90%), TdT (only rare cells)	CD20, CD34, EBER	CD10, CD19, CD20, CD45	CD5, CD34, TdT	Monotypic κ
11	B-LBL	Centroblastic	BCL2, BCL6, Ki67 (~100%), TdT		Increased forward scatter: CD10, CD19, CD45(very dim)	Increased forward scatter: CD5, CD20, CD34, TdT	Increased forward scatter: Negative for s/cy light chains
12 ^{AAA}	High grade BCL	Centroblastic	CD20, Ki67 (80-90%), TdT	CD34	CD10, CD19, CD20(dim), CD45, TdT	CD5, CD34, CD38	Monotypic κ
13	DLBCL	Centroblastic	CD10, CD20, BCL6, Ki67 (95%)	CD5, MUM1, EBER, TdT	CD10, CD19, CD20(dim), CD45(dim)	CD5	Negative for s/cy light chains

Shaded cases are THL; the remaining cases are DHL. Immunophenotypic findings are from evaluation of diagnostic site unless otherwise noted.

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Flow cytometry performed on bone marrow involved by lymphoma;
Flow cytometry performed on peripheral blood involved by lymphoma

Abbreviations: s (surface); cy (cytoplasmic); TdT (terminal deoxynucleotidyl transferase) ; BCL = B-cell lymphoma; B-LBL = B-lymphoblastic leukemia/lymphoma; DLBCL = diffuse large B cell lymphoma; BCLU = B cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma