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Follicular Lymphomas in children and young adults: A comparison of the pediatric variant with usual follicular lymphoma

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Abstract

Follicular lymphoma (FL), a common lymphoma in adults, occurs rarely in pediatric and young adult patients. Most pediatric cases have been described as Grade 3, but the criteria to distinguish the pediatric variant of FL (PFL) from usual FL (UFL) in adults are not well defined. We undertook a study of FL in patients under age 30. We identified 63 cases, which were analyzed by morphology, immunohistochemistry, and PCR analysis of *IGH*@ and *IGK*@ clonality. These data were correlated with clinical findings including stage, treatment, and outcome. Among the 63 cases, 34 cases were classified as PFL; 22 presenting in lymph nodes, 8 in Waldeyer's ring and 4 in testis. Clonal immunoglobulin gene rearrangement was detected in 97% of PFL cases, but FISH analysis showed an absence of the *BCL2/IGH*@ translocation in all cases tested. Twenty-nine cases were classified as UFL, 28 of which presented in lymph nodes. The nodal PFL cases were observed exclusively in males in both children and young adults with a median age of 15 years. They showed marked head/neck predilection, blastoid cytological features with a high proliferation rate, lack of *BCL2* protein and t(14;18), low clinical stage at presentation, and good prognosis. PFL involving Waldeyer's ring were distinguished by *MUM1* expression, 50% (3/6) of which carried *IRF4* breaks. *BCL2* expression was common (63%) in the absence of *BCL2/IGH*@ translocation. UFL cases were more common in females, exclusively in young adult patients (median age, 24 years) with no patients under age 18. 25/29 were grade 1–2, and four cases were classified as grade 3A. They exhibited a higher clinical stage at presentation. 83% expressed *BCL2*. Our results indicate that histological and immunophenotypic criteria can reliably separate PFL and UFL, and that UFL is exceptionally rare in the pediatric age group. PFL associated with particular anatomic sites have distinctive features, and should be evaluated separately in future clinical and biological studies.

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Keywords

follicular lymphoma; follicular lymphoma; *IRF4* (interferon regulatory factor 4); MUM1; BCL2; BCL6; Fluorescence *in-situ* hybridization (FISH); Waldeyer's ring; testis; pediatric

INTRODUCTION

Follicular lymphoma (FL) is the second most common lymphoma worldwide. It mainly affects adults and rarely occurs in the pediatric population and young adult patients.¹ Pediatric follicular lymphoma (PFL) is a newly recognized variant of FL included in the 2008 WHO Classification of Tumors of Hematopoietic and Lymphoid Tissues.¹ Limited studies have reported on the histologic, molecular, and clinical features of FL in young patients.^{2–8} PFL is reported to be characterized by high histologic grade, and the lack of BCL2 protein expression and t(14;18). Clinically, PFL predominantly occurs in males, often presents with localized disease, and carries good prognosis. (11–13) Currently, the pathogenesis of this entity is largely unknown, and differences between nodal PFL and PFL in other anatomic sites have not been explored. While differences in cytological grade have been noted with usual FL (UFL) as seen in adults, PFL is often a diagnostic challenge, and more precise histologic criteria have not been defined.

Although a conservative approach towards FL in pediatric patients had been proposed by Atrai et al. in 1998,² the optimal clinical management remains unclear. Furthermore, clinicopathologic features of FL in young adult patients have not been extensively studied. In this study, we examined the histologic features, immunophenotypic profiles, immunoglobulin gene rearrangement by PCR, cytogenetic characteristics by FISH, as well as the clinical follow up data of 63 FL cases in pediatric (≤ 18 year-old), and young adult (19–29 year-old) patients. We used both the WHO criteria and new observations to separate PFL from UFL in the young adult population, and to better delineate the clinical, histologic and immunophenotypic spectrum of PFL. These results will be of value in guiding the diagnosis and management of FL and the PFL variant.

