Article

Transformation of follicular lymphoma into classical Hodgkin lymphoma showing t(14;18)

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Abstract: Follicular lymphomas (FLs) are B-cell lymphomas with a generally indolent clinical course with the propensity to transform to higher grade lymphomas. Most commonly, FL transforms into diffuse large B-cell lymphoma (DLBCL), but transformation to other higher-grade B-cell lymphomas has also been described. Transformation to classical Hodgkin lymphoma (cHL) has been documented only anecdotally. Here we describe five patients diagnosed with FL and also subsequently or synchronously with cHL. For each patient, we demonstrate the clonal relationship between the FL and the cHL by the identification of t(14;18) in both the FL and in the Hodgkin-Reed-Sternberg (HRS) cells, if available. Aneuploidy and atypical expression of BCL2 was commonly seen in the HRS cells. After transformation to cHL, the lymphomas demonstrated a more aggressive clinical behavior. In conclusion, we report five cases of FL with clonal transformation to cHL and aggressive clinical behavior that typifies other more frequently encountered transformed B-cell lymphomas.

Keywords: Classical Hodgkin lymphoma, follicular lymphoma, t(14;18), transformation

Introduction

Follicular lymphoma (FL) is a generally indolent B-cell lymphoma arising from follicular germinal center B cells [1]. Composed of a mixture of centrocytes and centroblasts, FL is characterized in the majority of cases by the translocation t(14;18)(q32;q21), which is thought to occur early in the disease course [2]. The translocation results in a *IGHG1-BCL2* fusion (henceforth abbreviated as *IGH-BCL2*) with the resultant overexpression of the anti-apoptotic protein, BCL2 [3]. Although most patients with FL have widespread disease at diagnosis, they may be otherwise asymptomatic. In a subset of cases, FL will undergo histologic transformation to an aggressive lymphoma, with risk of transformation ranging from 11% to 17% at 5 years [4, 5] and approximately 30% at 10 years [6]. The transformed lymphomas are most frequently diffuse large B-cell lymphoma (DLBCL) and less commonly Burkitt lymphoma or B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma [6].

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Rare cases of blast transformation [7, 8] as well as co-occurrence with histiocytic/dendritic cell sarcomas have also been described [9].

A few cases of composite lymphomas consisting of both follicular and Hodgkin lymphomas, as well as follicular lymphoma with Hodgkin or Reed-Sternberg-like cells have previously been reported [10–17]. In addition, rare cases of true transformation of follicular lymphoma to Hodgkin lymphoma have been reported. In this report, we describe five patients who developed FL that subsequently or synchronously developed into distinct lymphomas that were morphologically and immunophenotypically consistent with classical Hodgkin lymphoma (cHL). This report is so far the largest series describing this atypical transformation of follicular lymphoma. The clonal relatedness of these two lymphomas was documented in each case by identification of the t(14;18) by fluorescence *in situ* hybridization (FISH).

Materials and Methods

Patient characteristics

Four patients with follicular lymphoma and classical Hodgkin lymphoma were from two different Veterans Affairs (VA) institutions, Tennessee Valley Healthcare System (Nashville, VA), and the Louisville VA. One patient was from Brigham and Women's Hospital (BWH, Boston, MA). This study has been approved by the IRBs of each institutions. Lymph node and bone marrow specimens were reviewed by practicing pathologists with hematopathology expertise (AK, CM, MN, and SS) during the course of routine clinical work and the de-identified data reviewed by all pathologists for the purpose of this manuscript.

