Clinicopathological Analysis of B Cell Lymphomas, Unclassifiable; with Features Intermediate Between Diffuse Large B-Cell Lymphoma and Burkitt Lymphoma in a Tertiary Care Hospital in Southern India

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Abstract B-cell lymphomas, unclassifiable; with features intermediate between large B-cell lymphoma and Burkitt lymphoma (BCLu-DLBCL/BL) is a new entity included in the recent World Health Organization (WHO) classification of Tumours of the Hematopoietic and Lymphoid Tissues (2008) to overcome the problems of difficulty in classifying certain lymphomas having overlapping morphological, immunophenotypical and genetic features. To study the clinicopathological profile of BCLu-DLBCL/BL. Cross-sectional study over 3 year period in the Haematology section of Department of Pathology in a large teaching hospital in Southern India from January 2011 to December 2013. All the cases reported as BCLu-DLBCL/BL were collected and the clinical, morphological and immunohistochemical parameters were analyzed. Descriptive statistics. There were seven cases, four males and three females, of age ranging from 20 to 70 years. Five cases had extranodal involvement. Four cases had Burkitt morphology with strong Bcl2 positivity and absent CD10 expression. One case had the morphology and immunophenotype that of typical BL, along with strong positivity to Bcl2 suggesting a double hit

hypothesis. Two cases had morphology and immunophenotype of BL with low Ki 67. Three patients on follow up had adverse outcome. BCLu-DLBCL/BL, a provisional category in WHO 2008 is useful in classifying the cases not meeting the criteria for classical BL or DLBCL. Each of these cases was interesting with different sites of involvement, different morphological features and immunophenotype with most of the patients on follow up ending with a grave prognosis.

Keywords BCLu-DLBCL/BL · Burkitt lymphoma · Diffuse large Bcell lymphoma · Intermediate features · WHO 2008

Key Message

Though BCLu-DLBCL/BL requires morphological, immunophenotypical and genetic features for definitive diagnosis, nevertheless it helped us to categorize seven cases with Burkitt morphology with atypical immunophenotype, in spite of lack of facilities for genetic studies.

Part of the study presented in 53rd Annual Conference of ISHBT organized by Odisha Hematology and Department of Clinical Hematology, SCB Medical College & Hospital Cuttack and held at Puri from 9th to 11th November 2012 by Dr. Rakhee as invited speaker in a case based panel discussion.

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Introduction

B-Cell lymphomas unclassifiable (BCLu) with features intermediate between diffuse large B-cell lymphoma (DLBCL) and Burkitt lymphoma (BL) are aggressive lymphomas that have morphological and genetic features of both DLBCL and BL, but for biological and clinical reasons should not be included in these categories [1]. Some of these cases were previously classified as borderline lymphomas, BCLu, atypical BL, Burkitt-like lymphomas, or gray zone lymphomas. The term “Gray Zone

Lymphoma” was first used in 1998 at the “Workshop on Hodgkin’s disease and related diseases” to designate lymphomas at the border of classical Hodgkin Lymphoma and other entities [2]. This term was then further extended to lymphomas with overlapping features between BL and DLBCL. We discuss here the clinical, morphological and immunohistochemical (IHC) features of seven cases that fits into this heterogeneous category.

Materials and Methods

This was a cross-sectional study conducted in the Haematology section of Department of Pathology in a large teaching hospital in Southern India from January 2011 to December 2013. All the cases reported as BCLu-DLBCL/BL by consensus opinion by two or more pathologists based on the complete morphological (H&E stained sections) and IHC workup (IHC markers-CD20, CD3, CD10, Tdt, Bcl2, Bcl6, Ki67) during this period were collected. The clinical details were collected from the medical records of the patients. The patients were closely followed up with regular hematological investigations and remission marrow biopsy was done wherever applicable. The clinical, morphological and IHC parameters were analyzed.