MATERIALS AND METHODS

Case selection

An electronic search of the pathology data base at the National Cancer Institute (NCI), National Institutes of Health (NIH), Bethesda, MD, was conducted for the diagnosis of FL, or consistent with FL, restricted to age < 30 , from 2000 to 2011. Sixty eight cases with material available for review were identified. Five cases were excluded for the following reasons: 1) revised diagnosis of follicular hyperplasia (1 case); 2) revised diagnosis of marginal zone lymphoma (1 case); 3) excluded based on a component of diffuse large B-cell lymphoma (3 cases). Sixty-three cases comprised the final study cohort. Cases of nodal FL (51) were classified according to the 2008 WHO Classification of Tumors of Hematopoietic and Lymphoid Tissues as PFL or UFL, without knowledge of the patient's age or other clinical data.¹ Criteria for nodal PFL included large expansile follicles, dim to absent BCL2 expression, and a high proliferative rate. This study was approved by the Institute Review Board of the National Cancer Institute. Clinical information and follow up were obtained from submitted patients records or referring physicians in accordance with medical practice guidelines. Ten pediatric nodal marginal zone lymphoma (PNMZL) cases were reviewed concurrently in order to compare histologic features of PFL with those of PNMZL.⁹

Histology and Immunohistochemistry

The morphologic and immunophenotypic features were studied on formalin-fixed and paraffin-embedded (FFPE) tissue sections of the diagnostic biopsies. Immunohistochemical stains were performed using an automated immunostainer (Ventana Medical Systems, Inc, Tucson, AZ) as described previously.¹⁰ In brief, antigen retrieval was performed using a Tender Cooker (Nordicware, Minneapolis, MN) with citrate buffer. The immunohistochemical panel included CD20, CD3, CD10, BCL2 (clone 124), BCL6, MUM1, IgD, CD21, CD23, MIB1(Ki-67), and PD-1(CD279). Appropriate controls were included in all cases. BCL2 and CD10 were scored as positive if more than 50% of tumor cells exhibited staining; for BCL6 and MUM1 the required value was 30%. The E17 clone reactive with the mutated form of BCL2 (Epitomics) was examined in a subset of the cases, negative for BCL2 with clone 124 (Dako).¹¹

PCR Analysis of Immunoglobulin Gene Rearrangement

PCR analysis of immunoglobulin gene rearrangement was performed as described previously.¹⁰ Genomic DNA was extracted from FFPE tissue sections using QIAamp DNA FFPE tissue kit (QIAGEN Inc, Valencia, CA) according to the manufacturer's instructions. For immunoglobulin heavy chain (*IGH*®) clonality assays, framework 3 and framework 2 were amplified by PCR using consensus primer sets reported as Segal et al¹² and Ramasamy et al¹³, respectively. For κ light chain (*IGK*®) clonality assays, *IGK*® was amplified by PCR using ABI Fluorescence Detection Kit (In VivoScribe Technologies, INC, San Diego, CA) according to the manufacturer's instructions. After amplification, the PCR products were analyzed using ABI 3130xl Genetic Analyzer (Applied Biosystem), and the results were interpreted based on the criteria developed in the laboratory.¹⁰ The tissue biopsies (lymph node, tonsil, testis) were extensively involved by the abnormal follicles, comprising the vast majority of the B-cells present; thus, enrichment for tumor cells was not required. In a single tonsillar case (Case 8), focal involvement was present; in this case macrodissection of the involved portion was performed to enrich for tumor DNA.

Construction of Tissue Microarray (TMA)

For PFL cases with available tissue blocks, a TMA was constructed to facilitate analysis of interphase cytogenetics, as described previously.¹⁴ Representative tumor areas were selected based on the corresponding H&E slides and marked directly on the donor block. Two tissue cores (diameter 1.5mm) were transferred from the selected region of each donor block and arrayed into the recipient block using TMArrayer (Pathology Devices, Westminster, MD). Following the TMA construction, 5 μ m sections were cut with a microtome. An H&E stain was obtained to confirm inclusion of diagnostic tissue.

FISH Analysis

Interphase fluorescence in-situ hybridization (FISH) analysis for the detection of breaks or gene fusion affecting the *IGH*, *BCL2*, *BCL6*LSI dual-color break apart probes and *IGH-BCL2* dual color dual-fusion translocation probe, Vysis- Abbott, IL, USA), and *IRF4* loci was carried out on formalin fixed paraffin embedded (FFPE) sections from tissue microarray (TMA) or whole tissue sections of the PFL cases as described previously.¹⁵ Breaks of the *CBFA2T3* gene, recently described to be translocated in pediatric germinal center derived lymphomas, were also investigated using previously described probes.¹⁶ FISH analysis for the detection of *IGH*®, *BCL6*, and *IRF4* breaks was performed on each case that had material available, whereas FISH analysis for *BCL2* and *CBFA2T3* breaks was carried out only on the cases that contained *IGH*® breaks. For the cases harboring breaks in both of *IGH*® and *IRF4* loci, *IGH-IRF4* gene fusion was confirmed by using fusion probes.¹⁶

RESULTS

Histologic and Immunophenotypic Findings

Thirty four of 63 FL cases were classified as PFL (Table 1), and 29 cases were classified as UFL (Table 2).

