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ORIGINAL ARTICLE

Frequent loss of CD10 expression in follicular lymphoma with leukaemic presentation

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Abstract

Introduction: Follicular lymphoma (FL) is usually a nodal lymphoma expressing CD10, rarely with leukaemic presentation (FL-LP). *Materials and Methods:* We searched for FL-LP in our institution from 2000 to 2018 and characterised the neoplastic cells by flow cytometry, immunohistochemistry and fluorescence *in situ* hybridization. Thirteen (6.1%) of 212 FL cases were FL-LP, all *de novo* neoplasms. The leukaemic cells were small in 12 cases and large in one. All had concurrent FL, mostly (92%; 12/13) low-grade. The single case with large leukaemic cells had a concurrent primary splenic low-grade FL and a double-hit large B-cell lymphoma in the marrow. *Results:* CD10 was expressed in the leukaemic cells in 38% (5/13) cases by flow cytometry and in 77% (10/13) cases in tumours (p= 0.0471). *IGH/BCL2* reciprocal translocation was identified in 85% (11/13) cases. Most patients were treated with chemotherapy. In a median follow-up time of 36 months, nine patients were in complete remission. The 2- and 5-year survival rates were at 100% and 83%, respectively. In this study, we characterised a series of *de novo* FL-LP in Taiwan. All patients had concurrent nodal and/or tissue tumours, which might suggest that these patients seek medical help too late. Conclusion: The lower CD10 expression rate by flow cytometry than by immunohistochemistry might be due to different epitopes for these assays. Alternatively, loss of CD10 expression might play a role in the pathogenesis of leukaemic change. The clinical course of FL-LP could be aggressive, but a significant proportion of the patients obtained complete remission with chemotherapy.

Keywords: B-cell leukaemia; CD10; fluorescence in situ hybridization; follicular lymphoma; leukaemic presentation; small B-cell leukaemia; Taiwan

INTRODUCTION

Follicular lymphoma (FL) is a malignant lymphoma of follicular centre B-cell origin, usually involves lymph nodes, but also spleen, Waldeyer's ring, bone marrow, and peripheral blood. FL with leukaemic presentation (FL-LP) is uncommon.[1-5] Beltran *et al*. reported seven cases plus a review of 24 additional cases from the literature and found that such patients tended to have higher risk disease.[4] Sarkozy *et al*. found that 7.4% (37/499) FL cases presented with leukaemia at the Lyon-Sud Hospital, France, and FL-LP was associated with poor prognosis.[5] They also found that those with circulating lymphoma cells > 4 x10^3/μL had a poorer outcome.[5]

Among various types of small B-cell lymphomas, CD10 is expressed almost exclusively by FL.[6] In flow cytometric immunophenotyping, a small B-cell leukaemia expressing CD5 and CD23 with dim CD20 expression is diagnostic for chronic lymphocytic leukaemia, while a small B-cell leukaemia expressing CD10 but not CD5 or CD23 is usually considered a leukaemic phase of FL. In this study, we characterised the clinicopathological features of 13 cases of FL-LP in Taiwan and found that

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the leukaemic cells in FL-LP frequently lost CD10 expression, which might be a potential diagnostic pitfall if only leukaemic cells were investigated without a tissue biopsy. To our surprise, the prognosis of our FL-LP cases was not associated with a poor outcome as compared to the previous studies.[4,5]

MATERIALS AND METHODS

We retrospectively searched for FL-LP in our institutions from January 2000 to December 2018. Only *de novo* FL cases were included. This study was approved by the Internal Review Board (approval code: 10112-004) at Chi-Mei Medical Center, Tainan, Taiwan.

We reviewed the peripheral blood smears, flow cytometric immunophenotyping, pathology, and clinical features of these patients. Immunohistochemistry and fluorescence *in situ* hybridization (FISH) using paraffin sections of the biopsy specimens were performed. All the immunohistochemical stains were performed in an autostainer (BOND-III, Leica BioSystems, Newcastle upon Tyne, UK) using a polymer-based detection system (Bond Polymer Refine Detection; Leica BioSystems). For CD10 immunohistochemistry, we pretreated the deparaffinised sections with BOND ER2 reagent for 30 minutes in the BOND III autostainer. Then we incubated the sections with anti-CD10 antibody (Clone 56C6 from Novocastra, Newcastle upon Tyne, UK; x100 dilution) for 30 minutes. DAB was used as chromogen. The FISH probes used included dual colour break apart rearrangement probes directed at *IGH*, *BCL2*, *BCL6* and *MYC* loci and dual colour reciprocal translocation probe for *IGH/BCL2* (Vysis/Abbott Laboratories Ltd, UK) as previously described.[7]