Histology/immunohistochemistry

Cases 1, 3, 4, and 5 were seen in consultation and exact information of the original histology and immunohistochemical (IHC) studies is unknown. Cases 1-3 had additional IHC stains performed at Vanderbilt University Medical Center (VUMC) and case 4 at Joint Pathology Center (JPC) as follows. Hematoxylin-eosin (H&E) and IHC stains were performed on 5 μm sections (VUMC) or 3 μm sections (JPC) of routinely formalin-fixed, paraffin embedded tissue (FFPE). The Leica Bond Polymer Refine Detection (DAB) Kit (Leica, Buffalo Grove, IL), mouse or rabbit, was used for antibody staining. IHC staining was performed on either the Benchmark Ultra (Ventana, Tucson, AZ) or Bond-III (Leica, Buffalo Grove, IL) automated stainer with standard heat or enzyme-induced epitope retrieval. The following monoclonal antibodies were used: CD3, CD20, and CD45 (Ventana, Tucson, AZ); BCL2, BCL6, CD5, CD10, CD15, CD30, PAX5, and TP53 (Leica, Buffalo Grove, IL), and c-Myc (Abcam, Cambridge, MA).

In situ hybridization

In situ hybridization (ISH) for EBER was performed on 5 μm FFPE sections using a Bond^{TM} Ready-to-Use *in situ* hybridization EBER probe (Leica, Buffalo Grove, IL). The Leica Bond Polymer Refine Detection (DAB) Kit, mouse or rabbit, was used for staining with a Mouse Anti-FITC secondary antibody (Leica, Buffalo Grove, IL).

Fluorescence in situ hybridization

FISH analysis was performed at VUMC (patients 1 and 2) and BWH (Patient 5) using Vysis LSI *IGH/BCL2* dual-color, dual fusion probes (Abbott Laboratories, Des Plaines, IL) on routinely processed 4 μm thick sections and processed according to the manufacturer's instructions. Details of the FISH methods used for patients 3 and 4 are not available. The signals from both follicular lymphoma and classical Hodgkin lymphoma areas were analyzed. FISH slides were reviewed for hybridization pattern as well as cellular morphology by hematopathologists AK and GG and cytogeneticists AY, FW, and AL.

Flow cytometry

Flow cytometry was performed for patient 2 using either an 8-color FACScanto II or FACSCalibur cytometer (Becton-Dickinson Biosciences, San Jose, CA) with antibodies to CD3, CD4, CD5, CD7, CD8, CD10, CD19, CD20, CD23, CD33, CD38, and immunoglobulin kappa and lambda light chains (all Becton-Dickinson). The other cases were obtained in consultation and flow cytometry data was provided by report only.

Results

The case histories for the five patients are described below. The clinical outcomes are summarized in Table 1 and the immunophenotypes of FL and cHL specimens are listed in Table 2.

Patient 1

A 63-year-old male Veteran presented with fatigue and hypermetabolic lymphadenopathy in the supraclavicular lymph nodes, mediastinum, mesentery, and retroperitoneum. A supraclavicular lymph node excision showed an enlarged lymph node (2.3 cm) with a fibrotic capsule. The lymph node architecture was effaced by scattered clusters of large atypical cells in a background of mostly small lymphocytes and few neutrophils and plasma cells without nodule-delimiting sclerosis. The atypical cells demonstrated Hodgkin-Reed-Sternberg (HRS) morphology, including irregular nuclear borders with occasional multinucleated forms, vesicular chromatin and one or more nucleoli (no definitive macronuclei) [Figure 1C]. Frequent lacunar cells and scattered mummified cells were noted. Within the attached adipose tissue, there were several additional small lymph nodes (<0.5 cm each) with numerous well-defined follicles characteristic of FL [Figure 1A]. Centroblasts numbered less than 5 per high power field (HPF).

IHC studies demonstrated the HRS cells to be positive for CD15 and CD30 [Figure 1D] with Golgi enhancement, MUM1 (strong), and PAX5 (dim). They also expressed CD20 (partial), CD45 (partial), CD79a (rare dim), BCL2, and CD3 (focal dim) and CD5. The HRS cells were negative for ALK1, CD10, and CD7. ISH for EBER was negative. Follicles present at the periphery of the enlarged lymph node and within the small adjacent lymph nodes were positive for CD20, CD10, and BCL2 [Figure 1B] with well-defined CD21 positive follicular dendritic meshworks. Flow cytometry showed a monoclonal CD10+ B-cell population with kappa light chain restriction. FISH analysis demonstrated the presence of t(14;18) in 87.5% of cells examined, both in the areas of FL and within the HRS cells in the areas of cHL where there were multiple fusions in a background of aneuploidy [Figure 1E]. *IGH* gene rearrangement studies demonstrated a single dominant clonal band.