PFL—Cases diagnosed as PFL presented in lymph node (22 cases), Waldeyer's ring (tonsils or tonsils and adenoids) (8 cases), and testis (4 cases). Differences in the histologic and immunophenotypic features were noted, depending on the anatomic site, and therefore these groups will be separately described. All cases of PFL would be considered Grade 3, based on the proportion of centroblasts/blastoid cells.

PFL involving tonsil (Fig.1) were characterized by large, closely-packed, expansile follicles composed predominantly of centroblasts (3/8 cases, Fig. 2A) or intermediate sized blastoid cells (5/8 cases, Fig. 2B). The blastoid cells had round to oval nuclei, finely clumped chromatin, small nucleoli, and scant cytoplasm. A "starry sky" appearance, a feature commonly seen in reactive follicles, was sometimes maintained. The subset involving tonsil was more likely to be positive for BCL2 protein (5/8, 63%), and notably all cases tested were positive for MUM1 (Table 3). CD10 was expressed in 63% (5/8) and BCL6 was positive in 100% (8/8) of the cases, confirming a germinal center derivation. BCL6 and CD10 positive cells were confined to the follicles. One case showed evidence of kappa light chain restriction by immunohistochemistry.

PFL involving lymph nodes showed effaced nodal architecture by large ill-defined follicles with attenuated lymphoid cuffs. (Fig. 3) A prominent starry sky pattern was present in all cases. The follicles were often serpiginous or irregularly-shaped. Occasional nodal PFL cases showed more uniform, closely packed follicles, similar to those seen in tonsillar PFL cases. (Supplemental digital content 1) A peripheral rim of paler cells suggesting monocytoid differentiation was sometimes present, but was a minor component. The cytological composition was similar in all cases, and was dominated by monotonous, small to medium sized blastoid cells with round to oval nuclei, finely dispersed chromatin, small nucleoli, and scant cytoplasm (Fig. 2C and 2D). Typical centroblasts with peripheral membrane-bound nuclei were infrequent. Centrocyte-like cells were sometimes present, but had somewhat dispersed chromatin.

PFL in lymph nodes were consistently and strongly positive for CD10 (100%), with CD10 highlighting the often irregular follicle centers (Fig.3). BCL6 was positive as well, with only 18% showing any expression of BCL2 protein, which was weakly positive. Eight cases of nodal PFL negative for BCL2 with clone 124 were examined with clone E17, and were negative as well. CD10 and BCL6 positive cells did not extend beyond the follicles. MUM1 was positive in 1/18 (5.5%) nodal PFL cases; this case was also positive for CD10, BCL6, CD5, and lambda light chain. Among the tonsillar or nodal MUM1 positive cases, cytologic features of plasmacytoid differentiation were not observed, even in the presence of light chain restriction by immunohistochemistry. Interfollicular B-cells were not markedly increased, helping in the distinction from PNMZL. In four cases with marginal zone differentiation, a perifollicular rim of B-cells could be identified.

PFL involving the testis contained back to back atypical small follicles composed of predominantly of centroblasts infiltrating between seminiferous tubules (Supplemental digital content 2). The follicles lacked well-defined mantle cuffs and did not show polarization. All cases were negative for BCL2 and MUM1, but were positive for BCL6 and CD10 in 4/4 (100%) and 3/4 (75%) of the cases, respectively. The follicles had a back to back arrangement, but interfollicular B-cells beyond the follicles were not present. The

follicular structures in all cases of PFL were highlighted by stains for CD21 and CD23. IgD identified thin and attenuated mantle zones. Tumor cells were negative for IgD. All PFL cases showed a high ($\geq 75\%$) or moderate to high (50–75%) proliferation rate assessed by MIB1 stain.

UFL—Twenty-nine cases were diagnosed as UFL, 25 classified as grade 1–2 and 4 classified as grade 3A. No cases of grade 3B were identified. All cases but one presented in lymph node; one case was diagnosed in thyroid, but the patient had disseminated disease. The histologic features were similar to those of adult UFL, in which the normal architecture was effaced or partially effaced by closely-packed atypical follicles that showed absent or attenuated mantle cuffs and lack of polarity (Supplemental digital content 3). A “starry sky” appearance was no longer present. Centrocytes comprised the majority of the cells present in the grade 1–2 UFL cases, whereas greater than 15/HPF centroblasts were present in grade 3a UFL cases. However, nodal UFL lacked the monotonous blastoid cytology that dominated nodal PFL.