RESULTS

During this study period, there were a total of 1,684 cases of lymphoma diagnosed in our institutions, and there were 212 cases (12.60%) of FL. Among these FL cases, 13 (6.1%) presented initially with leukaemia (or FL-LP). All these cases were *de novo* neoplasms, without a prior history of lymphoma. Table 1 lists the pertinent clinicopathological features of these cases. There were 7 males and 6 females with a median age of 52 (range, 34-75). All cases had concurrent tumours: one had only marrow disease (Case no. 5), the remaining 12 patients had involvement of multiple organs including lymph node (n=11), bone marrow (n=11), spleen (n=8), nasopharynx (n=2), and other rare sites.

The median leukaemic cell count was 11.40 x 10^3/μL (range, 6.08-154.6 x 10^3/μL). Two of these cases have been previously reported.[8,9] In one case (Case no. 12),[9] the leukaemic cells were large with vesicular nuclei and open chromatin, while that in the remaining 12 cases the leukaemic cells were small with irregular nuclear contours and condensed chromatin (Fig. 1).

Table 2 lists the pertinent pathological features, immunophenotype, and FISH findings of these cases of FL-LP. Histologically, the majority of concurrent FLs were low-grade (n=12; 92%), with the remaining case had grade 3A disease (Case no. 6). Interestingly, the case with large leukaemic cells (Case no. 12) had a concurrent primary splenic low-grade FL and a double-hit (rearranged *BCL2* and *MYC* genes) large B-cell lymphoma, of germinal centre B-cell phenotype and also a double-expresser (positive for both bcl-2 and MYC proteins), in the marrow.[9] CD10 was expressed in the leukaemic cells in 38% (5/13) cases by flow cytometry and in 77% (10/13) cases in the corresponding tumour tissues (p= 0.0471).

FISH assay showed that both *IGH* and *BCL2* loci were rearranged in 77% (10/13) cases, and all these rearranged cases had *IGH/BCL2* reciprocal translocation by dual fusion probes.

Bone marrow karyotyping was available in four cases. In Case no. 5, the result was 46,X. del(X)(p22.1)[cp14]/46,XX[cp6], probably representing normal karyotype with mosaicism for Turner syndrome. In Case no. 12, the karyotype was complex: 48,XY,der(2q),der(3q),der(6q),+7,+8,t(8;14)(q24;q32),der(13q),der(18q)[cp19]/46,XY[1], indicating rearranged *MYC* and *IGH* loci. As detailed in our prior case report that the tumour cells also exhibited *BCL6* rearrangement, this patient had a primary low-grade splenic FL and a concurrent leukaemic presentation by large neoplastic cells, indicating a double-hit lymphoma.[9] In the remaining two cases (Case nos. 1 and 4), the karyotype was normal.

Excluding one case with incomplete data, the FLIPI scores were 1 (n=2), 2 (n=2), 3 (n=3), 4 (n=4), and 5 (n=1), respectively. The primary treatment included eight patients with cyclophosphamide, vincristine, and prednisolone (COP), with or without rituximab (R) or obinutuzumab (G), three with R or G-CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisolone), and one case

TABLE 1: Clinical and laboratory features of follicular lymphoma with leukaemic presentation