The patient was treated with eight cycles of rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone (R-CHOP), resulting in resolution of his chest disease but continued mesenteric adenopathy. A mesenteric lymph node biopsy 1.5 years after diagnosis showed persistent involvement by cHL, but no evidence of FL. A bone marrow biopsy was negative for lymphoma. The patient was treated with doxorubicin, bleomycin, vinblastine and dacarbazine (ABVD) for six cycles then followed by two cycles of brentuximab. He initially showed a good response, but then demonstrated hypermetabolic mesenteric lymphadenopathy by positron emission tomography (PET), consistent with recurrence. At his last follow-up visit nearly three years after diagnosis, the patient had not responded to ifosfamide, carboplatin, etoposide (ICE).

Patient 2

A 63-year-old male Veteran presented with severe anemia to an outside hospital and was diagnosed with follicular lymphoma, stage 4 (original slides not available for review). Over the following three years, the patient was first treated with rituximab, cyclophosphamide, vincristine, and prednisone (R-

Table 1: Clinical characteristics, clinical course, and outcomes of patients.

Patient	Age/sex	Sites of FL	FL grade	Sites of cHL	cHL subtype	Disease interval between FL & cHL	Clinical course
1	63/M	Supraclavicular, mediastinal, mesenteric and retroperitoneal LNs	1-2	Mesenteric LN	MC	Concurrent	Alive, 34 mo after diagnosis with cHL refractory to salvage chemotherapy
2	63/M	*	NR	Retroperitoneal LNs	n/a	6 years	Alive, recent diagnosis of cHL
3	53/M	Pulmonary LN, BM	3	Mediastinal LN	NS	5.5 years	Auto-SCT after diagnosis of cHL with subsequent development of t-MDS and death
4	48/M	Inguinal, supraclavicular, retroperitoneal LNs	1-2	Paraaortic, retroperitoneal, peribiliary LNs	NS	2 years	Dead, 2 years after FL diagnosis from widespread disease; LNs on autopsy showed cHL
5	68/M	Inguinal LNs	1-2	Axillary LN	NS	1 year	Alive, 3 years after cHL diagnosis, with refractory disease

Abbreviations: FL, follicular lymphoma; cHL, classical Hodgkin lymphoma; LAD, lymphadenopathy; LN, lymph node; BM, bone marrow; MC, mixed cellularity; n/a, not available; NR, not reviewed; NS, nodular sclerosing; SCT, stem cell transplantation

*Slides of the FL diagnosis not available for review for Patient 2.

CVP), then with 6 cycles of bendamustine and rituximab, resulting in clinical remission by computed tomography (CT) scan. A follow-up PET scan a year later showed recurrent disease. Despite multiple subsequent therapies within a 1.5 year period, the patient showed progressive disease with enlarging retroperitoneal lymph nodes.

A para-aortic lymph node core biopsy performed 6 years after diagnosis demonstrated fragments of fibrous soft tissue infiltrated by mostly small and morphologically unremarkable lymphocytes with admixed large cells with large irregular nuclei, vesicular to hyperchromatic chromatin, scant cytoplasm, and occasional prominent nucleoli, including inclusion-like macronucleoli. These large cells focally formed large aggregates [Figure 1F]. There was no significant infiltrate of plasma cells, neutrophils or eosinophils. The large cells were positive for CD30 [Figure 1G], MUM1, BCL6 (dim), with partial staining with BCL2 and c-MYC (>40% of large cells), and an increased Ki67 proliferation index (70-80%). The large cells were negative for CD45, CD15, CD3, CD20, CD10, and EBER ISH. The background small lymphocytes were predominantly composed of CD3-positive T cells with few B cells. CD21 was negative throughout the specimen. FISH studies identified fusion signals for *IGH-BCL2* probes as well as multiple copies of each of the probes in the HRS cells [Figure 1H].