All cases were positive for CD20 and BCL6. Of UFL classified as grade 1–2, 100% (21/21) were CD10 (+) and 83% (20/24) were BCL2(+). All but two grade 1–2 UFL cases showed low ($\leq 25\%$) or low to moderate (25–50%) proliferation rates by MIB1 stain, with higher rates seen in two cases. Of the four grade 3A UFL cases, 2 of 4 (50%) were CD10 (+), and 2 of 3 (66.7%) cases tested were BCL2 (+). All grade 3 UFL cases showed a high proliferation rate ($>75\%$).

PNMZL—Ten pediatric nodal marginal zone lymphoma cases were reviewed concurrently in order to compare the morphologic features between PFL and PNMZL (Table 4). The cases showed characteristic histologic and immunophenotypic features as described previously,⁹ such as increased interfollicular B cells and fragmentation of residual germinal centers resembling progressive transformation of germinal centers (PTGC). (Supplemental digital content 4) Residual hyperplastic germinal centers maintained a starry sky appearance, showed evidence of polarization best visualized with immunohistochemistry for Ki-67, and had a heterogeneous cellular composition. The follicles were widely spaced, with less prominent CD10 positivity.

PD-1 staining pattern—To further facilitate the distinction between PFL and PNMZL, staining for PD-1 (CD279) was performed on PFL (24), UFL (16), and PNMZL (9) cases. Distinctive patterns of PD-1 staining in the follicles were observed among tonsillar PFL, nodal PFL, nodal UFL, and PNMZL cases. (Supplemental digital content 5). In reactive follicles PD-1 positive lymphocytes were abundant and more numerous in the light zone. This polarity was absent in the atypical follicles involved by PFL, UFL, and PNMZL. Generally, tonsillar PFL cases showed absent or a few scattered PD-1 positive cells. PD-1 positive cells were somewhat more frequent in nodal PFL, but tended to be concentrated in the periphery, giving an impression that these cells were pushed aside by the neoplastic cells. PD-1 positive cells were more abundant in the germinal centers of PNMZL, as compared with nodal PFL. UFL cases showed large numbers of PD-1 positive cells with a loss of polarity in the atypical follicles in comparison to that seen in hyperplastic follicles.

PCR and FISH Findings

A majority of the PFL cases demonstrated clonal immunoglobulin gene rearrangement by PCR analysis (Table 3). The percentage of positive cases varied slightly among different anatomic sites, but the average rate of positivity was 97%. Four cases were negative with primers to Fr2 and Fr3, but positive with primers to the Ig κ gene segments.

FISH analysis to identify breaks in the *IGH@*, *IRF4*, and *BCL6* loci was performed on FFPE sections of the PFL cases with available material. FISH analysis for *BCL2* and *CBFA2T3* breaks was carried out only if the case had an *IGH@* break. *IGH@* breaks were identified in 21% (5/24) of the PFL cases, including 3 Waldeyer's ring PFL cases, and 2 nodal PFL cases. Among the 22 PFL cases tested for *IRF4* breaks, three Waldeyer's ring cases (case 5, 7, and 8) carried *IRF4* breaks. One of the cases tested also showed an *IGH* break, and an *IGH@-IRF4* fusion was confirmed by FISH (Fig. 4). FISH analysis for *IGH@* breaks could not be performed on the third case due to technical reasons. None of the PFL cases had *BCL2*, *BCL6* or *CBFA2T3* breaks.

Clinical Findings

The clinical data of the FL cases are summarized in Tables 1, 2, and a comparison of the features between UFL and PFL is summarized in Table 5. The PFL cases occurred in both pediatric and young adult patients with marked male predominance (M:F = 29:5) and a median age of 14 years (age range 3–25) (Tables 1, 5). All of the Waldeyer's ring and testicular PFL cases were from pediatric patients, whereas 68% (15/22) of the nodal PFL cases were in the pediatric age group (age range 3–18), and 32% were in young adult patients (age range 19–25). Nodal PFL was seen exclusively in males, and presented predominantly in the head/neck region (77%). Four PFL cases showed a concurrent diffuse large B cell lymphoma (DLBCL) component (2 Waldeyer's ring cases, 2 nodal cases). All PFL cases presented with localized disease and were clinical stage I (E) or II (E). The treatment regimens varied among the patients, including surgical excision only and excision with chemotherapy with or without local radiation. The components of the chemotherapy regimens also varied, but R-CHOP (R, Rituximab; CHOP, cyclophosphamide, adriamycin, vincristine, prednisone) was the most common regimen. All PFL patients achieved complete remission and showed no evidence of disease (NED) upon completion of treatment for various lengths of follow up periods. The median follow up period was 18 months (ranging from 1 to 120 months). A few patients achieved long-term disease free survival (patient #2, #9, and #25) following surgical excision only. Several recently-diagnosed PFL patients elected to be managed by surgical excision with close clinical follow up.