Results

There were seven cases in total with the diagnosis of BCLu-DLBCL/BL during the study period. The age of the patients ranged from 20 to 70 years with mean age of 49 years. Four (57.14 %) out of seven cases were males, and three (42.86 %) were females. Five (71.4 %) of them had extranodal involvement, one case involving the small intestine, one involving perinephric region, one involving ovary, one involving retroperitoneum and the other involving liver and bone. One case had ascites positive for lymphoma cells with Burkitt like morphology.

On histopathological examination all the seven cases had Burkitt morphology with intermediate sized atypical lymphoid cells with increased mitosis. Six cases showed increased apoptosis. Three cases showed starrsky pattern. On IHC all cases were positive for CD20, two cases showed CD3 positivity in reactive T cells, only three cases showed CD10 positivity, Tdt was negative in all cases, Bcl2 was moderate to strongly positive in six cases, Ki67 was high in five cases ranging from 90 to 100 %, and low in two cases where Ki67 was 80 and 85 %. IHC with Bcl6 could be done in three cases and was weakly positive in two cases; c-myc was done in two cases and was positive in one case.

Correlating morphology with IHC features four cases (57.14 %) had Burkitt morphology, high Ki 67 index,

strong Bcl2 positivity and absent CD10 expression. One case (14.28 %) had the morphology of BL with the immunophenotype that of typical BL, in addition with strong positivity to Bcl2 suggesting a double hit hypothesis. Two cases (28.58 %) had morphology and immunophenotype of BL with low Ki 67.

None of the cases showed peripheral blood involvement. One case (14.28 %) showed bone marrow infiltration. Among the seven, two patients opted for treatment outside and two patients lost to follow up and only three patients were on regular follow up. And among the three who were on regular follow up, one case relapsed with marrow involvement, two cases expired during the course of chemotherapy. The follow up period for these patients ranged from 8 to 35 months with mean follow up survival time of 8 months for two patients who expired on the course of chemotherapy and disease free survival of 30 years for one patient who completed chemotherapy. The individual case details including clinical, radiological and investigation findings are tabulated in Table 1.

Discussion

The current classification of lymphoid neoplasms is based on clinical information, morphology, immunophenotype, and molecular genetic characteristics. Most lymphomas can be accurately classified, others are more complex and can easily lead to much confusion. Since clinicians prefer to treat patients according to clear protocols for defined diseases; this borderline category is obviously unattractive.

BCLu-DLBCL/BL is relatively rare and mainly diagnosed in adults. They represent up to 5 % of adult aggressive B-cell lymphomas and usually occur in extranodal sites sometimes associated with leukemic involvement [3]. In this study also all were adults with five out of seven cases having extranodal involvement without peripheral blood involvement.

According to WHO (2008) the diagnosis BCLu-DLBCL/BL is based on morphology and immunophenotype/genetic features. The morphologic features include variable cellular forms, such as those smaller than typical DLBCL, those resembling BL cells, those larger than typical BL, and those resembling DLBCL cells, presence of starry sky macrophages, many mitotic figures and apoptosis. Some case are morphologically consistent with BL but with atypical immunophenotype/genetic features. Some has immunophenotype consistent with BL but with variable nuclear size. However it should be emphasized that cases which are morphologically DLBCL with high proliferation index is not included in this category [1, 4].

The immunophenotype shows positivity for B—cell markers like CD19, CD20, CD22, CD79a, and germinal

Table 1 Clinical, radiological, morphological and immunohistochemical details of cases