Patient 3

A 53-year-old male Veteran was diagnosed with stage 4 FL (grade 3) and treated with eight cycles of R-CHOP followed by maintenance rituximab for approximately 1.5 years, achieving a complete remission. Four years after diagnosis, a chest CT showed a 2.9 cm enlarging ground glass lesion in the lower lobe of the right lung. Wedge resection of this lesion revealed a central lymphoid nodule within the lung parenchyma consisting of crowded follicles characteristic of FL [Figure 1I]. Centroblasts averaged approximately 10 per HPF. Surrounding the nodule, small lymphocytes diffusely trickled into the surrounding, otherwise unremarkable, lung parenchyma in a lymphangitic pattern. IHC studies demonstrated that the centrocytes within the crowded follicles as well as in the peripheral areas of diffuse lymphangitic spread were positive for CD20 and CD79a with co-expression of CD10, BCL6, and

Table 2: Immunophenotype of follicular lymphoma and classical Hodgkin lymphoma.

Patient	Cell type	CD45	CD20	CD10	BCL2	CD30	CD15	EBV[a]
1	FL	+	+	+	+	-	-	-
	cHL	+(PD)	+ (focal)	-	+	+	+	-
2[b]	cHL	-	-	-	+ (partial)	+	-	-
3	FL	+	+	+	+	-	-	-
	cHL	+ (focal)	+ (variable)	-	+ (weak)	+	-	-
4	FL	+	+	+	+	-	-	-
	cHL	-	-	NA	NA	+	+	-
5	FL	+	+	+	+			
	cHL	-	-	-[c]	+[c]	+	-	-

Abbreviations: EBV, Epstein-Barr virus; FISH, fluorescence *in situ* hybridization; FL, follicular lymphoma; cHL, classical Hodgkin lymphoma (specifically on the Hodgkin-Reed-Sternberg cells); PD, partial dim; NA, not available.
[a]: EBER *in situ* hybridization used for Patients 1-3 and 5; EBV LMP-1 used for Patient 4.
[b]: FL not reviewed at our institution.
[c]: CD10 and BCL2 performed on a subsequent specimen also involved by the same process.