Twenty nine of the 63 cases were classified as UFL, including 25 grade 1–2 cases and 4 grade 3A cases (Tables 2, 5). The median age of this group was 24 year-old (range 18–29) with a female predominance (M:F=9:20). Interestingly, all four grade 3A UFL cases were diagnosed in females. Notably no case of UFL was diagnosed under age 18. All UFL cases but one were diagnosed in lymph node biopsies. The single case with extranodal involvement (thyroid) had disseminated disease (case 39). 74% of the patients presented with multiple sites of lymphadenopathy with or without hepatosplenomegaly and/or presented as stage III or VI disease. For the cases that had available clinical staging information, 57% presented with clinical stage III-IV disease and 38% presented with stage I disease (5/14 stage IV, 3/14 stage III, 6/14 stage I). Most patients were treated with various regimens including chemotherapy with or without radiation, or radiation only. A few patients were managed by close clinical follow up only. A majority of the patients achieved complete remission upon treatment except one patient, who showed recurrent disease 3 years after initial treatment (case 53).

DISCUSSION

In this study we analyzed the clinicopathologic features of FL in pediatric and young adult patients. Using published criteria and new observations based on our analysis of these cases, we recognized two groups, usual FL (UFL) resembling FL as seen in older adults, and PFL, lesions with distinctive histologic, immunophenotypic and molecular features. Clinically, the UFL cases were seen exclusively in young adult patients, with no case diagnosed under

age 18. Patients with UFL showed a female predominance and had more advanced clinical stage at presentation. In contrast, the nodal PFL cases were seen in both pediatric and young adult patients, exclusively in males, with a predilection for the head/neck region and low clinical stage at presentation. All of the PFL patients achieved complete remission following various treatment regimens, including surgical excision alone. These data suggest that PFL is a localized disease with good prognosis. For these patients, “Watch and wait” may be the best course of action after surgical excision, as previously suggested.²

PFL and UFL show significant differences in clinical presentation, and appear to differ in clinical outcome. Therefore, correct pathologic diagnosis is essential in guiding the optimal clinical management. In addition to the recognized architectural and cytological features of nodal PFL delineated in the WHO classification (broad expansile follicles, high histological grade), our study highlighted more subtle differences in cytological composition. While PFL cases have been generally considered Grade 3 based on a high proliferation rate, we observed that the neoplastic cells in nodal PFL were neither typical centrocytes nor centroblasts in most cases. The cells were often medium in size, monotonous in cytological composition, with dispersed chromatin and inconspicuous nucleoli. This blastoid appearance correlated with a high proliferative rate. Understandably these cases have been categorized as grade 3, but diagnosis is facilitated by the appreciation of these distinctive cytological features.

At times, making the distinction between nodal PFL and florid follicular hyperplasia with monotypic light chain expression and clonal immunoglobulin gene rearrangement by PCR,¹⁷ can be extremely difficult. The retained “starry sky” appearance characteristically seen in nodal PFL cases further complicates the matter. In these situations, recognition of a monotonous, blastoid cytological composition without polarization, or typical centroblasts and centrocytes is helpful. The extent of the architectural alteration is also useful. PCR for detection of a clonal B-cell population appears to be a very sensitive test in PFL, a finding enhanced by the use of multiple primer sets.¹⁸ Four cases negative for clonality by PCR with primers to the immunoglobulin heavy chain genes were positive when kappa probes were also employed.

A notable observation was uniform positivity for MUM1 in all evaluable cases of PFL involving Waldeyer’s ring. In contrast, MUM1 was expressed in only 5.5% of nodal PFL and no case of testicular PFL. All of the MUM1 positive cases expressed BCL6, and 57% expressed CD10 (4/7, Table 6). BCL2 protein was expressed in 63% of the tonsillar cases as well. Prominent cytologic features of plasmacytic differentiation were not evident on H&E sections, and restricted light chain expression was rarely observed.