Case no.	Case 1	Case 2	Case 3	Case 4	Case 5	>Case 6	Case 7
Date of diagnosis	11/4/2012	13/3/2012	27/1/2011	12/3/2011	10/1/13	20/6/13	2/9/13
Age (years)	20	30	70	45	65	50	62
Sex	Male	Male	Male	Female	Female	Male	Female
Clinical details	Left cervical lymphadenopathy	Left lower abdominal pain and mass	Left cervical lymphadenopathy	Abdominal pain	Abdominal pain	Axillary & cervical lymphadenopathy, ascites	Retropertitoneal mass
Radiological findings	USG Multiple intrabdominal lymph nodes seen. Small bowel thickening in RIF	CT Scan 17 × 17 cm heterogenous mass with central scar in left perinephric region	–	USG Multiple lesions in right and left lobe of liver BONE SCAN Multifocal lesions	USG Heterogenous hypoechoic mass in right adnexa	–	–
Peripheral Smear	No atypical cells	No atypical cells	No atypical cells	No atypical cells	No atypical cells	No atypical cells	No atypical cells
Cytology	–	–	–	<i>Liver</i> — Hematolymphoid malignancy	–	<i>Lymph node, ascitic fluid</i> —High grade NHL with Burkitt like features (Fig. 4a, b)	High grade NHL with burkitt like features
Biopsy	<i>Lymph node</i> BCLu-DLBCL/BL (Fig. 1a,b)	<i>Perinephric mass</i> BCLu-DLBCL/BL (Fig. 2a, 2b)	<i>Lymph node</i> BCLu-DLBCL/BL	<i>Bone marrow</i> High grade NHL— Burkitt like	<i>Ovary</i> BCLu-DLBCL/BL (Fig. 3a, b)	<i>Lymph node</i> BCLu-DLBCL/BL	<i>Retropertitoneal mass</i> BCLu-DLBCL/BL
CD20	Positive (Fig. 1c)	Positive (Fig. 2c)	Positive	Positive	Positive (Fig. 3c)	Positive	Positive
CD3	Reactive T cells (Fig. 1d)	Negative	Reactive T cells	Negative	Negative	Negative	Negative
Ki 67	97 % (Fig. 1e)	95 % (Fig. 2d)	100 %	80 %	95 % (Fig. 3d)	85 %	90 %
Bcl2	Positive (Fig. 1f)	Strongly positive (Fig. 2e)	Positive	Negative	Positive (Fig. 3e)	Positive	Positive
CD10	Negative	Negative (Fig. 2f)	Positive	Positive	Negative (Fig. 2f)	Positive	Negative
Tdt	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Bcl6	–	Focal weak positivity	–	–	Weakly positive	Negative	–
c-myc	–	–	–	–	Negative	Positive (80 %)	–

Table 1 continued

Case no.	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7
Bone marrow	No evidence of infiltration	–	–	NHL infiltration—Burkitt like	No evidence of infiltration	No evidence of infiltration	No evidence of infiltration
Treatment & Follow up	Completed chemotherapy and Relapsed with bone marrow infiltration after 30 months. Restarted chemotherapy and is on follow up for past 5 months	Opted for treatment outside	Opted for treatment outside	Started chemotherapy Expired within 8 months	Lost to follow up	Lost to follow up	Started chemotherapy and expired within 8 months

center-associated molecules, CD10 and Bcl6. Bcl2 expression may be absent, weak, or strong, IRF/MUM1 should be negative and the Ki67 labeling index is usually high showing varying positivity from 50 to 100 %. In a case with Burkitt morphology weak positivity for Bcl2 is accepted, but strong expression of Bcl2 and a proliferation fraction below 90 % are strong contraindications for a diagnosis of BL. Bcl2 positivity in an otherwise BL immunophenotype suggests the possibility of double hit lymphoma [1, 4].

Genetically, 35–50 % of cases of intermediate DLBCL/BL show 8q24/*MYC* translocations but often with atypical features, including one or more of the following: (1) rearrangement with a non-*IG* partner, (2) part of a complex karyotype, and (3) concurrent rearrangements of the *Bcl2* and/or *Bcl6* genes, suggesting a “double-hit” or “triple-hit” lymphoma. Double-hit lymphomas are characterized by a second translocation in addition to t(8;14), t(8;22), or t(2;8) [3, 5–7]. Importantly, lymphomas with a typical DLBCL morphology that have a *MYC* breakpoint are excluded from the category of BCLu-DLBCL/BL. Up to 15 % of DLBCL have *MYC* translocations [8, 9], and they are generally associated with an inferior outcome [10, 11]. Genetic analysis was not done in our institutes it is a resource limited setting where there are no facilities for genetic studies and where patients are poor who cannot afford additional outsourced molecular investigation.