No./Sex/Age	WBC	Lymphocytes	Bx site	Other involved organs	Grade	FLIPI	C/T	FU (m)
1/F/75	166.7K	92.75% (154.6K)	LN, left axilla	LN, BM	1	4	COP x6	DOD (61)
2/F/38	27.8K	34.50% (9.59K)	LN (site unspecified)	LN, spleen, BM	1	3	R-COP, R maintenance	NED (129)
3/M/52	19.5K	78% (15.21K)	LN, left axilla	LN	1	1	R-COP x8	NED (129)
4/M/43	15.3K	79% (12.09K)	LN, right neck	LN, BM	1	4	HyperCVAD	AWD (90)
5/F/69	15.0K	76% (11.40K)	BM	BM	1	4	Rituximab	NED (72)
6/F/56	10.3K	59% (6.08K)	LN, right axilla	LN, NP, spleen, lungs, bone, BM	3A	4	R-CHOP, ICE for relapse	DOD (33)
7/F/52	19.0K	74% (14.06K)	spleen	LN, spleen, BM	1	1	G-COP	NED (41)
8/M/69	12.8K	55% (7.04K)	LN, right inguinal	LN, spleen, BM	2	5	R-COP x8	NED (36)
9/M/38	17.3K	79% (13.68K)	LN, right inguinal	LN, spleen, BM	2	NA	R-COP x6->PR -> relapse -> R-CHOP x4	NED (32)
10/F/45	11.4K	62.30% (7.10K)	LN, right neck	LN, spleen, BM	1	2	R-COP x8	NED (25)
11/M/58	14.0K	48.50% (6.79K)	Nasopharynx	LN, NP, spine, ST, BM	2	3	R-COP x8, R maintenance	NED (19)
12/M/68*	31.4K	48.00% (18.33K)	Spleen	Spleen, BM	2	3	R-CHOP x3	DOUD (3)
13/M/34	16.9K	62% (10.48K)	LN, left neck	LN, spleen, BM	2	2	G-CHOP x8. G maintenance	NED (12)

Lymphocyte, lymphocyte counts presented as percentage among all WBC and absolute lymphocyte counts. *Case 12 presented as large cell leukaemia/transformation while the primary splenic tumour was a low-grade FL. The patient died of dengue fever after the third course of R-CHOP chemotherapy.
Abbreviations: AWD, alive with disease; BM, bone marrow; COP, cyclophosphamide, vincristine, and prednisolone; C/T, chemotherapy; DOD, died of disease; DOUD, died of unrelated disease; FLIPI; follicular lymphoma international prognostic index; G, Gazyva (obinutuzumab); G-CHOP, obinutuzumab plus cyclophosphamide, doxorubicin, vincristine, and prednisolone; HyperCVAD: hyperfractionated cyclophosphamide, vincristine, doxorubicin, dexamethasone, cytarabine, and methotrexate; ICE, ifosfamide, carboplatin and etoposide; LN, lymph node; NED, no evidence of disease; NP, nasopharynx; R, rituximab; ST, soft tissue.