In view of the frequent expression of MUM1, we were interested in examining PFL for breaks involving *IRF4/MUM1*. Of the Waldeyer’s ring cases tested, *IRF4* breaks were detected in 3 of 6 cases, and an *IGH/IRF4* fusion was confirmed in 1 of 2 cases tested. These data suggest that while *IGH/IRF4* fusion may represent one possible mechanism for the up-regulation of MUM1 expression, other mechanisms probably also exist. Recent studies have highlighted the significance of MUM1 expression in B-cell lymphomas in children and young adults. Salaverria et al.¹⁵ identified recurrent *IGH/IRF4* fusions in both diffuse large B-cell lymphomas of the germinal center B-cell type (GCB), and grade 3 FL. Sixteen of the twenty positive cases in the published study were diagnosed in patients younger than age 30, and with frequent involvement of Waldeyer’s ring (80%) as in our study. Both series identified coexpression of MUM1 with BCL6, which is an aberrant immunophenotype as these proteins are reciprocally expressed in normal germinal centers.¹⁹ Salaverria et al. also reported that the cases harboring an *IGH/IRF4* translocation had frequent alterations in the *BCL6* locus, including breaks and mutations.¹⁵ While we did not identify chromosomal

breaks in the *BCL6* gene in any of our PFL cases, we did not perform sequence analysis to determine if there were mutations in the *BCL6* gene resulting in disruption of the BCL6-IRF4-PRDM1 regulatory pathway.

Originally identified by cloning the chromosomal breakpoints of a multiple myeloma cell line with t(6;14), the *IRF4* gene is a member of the interferon-regulatory factor family of transcriptional factors. MUM1 shows a biphasic expression pattern in B cells, i.e., it is expressed in immature B cells in bone marrow, absent in centroblasts of the germinal centers, and is again expressed in a subset of centrocytes in the light zone of germinal centers, and plasma cells.^{14,20} It is generally believed that the expression of IRF4/MUM1 and BCL6 in B cells is mutually exclusive in normal germinal centers, but the positive cases of PFL aberrantly co-expressed both proteins.²¹ UFL typically lacks MUM1 expression.¹ However, the reported positive cases are usually CD10 negative, of high histologic grade, clinically more aggressive, and interestingly occur in an older patient population.²²

In comparison to PFL in other anatomic sites, several notable differences were observed in PFL involving Waldeyer's ring. In addition to uniform positivity for MUM1, these cases were more often BCL2 positive (63%), had a higher frequency of *IGH@* breaks (50%), and lacked a male predominance. These data suggest that PFL of Waldeyer's ring may have a different molecular pathogenesis from other forms of PFL.

As expected, the nodal PFL cases in our series were generally negative for BCL2 protein (18% positivity and generally weak). These data are consistent with the previously published studies in which BCL2 positivity was reported to be 25%–55%.^{3,4} Although advanced disease at presentation and resistance to combination chemotherapy have been reported to be associated with BCL2 expression,³ a relationship between BCL2 expression and clinical stage or outcome was not observed in our study. The PFL patients in our series consistently presented with early clinical stage (I or II) and all achieved complete remission without recurrence during the available clinical follow up. Our data are in keeping with another recently published study.⁴ Earlier studies also have demonstrated the lack of t(14;18) in PFL,^{3,4} a finding confirmed in our series.

The distinction between PFL and PNMZL also can be problematic, given the overlapping features of these two entities. However, characteristic features seen in PNMZL,⁹ such as expanded interfollicular areas by B cells and PTGC-like changes, were usually absent in PFL cases. Residual hyperplastic germinal centers may be present, and show the expected cytological composition and polarization. PD-1(programmed death-1), a member of the CD28 costimulatory receptor family, is expressed by follicular helper T cells (TFH).²³ We sought to use the PD-1 staining pattern to help distinguish PFL and PNMZL. Overall, the abnormal follicles of nodal PFL contained many fewer PD-1 positive T-cells than the disrupted follicles of the PNMZL cases. Additionally, the distribution of the PD-1 positive lymphocytes was abnormal in nodal PFL, being pushed to the peripheral rim of the atypical follicles. Muenst et al. also studied the distribution of PD-1 positive lymphocytes in B-cell lymphomas.²⁴ They observed fewer PD-1 positive lymphocytes in Grade 3 FL as compared with Grade 1–2 FL, in keeping with our observations, but their study did not include nodal MZL.

Utilizing the published and expanded criteria for the diagnosis of PFL and UFL, lymph nodes involved by FL in patients under the age of 30 clearly segregate into two groups. UFL was not observed under age 18, and is much more likely to present with disseminated disease. In addition, the data in our study indicate substantial differences in PFL involving Waldeyer's ring from those presenting in lymph nodes or testis. Up regulation of MUM1 expression and/or *IRF4* breaks imply a possible pathogenetic mechanism for PFL located in

Waldeyer's ring. A prior publication²⁵ had emphasized the unique features of testicular FL, which rarely if ever show evidence of extratesticular disease.