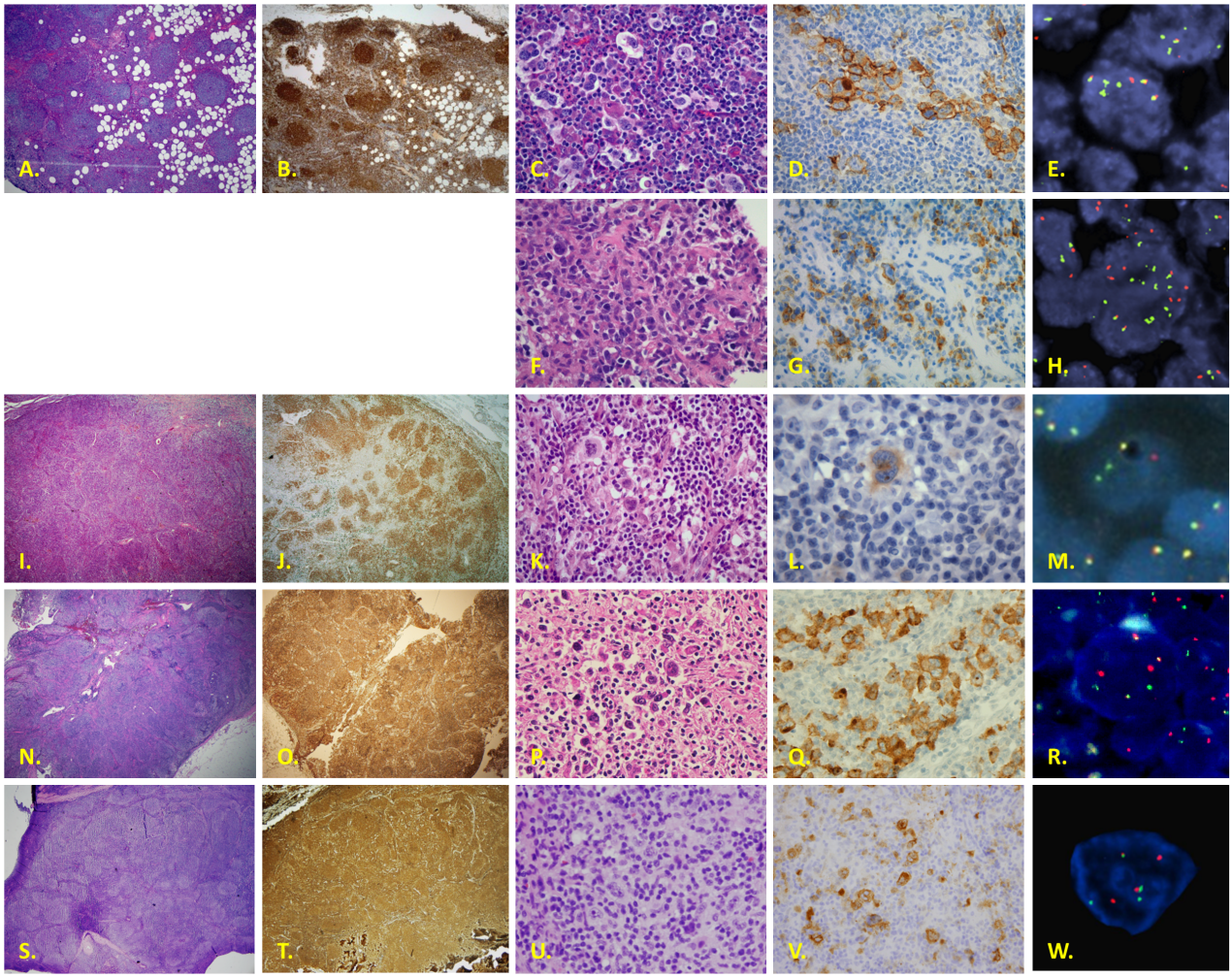

Figure 1: Representative images of follicular lymphoma (FL) and classical Hodgkin lymphoma (cHL). A-E Patient 1; F-H Patient 2; I-M Patient 3; N-R Patient 4; S-W Patient 5. FL images: A, I, N, S (H&E, original magnification 20x); B, J, O, T (IHC for BCL2, original magnification 20x). cHL images: C, F, K, P, U (H&E, original magnification 400x); D, G, Q, V (IHC for CD30, original magnification 400x); L (IHC for CD30, original magnification 600x); E, H, M, R, W (FISH images: t(14;18) (*IGH/BCL2* fusion) in HRS cells: (E: nuc ish (IGH, BCL2)x2-4, (IGH con BCL2x1-3) [175/200], H: nuc ish (IGH, BCL2)x1-7, (IGH con BCL2x1-3) [112/200], W: nuc ish (IGH, BCL2)x3-7, (IGH con BCL2x1-3)[48/50], detailed ISCN nomenclature for M and R not available).

BCL2 [Figure 1J]. FISH analysis demonstrated an atypical signal pattern, including *IGH/BCL2* fusion, one intact *BCL2*, and an extra copy of *IGH*.

Because the patient was otherwise asymptomatic, he was managed with observation. However, a CT scan six years after diagnosis demonstrated interval enlargement of pretracheal/subcarinal lymph nodes that upon biopsy revealed a vaguely nodular infiltrate with extensive sclerosis and fibrosis. There were frequent atypical large cells, some binucleate, with vesicular chromatin, pale cytoplasm and macronucleoli, consistent with HRS cells [Figure 1K]. By IHC, the large cells expressed PAX5 (dim), CD30 (cytoplasmic and Golgi) [Figure 1L], CD20 (variable), BCL2 (weak), and BCL6 (weak). The HRS cells were predominantly negative for CD45, and negative for

CD15 and CD10. The background small lymphocytes were predominantly CD3 positive T cells. Flow cytometry did not identify an atypical or monotypic B-cell population. FISH studies demonstrated that the HRS cells showed the same aberrant signal pattern as seen in the patient's previously diagnosed follicular lymphoma [Figure 1M].