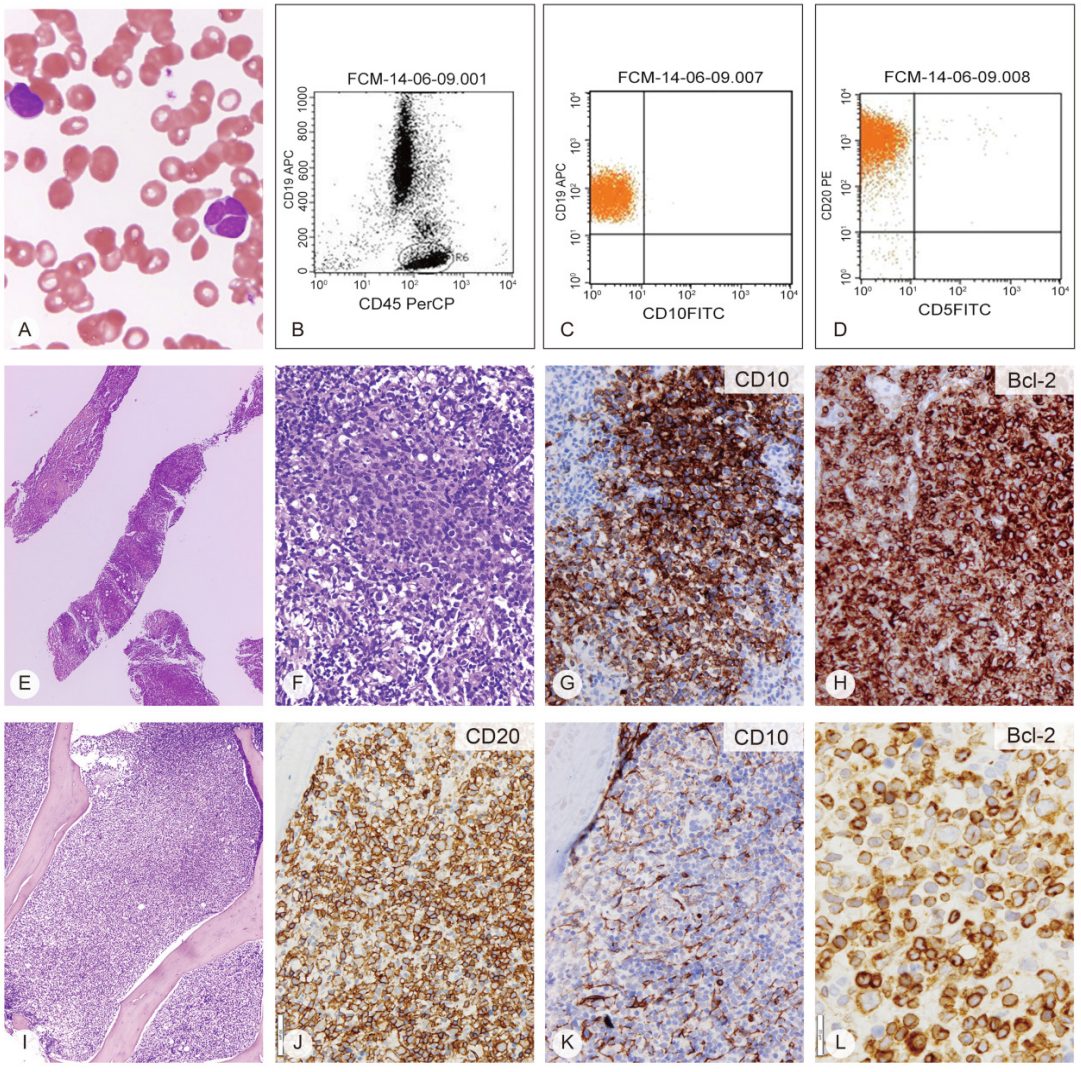

FIG. 1: A representative case of FL with leukaemic presentation (Case no. 6). A, A leukaemic cell with condensed chromatin and convoluted nuclear shape on the right-hand side. B-D, Flow cytometric immunophenotyping of these leukaemic cells shows an expression of CD19 and CD20 but not CD5 or CD10. E-H, Core biopsy of the left inguinal lymph nodes reveals a grade 3A FL with nodular lesions comprising of many centroblasts (F, x400) expressing CD10 (G) and bcl-2 (H). I-L, Bone marrow biopsy shows extensive marrow involvement by lymphoma cells expressing CD20 (J, x400) and bcl-2 (L, x1,000). Please note that the stromal cells but not the neoplastic cells express CD10 (K, x400).

each with HyperCVAD (hyperfractionated cyclophosphamide, vincristine, doxorubicin, dexamethasone, cytarabine, and methotrexate) and rituximab alone. In a median follow-up time of 36 months (range, 3-129), nine patients were in complete remission, one alive with disease, two died of disease, and one died of dengue fever after the third course of chemotherapy. The 2- and 5-year survival rates were 100% (10/10) and 83% (5/6), respectively.

DISCUSSION

FL-LP is a rare event among patients with FL and this phenomenon has only been occasionally reported.[1-5] In this study, we characterised the clinicopathological, immunophenotypic, and genetic features of a series of *de novo* FL-LP in Taiwan. All patients had concurrent nodal and/or tissue tumours, without any cases with leukaemic phase alone, which might suggest that these patients seek medical help too late. The relative frequency of FL-LP at 6.1% is

TABLE 2: Pathological features, immunophenotype, and FISH findings.

				Immunohistochemistry			FISH				
No./Sex/Age	**Bx site**	**Grade**	**CD10 by FCM**	**CD10**	**bcl-2**	**bcl-6**	***IGH***	***BLC2***	***IGH/BCL2***	***BCL6***	***MYC***
1/F/75	LN, left axilla	FL, gr 1	+	+	+	+	-	-	-	-	ND
2/F/38	LN (neck or axilla)	FL, gr 1	-	+	+	+	+	+	+	-	ND
3/M/52	LN, left axilla	FL, gr 1	-	-	+	+	-	-	-	-	ND
4/M43	LN, right neck	FL, gr 1	-	+	+	+	+	+	+	-	ND
5/F/69	BM	FL, gr 1	-	-	+	+	-	-	ND	ND	ND
6/F/56	LN, right axilla	FL 3A	-	-	+	+	+	+	+	-	ND
7/F/52	Spleen	FL, gr 1	+	+	+	-	+	+	+	ND	ND
8/M/69	LN, right inguinal	FL, gr 2	+	+	+	+	+	+	+	-	ND
9/M/38	LN, right inguinal	FL, gr 2	-	+	+	+	+	+	+	?+	ND
10/F/45	LN, right neck	FL, gr 1	-	+	+	+	+	+	+	-	ND
11/M/58	Nasopharynx	FL, gr 2	+	+	+	+	+	+	+	-	ND
12a/M/68	BM	Double-hit LBCL*	-	+	+	+	failed	+	+	failed	failed
12b/M/68	Spleen	FL, gr 2	-	+	+	-	+	+	+	-	ND
13/M/34	LN, left neck	FL, gr 2	+	+	+	+	+	+	+	ND	+