In a recent paper Siebert et al. [12], reviewed the “grey zone” between BL and DLBCL from a genetic perspective. This paper aims to clarify the different definitions of intermediate lymphomas and to propose a subclassification based on genetic aberrations. The “intermediate lymphoma” group from gene expression profile (GEP) studies and the BCLu-DLBCL/BL from the WHO classification though similar are by far not identical. The ‘intermediate group’ of GEP is defined as a group of lymphomas not meeting the profiling of either molecular BL or for molecular DLBCL. Therefore, this category represents a wastebasket for all lymphomas, which do not fit into the two molecularly defined entities. On the other hand, the BCLu-DLBCL/BL entity defined by the WHO contains all lymphomas, which does not meet the criteria of either BL or DLBCL on morphologic, IHC/classical genetic grounds and represents a heterogeneous group of diseases.

In a setting where there are no facilities for molecular studies, the criteria defined by WHO classification plays significant role in diagnosing the intermediate lymphomas based on morphology and immunohistochemistry. In this study the first, second, fifth and seventh case had Burkitt morphology and high Ki 67 index but classified as intermediate between DLBCL/BL based on the strong IHC positivity for Bcl2 and absence of CD10. In case three the

Fig. 1 **a, b** Cervical lymph node with Burkitt morphology with starry sky pattern (40 $\times$, 1000 $\times$ H&E); **c** CD20 positivity (100 $\times$, IHC); **d** CD3 positivity in scattered reactive T cells (100 $\times$, IHC); **e** High Ki 67 (100 $\times$, IHC) and **f** Bcl2 positivity (100 $\times$, IHC)

Fig. 2 **a, b** Perinephric tissue showing infiltration by lymphoma with Burkitt morphology with increased mitosis and apoptosis (40 $\times$, 400 $\times$; H&E); **c** CD20 positivity (100 $\times$, IHC); **d** High Ki 67 (100 $\times$, IHC); **e** Strong Bcl2 positivity (100 $\times$, IHC); **f** CD10 Negative (100 $\times$, IHC)

morphology was that of BL with the immunophenotype that of typical BL, in addition with strong positivity to Bcl2 which favored the diagnosis of intermediate DLBCL/BL suggesting a double hit hypothesis. In case four and six again though the morphology and immunophenotype

favoured BL, the low Ki 67 precludes the diagnosis of BL and places in the category of BCLu-DLBCL/BL. Also this case is interesting in the sense that the patient presented with multiple liver and bony lesions suggesting secondaries which later turned out to be lymphoma.

Fig. 3 **a, b** Ovary showing infiltration by lymphoma with Burkitt morphology with starry sky appearance (100 $\times$, 400 $\times$; H&E); **c** CD20 positivity (100 $\times$, IHC); **d** High Ki 67 (100 $\times$, IHC); **e** Bcl2 positivity (100 $\times$, IHC), **f** CD10 Negative (100 $\times$, IHC)

Fig. 4 **a** FNAC from cervical lymph node showing atypical cells with cytoplasmic vacuoles suggestive of Burkitt morphology (400 $\times$, MGG); **b** Centrifuge smear from ascitic fluid showing large atypical cells with Burkitt morphology (400 $\times$, MGG)

Thus in cases with Burkitt morphology, the diagnosis of BCLu-DLBCL/BL can be made if there is an atypical immunophenotype like absent CD10, moderate/severe Bcl2 expression or low Ki 67 index (<95 %). The necessity for genetic workup in these cases is just adjunctive and confirmative to morphology and IHC. But in cases with morphological DLBCL, the genetic study becomes essential since DLBCL can show wide immunophenotype, also DLBCL with high proliferation index is not included in the intermediate category. This study did not have any cases with DLBCL morphology.