The patient was subsequently treated with two cycles of chemotherapy prior to undergoing an autologous stem cell transplant in April 2008. Unfortunately, within two years, he developed a therapy-related myelodysplastic syndrome and died shortly after an admission to the hospital for pneumonia.

Patient 4

A 48-year-old male Veteran presented with left inguinal lymphadenopathy. Lymph node resection showed partial effacement by FL with extension into perinodal adipose tissue and notable angioproliferation [Figure 1N]. Centroblasts numbered less than 5 per HPF. By IHC, the lymphoma cells were positive for CD20, PAX5, BCL2 [Figure 1O], and BCL6. The patient was treated with rituximab and bendamustine for 4 cycles and R-CHOP for 6 cycles. During this time period, he continued to have splenomegaly with progressive supraclavicular, inguinal, and retroperitoneal lymphadenopathy, the latter encasing the abdominal aorta and common iliac arteries. A left inguinal lymph node biopsy and tandem bone marrow biopsy did not reveal a neoplastic B-cell process. However, a month later, the patient presented with dysarthria, confusion and dysphagia. Cerebrospinal fluid was negative for lymphoma, but grew out *Cladophialophora carrionii*, *Chromoblastomycoses* species. The patient expired shortly after admission to hospice.

At autopsy diffuse lymphadenopathy was noted that was especially impressive in the retroperitoneal area (25 cm in greatest dimension). Samples of the paraaortic, retroperitoneal, and peribiliary lymph nodes were unexpectedly involved by cHL, nodular sclerosis pattern, in a background of FL (grade 2). The representative lymph nodes demonstrated thin bands of fibrosis and a background of mainly lymphocytes with fewer neutrophils and rare eosinophils. Admixed with the inflammatory cells were large atypical cells with abundant basophilic or amphophilic cytoplasm, irregular nuclear membranes, single to multiple nuclei, and inclusion-like macronucleoli [Figure 1P]. IHC stains for CD30 [Figure 1Q] and CD15 highlighted the diagnostic HRS cells (membranous and Golgi), which also showed positivity for PAX5 (dim). They were negative for EBV LMP1, BCL6, CD20 and CD45. Large foci of necrosis were also noted.

PCR for *IGH* gene rearrangement was performed using DNA extracted from FFPE tissue of the patient's original FL and the Hodgkin transformation with background FL obtained at autopsy. Identical dominant monoclonal bands were present in the pre-mortem and post-mortem samples. FFPE tissue from pre-mortem and autopsy blocks were also used to perform FISH assays for the presence of the t(14;18)(q32;q21) translocation. The translocation was detected in both samples, including the HRS cells [Figure 1R].

Patient 5

A 68-year-old male presented with inguinal lymphadenopathy and B symptoms. An excisional inguinal lymph node biopsy showed low grade FL (Grade 1-2) consisting of numerous tightly packed follicles of centrocytes with less than 15 centroblasts per HPF [Figure 1S]. IHC and flow cytometry showed the neoplastic cells to be positive for CD20, CD10, BCL6, and BCL2 [Figure 1T] with monotypic staining for kappa light chains, and negative for CD5, Cyclin D1 and CD3. The Ki-67 proliferation index was approximately 30% in the neoplastic follicles.

The patient was subsequently treated with rituximab and bendamustine followed by ibritumomab tiuxetan for consolidation. PET imaging performed three months after completing consolidation showed persistent/progressive lymphadenopathy. Exci-

sional biopsy of an axillary lymph node demonstrated cHL, nodular sclerosis type, consisting of fibrotic bands with a mixed infiltrate of small lymphocytes, plasma cells, and histiocytes with admixed large atypical cells with irregular nuclei, dispersed chromatin, prominent nucleoli, and abundant cytoplasm, consistent with HRS cells [Figure 1U]. IHC showed the large atypical cells to be positive for PAX5 and CD30 [Figure 1V] and negative for CD15, CD45, CD20, CD3, ALK, and EBER. Molecular analysis showed a clonal *IGH* gene rearrangement of identical size to that found in the patient's original follicular lymphoma sample. FISH studies demonstrated *IGH/BCL2* rearrangement in the HRS cells in a background of polysomy for both loci [Figure 1W].