Abbreviations: BM, bone marrow; FCM, flow cytometric immunophenotyping; LBCL, large B-cell lymphoma; LN, lymph node, ND, not done. *In Case 12a, bone marrow karyotyping revealed 48,XY,der(2q),der(3q),der(6q),+7,+8,t(8;14)(q24;q32),der(13q),der(18q)[cp19]/46,XY[1], indicating translocation involving *IGH* and *MYC* genes. Together with *BCL2* rearrangement by FISH, the BM specimen of this case was diagnosed as double-hit large B-cell lymphoma.

comparable to, but slightly lower than the 7.4% from the French series by Sarkozy *et al*.[5] The median lymphocyte count in that study was 4.0 x 10^3/μL (range 1-130 x10^3), while that in our study the value was higher at 11.40 x 10^3/μL.

Flow cytometric immunophenotyping is considered to be more sensitive than immunohistochemistry in surface antigen detection. However, in our study, the CD10 expression rate by the leukaemic cells using flow cytometry was significantly lower as compared to their tissue counterpart by immunohistochemistry (38% vs. 77%; p= 0.0471, Chi-squared test). This phenomenon might be due to the effects of different microenvironment for the neoplastic cells (serum vs. tissue), or might be the results of the different epitopes for antigen detection between flow cytometry and immunohistochemistry. Alternatively, loss of CD10 expression might play a role in the pathogenesis of leukaemic change.

Jacob *et al* had previously reported a similar finding of CD10 downregulation in leukaemic phase of FL.[3] In their report, seven (54%) of 13 FL-LP cases were negative for CD10 by the leukaemic cells; in contrast, nodal biopsies in six of these seven patients were performed, and the neoplastic cells in all six cases were positive for CD10 by immunohistochemistry. Interestingly, the neoplastic cells in the marrow trephine were negative for CD10 by immunohistochemistry in five of six cases. Their findings revealed the discrepant CD10 expression rates of FL in distinct organs/microenvironment. Again, the lower than expected CD10 expression rate by leukaemic cells of FL might pose a diagnostic challenge in flow cytometric diagnosis of small B-cell leukaemias.

In our prior study of FL in Taiwan, only 63% (36/57) cases of low-grade FL exhibited *IGH/BCL2* reciprocal translocation.[10] In that study, we also showed that the relative frequency of FL with *IGH/BCL2* reciprocal translocation was lower in East Asian populations as compared to the Western ones (50-80% vs. 85-95%).[10] In the current study, 77% (10/13 cases) of FL-LP cases were positive for *IGH/BCL2* reciprocal translocation, comparable to most Asian FL cases, but slightly lower than the Western FL series.[10-12]

In so-far the largest series by Sarkozy *et al*. from the Lyon-Sud Hospital France, FL-LP patients were matched with 111 newly diagnosed FL without LP. Presence of FL-LP was associated with shorter progression-free survival and overall survival (OS). Presence of FL-LP and high FL International Prognostic Index (FLIPI) score remained independent prognostic factors in a Cox model for time to progression (TTP). A number of circulating lymphoma cells (CLC) > 4.0 x 10^3/μL was the most significant predictor for a shorter TTP in this Cox model. They estimated that the overall survivals at 5 and 10 years of their 37 patients were 86% and 68%, respectively.[5] The clinical course of patients with FL-LP could be more aggressive than those without; however, excluding Case no. 12 (with large cell leukaemia and died of dengue fever), half (6/12) of our patients achieved complete remission after chemotherapy with a median follow-up time at 70 months (range, 28-129). The 2- and 5-year OS rates were 100% (7/7) and 83% (5/6), respectively. Although our case number was small, our actual 5-year OS rate at 83% was comparable to that of 86% as estimated by Sarkozy *et al*.[5] We speculated that FL-LP was not associated with a poor prognosis in Taiwan; however, studies on larger numbers of patients are warranted for a better understanding of the impact of leukaemic presentation in patients with FL.

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Conflict of interest: The authors declare they have no conflict of interests.

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