The clinical evolution of patients with double-hit lymphomas is dramatic with frequent involvement of peripheral blood, bone marrow and CNS with a median survival of 4.5 months, and they are usually resistant to either conventional CHOP-like regimens or to intensive therapy used to treat BL. This study had one case with IHC features suggestive of double hit lymphoma with no involvement of

peripheral blood who was lost to follow up. However, factors associated with a better survival have been identified, which include non-IGH MYC partner, BCL2 protein expression, and rituximab inclusive chemotherapy [13].

In children, high cure rates are achieved with treatment strategies similar or identical to those for BL and DLBCL [14]. The gray zone between BL and DLBCL currently does not impact therapy decision or outcome in childhood lymphomas [15]; and either intermediate lymphomas according to both GEP and WHO classification are infrequent in patients under the age of 18 years and do not seem to have an adverse prognosis [12, 16]. Therefore, it seems that in children “intermediate lymphomas” represent rather true biologic BL, which were classified as “intermediate” in the GEP due to secondary aberrations. In adult patients the situation is rather different and the subclassification of aggressive B-cell lymphomas has a true impact on treatment decisions as it is usually associated with poor

response to chemotherapy and grave prognosis. In this study also the 20 years old patient tolerated the chemotherapy and had a disease free survival of 30 months when compared to the other two patients of old age who expired at 8 months indicating a better response by younger patients to chemotherapy than the elderly as mentioned in literature.

Perry et al. [17] reported 39 cases of BCLu-DLBCL/BL over a period of 15 years. In his study majority of patients presented with advanced-stage disease (62 %) with median overall survival (OS) of only 9 months and the 5-year OS was 30 %. He concluded that these cases were genetically heterogeneous and none of the IHC or genetic features was predictive of survival. New and better treatments are needed for this aggressive lymphoma.

Bürgesser et al. [18] studied the clinicopathological features of 30 cases of DLBCL, 8 cases of BL and 6 cases of BCLu-DLBCL/BL in Argentina and found the mean follow-up survival time of BCLu-DLBCL/BL was 6.6 months, which was significantly shorter in comparison to DLBCL (31 months) and BL (30 months), respectively with different clinical course and poor prognosis. The mean follow up survival time for BCLu-DLBCL/BL in this study is 8 months for the two patients who expired on chemotherapy and the disease free survival is 30 months for one patient who completed chemotherapy.

Edwards et al. [19] conducted a retrospective study over 10 years and studied the clinical outcome and treatment response of 34 BCLu-DLBCL/BL, 9 BL, and 97 DLBCL and found that BCLu-DLBCL/BL patients present with more advanced disease, progress while on treatment and had a poorer survival. In this study all the three patients who had follow up showed adverse outcome in the form of relapse or death indicating failure to respond to chemotherapy as described in literature.

Jaradat et al. [20] reported a case of BCLu-DLBCL/BL involving the oral cavity. Ahn et al. [21] reported a case report of BCLu-DLBCL/BL involving the femur neck in a Korean child. Zhang et al. [22] reported two cases in paediatric population. There are no case series of BCLu-DLBCL/BL available from Indian literature after extensive search to the best of our knowledge. Although studies are available in western literature they have focused mainly on clinical outcome and survival; the morphological and IHC profile of this lymphoma is not studied in detail.

Conclusion

BCLu-DLBCL/BL is a rare and heterogeneous category not considered as a distinct disease entity in WHO 2008 but is useful in classifying the cases not meeting the criteria for classical BL or DLBCL. Though BCLu-DLBCL/BL requires

morphological, immunophenotypical and/or genetic features for definitive diagnosis, in a resource limited setting where there are no facilities for genetic studies, this intermediate category helped us to club seven such cases with Burkitt morphology with atypical immunophenotype based on morphology and immunohistochemistry. Each of these cases was interesting with different sites of involvement with different morphological features and immunophenotype with most of the patients on follow up ending with a grave prognosis.

Conflict of interest None declared.

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