Following the diagnosis of cHL, the patient was treated with ABVD. After three cycles, however, he experienced worsening symptoms and had PET scan showing persistent/progressive disease. He was subsequently treated with brentuximab and then gemcitabine, navelbine, and doxorubicin but again had progressive disease. Core biopsy of an inguinal lymph node showed low grade FL. The patient was started on RICE chemotherapy and experienced a good response based on PET imaging. Planning for an autologous stem cell transplant was initiated, but the patient again experienced recurrent symptoms along with a new hypermetabolic liver lesion, which showed involvement by cHL on core biopsy. The patient then received three additional cycles of brentuximab but had evidence of disease progression on imaging. Due to multiply refractory disease, the patient was then placed on dual immune checkpoint blockade with ipilimumab and nivolumab. Since that time, the patient has had a positive clinical and imaging response and is currently alive with disease approximately three years after the initial diagnosis of FL.

Discussion

In this case series, we describe five cases of FL with apparent transformation into cHL (Table 1). The t(14;18) was demonstrated by FISH in the initial FL for patients 1, 3, and 4 where tissue was available; in patient 5, the original follicular lymphoma was BCL2 positive by IHC stains, likely due to the presence of t(14;18). The initial FL of patient 2 was by report only. In patient 1, the FL and cHL were diagnosed concurrently, and in the remaining four cases the patients were initially diagnosed with FL with a subsequent diagnosis of cHL ranging from two to six years later. In three cases, the follicular lymphoma was no longer present at the time of cHL diagnosis. In all of the cHL specimens the t(14;18) was identified by FISH within the HRS cells.

While FL is known to carry a risk of transformation from an indolent disease into a higher grade lymphoma, transformation to cHL is uncommon. HRS-like cells have been noted in FL previously [10–17]. However, in many cases the HRS-like cells are scattered within a background of FL and express strong uniform CD20 and CD45 [15–18]. Thus, the immunophenotype of the HRS-like cells is not that of a true cHL, despite the atypical morphology of individual cells. In these cases, a more appropriate diagnosis to consider may be FL with HRS-like cells. As shown in a subset of the studies, the HRS-like cells are clonally related to the FL. For example, Bayerl *et al*. described two cases of FL with CD30-positive RS-like cells and established a clonal relationship between the FL cells and RS cells by demonstrating identical sequences of *IGH* gene rearrangements [18].

The occurrence of a true cHL, with an immunophenotype typical for that diagnosis, subsequent to a FL, as well as composite lymphomas with regions of both cHL and FL, have been reported previously in the literature. Zarate-Osorno described nine patients who initially developed a non-Hodgkin lymphoma (seven of which were categorized as of follicular origin by the Working Formulation but included large cell variants) and subsequently developed Hodgkin lymphoma within a median interval of 5 years [14]. Similarly, Nakamura *et al*. described a case of FL followed 4 years later by cHL with

demonstration of clonality by identically sized PCR products of the *IGH/BCL2* fusion and nearly identical nucleotide sequences from both specimens [11]. Küppers *et al*. described a composite cHL and FL in a splenic hilar lymph node [12]. The two lymphomas had clonally related variable genes as well as distinct somatic mutations. Marafioti *et al*. described a patient who initially developed cHL without known involvement by FL, but was subsequently diagnosed with FL two years later [13].

Although these cases of cHL are immunophenotypically more consistent with cHL than the cases of FL with HRS-like cells, they may still represent a continuum of the transformation process. There does not appear to be a particular cHL subtype to which FL has a predilection to transform: the nodular sclerosis and mixed cellularity subtypes are the most frequently encountered in the literature, but this finding may be attributed to the high incidence of the two subtypes in cHL in general.