Correct diagnosis of FL in these patients requires careful examination of histologic, immunophenotypic, cytogenetic features, and clinical features, but the optimal clinical management is still unresolved. While monoclonality is demonstrated for these lesions, many cases appear to have a very limited potential for progressive disease. There is an increasing recognition in the literature of clonal lymphoid proliferations with limited potential for clinical aggressiveness.²⁶ In the future nodal and testicular PFLs may be grouped with other clonal B-cell proliferations of undetermined significance, such as monoclonal gammopathy of undetermined significance or MGUS, follicular lymphoma in situ, and mantle cell lymphoma in situ.^{26,27} However, as PFL involving Waldeyer's ring are associated with a recurrent cytogenetic abnormality, they may have potential for a more aggressive clinical course.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Histologic and immunophenotypic features of PFL in Tonsil, case 1. The architecture is focally effaced by large, expansile atypical follicles (A 20X). A few reactive follicles are seen on the left adjacent to the lesion. The neoplastic cells are positive for CD20 (B, 20X; I, 400X), CD10 (weak, C, 20X), BCL6 (D, 20X; J, 400X), MUM1 (E, 20X; K, 400X), and weakly positive to negative for BCL2 (F, 20X). CD3 stains the T cells in the periphery of the follicles (G, 20X). MIB1 indicates a moderate to high proliferation rate (H, 20X; L, 400X).

Figure 2.

Comparison of cytologic Features of PFL in Tonsil and Lymph Node (1000X). Centroblasts comprised the dominant cell type in 3 of the 8 tonsillar PFL cases (A, case 1), and while the remaining 5 tonsillar PFL cases contained a predominance of monotonous, medium-sized blastoid cells in the atypical follicles (B, case 6). An arrow identifies a follicular dendritic cell nucleus, which is similar in nuclear diameter to the neoplastic cells. C. Small to medium-sized blastoid cells in some nodal PFL (case 18) resembled centrocytes, but exhibited a high proliferative rate with more dispersed chromatin. D. In most cases of nodal PFL the blastoid cells were medium to large, with finely clumped chromatin and small nucleoli (case 22). Compare with centroblasts shown in A.

Figure 3.

Histologic and immunophenotypic features of nodal PFL, case 22 (200×) A. The nodal architecture is effaced by ill-defined follicles. A starry sky pattern is evident, but without polarization characteristic of reactive germinal centers. (200 X). B. The IgD stain shows thin and attenuated mantle zones. C. CD79a shows the back to back follicles, often with a serpiginous configuration. Small numbers of interfollicular B-cells are present. D. The irregular follicles are strongly CD10 positive, but CD10 positive cells do not extend to the interfollicular region.

Figure 4.

FISH analysis of a MUM1-positive tonsillar PFL. *IGH@-IRF4* fusion is shown using a locus-specific probe consisting of BAC clones labeled in spectrum orange (*IRF4*) and spectrum green (*IGH@*). The *IGH2-IRF4* fusion is a reciprocal fusion, in which in most cells will display two fusion signals (Arrows). The presence of a single fusion signal in one cell (right arrow) is likely related to sectioning artifacts or differences in the focal plane.

TABLE 1

Summary of Clinical Features of PFL Patients

Patient	Age(y)/Sex	Biopsy Site	Clinical Stage	Treatment	NED (m)
1	5/M	Tonsil	III	Tonsillectomy	4
2	5/F	Tonsil	IE	Tonsillectomy	32
3	7/M	Tonsil/adenoids	III	Chemotherapy	~120
4	11/F	Tonsil	NA	NA	39
5	18/M	Tonsil	IE	C, Anthracyclin	62
6	18/F	Tonsil	IE	RCHOP, Methotrexate	1
7	15/F	Tonsil	IE	Await for Tx	NA
8	3/M	Tonsil	NA	NA	NA
9	3/M *	Testis	I	Orchiectomy	~96
10	4/M	Testis	I	CHOP, Methotrexate	66
11	7/M	Testis	II	Chemotherapy	8
12	10/M	Testis	NA	NA	NA
13	5/M	LN, femoral	I	NA	NA
14	8/M	LN, neck	I	Watch	6
15	12/M	LN, post. auricular	II	CHOP	36
16	12/M	LN, neck	NA	NA	NA
17	12/M	LN, submandibular	NA	NA	NA
18	14/M	LN, submental	NA	NA	NA
19	14/M	LN, inguinal	I	RCHOP	14
20	14/M	LN, neck	I	R, C, doxorubicin	8
21	14/M	LN, neck	I	RCHOP	4
22	15/M	LN, axilla	NA	NA	NA
23	15/M	LN, neck	I	Watch	9
24	16/M	LN, neck	NA	NA	NA
25	17/M	LN, submandibular	I	Excision	71
26	17/M	LN, neck	I	CHOP	30
27	18/M	LN, neck	I	RCHOP	3