In approximately 80% of cases, FL is marked by the presence of a t(14;18) translocation, which results in overexpression of the anti-apoptotic BCL2 protein. This translocation is absent in cHL, but may occur in cHL arising in association with FL [19]. Yoshida *et al*. reviewed 428 cases of cHL and identified four cases (0.95%) with associated FL, three of which showed t(14;18) in the HRS cells [20]. All but one of the 11 sporadic cHL cases used as control cases were negative for the translocation. It has been proposed that when t(14;18) is detected in cHL, it tends to occur in cases associated with a previous or concurrent FL [21]. In addition, BCL2 expression was seen in all of the published cases of FL transformed to cHL [10, 11, 20, 21], while BCL2 is positive in only 33% of cases of *de novo* cHL lacking t(14;18). In accord with the published data, all cHL cases in our series with available IHC expressed BCL2 (Table 2). BCL2 overexpression may lay the initial groundwork for later superimposed events specific to development of cHL. In addition, in four of our five cases, the HRS cells contained either multiple copies of the *IGH/BCL2* fusion and /or multiple copies of the un-rearranged loci, suggesting the development of aneuploidy as a potential progression event. The fifth case (patient 3) had an atypical fusion in both the original FL and subsequent cHL.

Epstein-Barr virus (EBV) has been postulated as a player in the pathogenesis of cHL. EBV infection and gene expression is known to lead to suppression of apoptosis that should result from the crippled state of the germinal center B cell in cHL [22–24]. This phenomenon is related to induction of BCL2 expression by virus-encoded LMP-1 [25]. Menon *et al*. reported a case of low grade FL transforming to a grade 3A FL with HRS-like cells showing positive EBER ISH staining [10]. However, the cHL cases in our current series were not associated with EBV infection. Similarly, there was rare to no staining for EBV within the HRS cells of other previously mentioned case series. It is unlikely, therefore, that EBV is a major contributor to the pathogenesis of cHL transformation from prior FL. In these cases, the over-expression of BCL2 that results from t(14;18) likely promotes survival in the cHL cell in lieu of EBV infection.

The outcome of FL transformed to cHL is not well known. In general, transformation of FL to a high-grade lymphoma is usually associated with a rapidly progressive clinical course and death [1]. Like FL, chronic lymphocytic leukemia (CLL) may also transform to large cell lymphoma (Richter transformation), most commonly as a diffuse large B-cell lymphoma, but rarely as a cHL that may be clonally related to the preceding CLL in a subset of cases. Those patients with CLL transformed to a cHL likely experience worse outcome than patients with *de novo* cHL, with poor response to treatment [26, 27].

In our case series, patient 4 died of his lymphoma and patient 3 died from a therapy-related myeloid neoplasm after autologous stem cell transplantation. Patients 1 and 5 are currently alive, but with disease refractory to multiple cycles of various chemotherapy regimens. There has been insufficient time since the diagnosis of patient 2 for any discussion of outcomes. Of the previously mentioned reports

in the literature with longer clinical follow-up, all patients died from sequelae of their transformed FL [10, 13, 28]. Thus, even though patients with de novo cHL are cured in up to 85% of cases, it is apparent that secondary cHL transformed from FL carries a more aggressive clinical course and poor overall prognosis. It should be noted that four of the patients in our series were Veterans, but no common specific exposure history was noted.

In conclusion, we describe five cases of FL with immunophenotypic and genetic evidence of transformation to cHL. The HRS cells were positive for CD30 and lacked uniform CD20 and CD45 expression. In addition, the HRS cells were positive for BCL2 and demonstrated a t(14;18) by FISH, which establishes the clonal relatedness of the underlying FL and the transformed cHL in each patient. EBV did not play a role in the transformation of these cases. In several patients in our series, transformation to cHL also appeared to correlate with a more aggressive clinical course, which is consistent with other reports in the literature. Although further study is no doubt required, it appears likely that cHL arising from FL ("secondary" cHL) exhibits unique clinical and genetic features relative to *de novo* cHL and may necessitate modified treatment approaches.

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