Patient	Age(y)/Sex	Biopsy Site	Clinical Stage	Treatment	NED (m)
28	19/M	LN, neck	NA	NA	NA
29	19/M	LN, neck	I	CHOP, Rad	39
30	20/M	LN, thigh	I	RCHOP, Rad	78
31	20/M	LN, neck	NA	NA	NA
32	20/M	LN, neck	I	Watch	2
33	21/M	LN, occipital	I	RCHOP	18
34	25/M	LN, inguinal	II	RCHOP, Rad	4

C, cyclophosphamide; R, Rituximab; CHOP, cyclophosphamide, adriamycin, vincristine, prednisone; Rad, local radiation; NED, no evidence of disease; NA, not available;

* this case has been published in a previous study (7)

TABLE 2

Summary of Clinical Features of UFL Patients

Patient	Age(y)/Sex	Biopsy Site	Histologic Grade	Clinical Stage/ Presentation	Treatment	NED (m)
35	18/M	LN, neck	1-2	III	RCHOP	11
36	19/F	LN, neck	1-2*	IV	RCHOP	19
37	19/F	LN, axilla	1-2	NA	NA	NA
38	19/F	LN, neck	1-2	I	None	NA
39	19/M	LN, periauricular	1-2#	I	Awaiting for treatment	2
40	19/F	Thyroid	1-2	Generalized LAD, SM	NA	NA
41	21/F	LN, neck	1-2	Generalized LAD	NA	NA
42	22/F	LN, inguinal	1-2	I	Rad	15
43	22/F	LN, epitrochlear	1-2	I	Rad	46
44	23/F	LN, mesenteric	1-2 with features of FLIS	I	None	12
45	24/M	LN, inguinal	1-2	NA	NA	NA
46	24/M	LN, neck	1-2	NA	NA	NA
47	24/F	LN, neck	1-2	IV	RCHOP, Rad	12
48	24/F	LN, axilla	1-2	III	RCHOP, Rad	17
49	24/F	LN, axilla	1-2	IV	RCVP	14
50	25/M	LN, unknown	1-2	Generalized LAD, HSM	NA	NA
51	26/M	LN, neck	1-2	NA		
52	26/F	LN, neck	1-2	NA	RCHOP	4
53	27/M	LN, neck	1-2	IV	NA	NA
54	27/F	LN, inguinal	1-2	IV	Chemotherapy	Recurred
55	27/M	LN, para-aortic	1-2	NA	NA	NA
56	27/F	LN, neck	1-2	III	No Tx for a year, followed by R	Persistent LAD, BM+
57	29/F	LN, axilla	1-2	Multiple peripheral LAD	NA	NA
58	29/F	LN, groin	1-2	NA	NA	NA
59	29/M	LN, mediastinal	1-2	Generalized LAD	NA	NA
60	21/F	LN, inguinal	3	NA	NA	NA
61	26/F	LN, unknown	3	Generalized LAD, HSM	NA	NA

Patient	Age(y)/Sex	Biopsy Site	Histologic Grade	Clinical Stage/ Presentation	Treatment	NED (m)
62	27/F	LN, inguinal	3	I	RCHOP	4
63	28/F	LN, submental	3	NA	NA	NA

RCVP, Rituximab, cyclophosphamide, vincristine, prednisone;

^{*} histologic grade 1–2 with high proliferation rate;

[#] unusual morphology with irregular follicles; FLIS, FL in situ

TABLE 3

Immunophenotypic Features, Clonality by PCR, and FISH Results of PFL Cases

Site	PCR/IG+ (%, +/-tested)	CD10+ (%, +/-tested)	Bcl-2+ (%, +/-tested)	MUM1+ (%, +/-tested)	FISH for Breaks (positive cases/cases tested)		
					IGH@*	IRF4	BCL6
Tonsil	83 (5/6)	63 (5/8)	63 (4/8)	100 (5/5)	3/5	3/6	0/4
Testis	100 (4/4)	75 (3/4)	0 (0/4)	NA	0/3	0/2	0/2
LN	100 (21/21)	100 (22/22)	18 (4/22)	5 (1/18)	2/16	0/14	0/14

* FISH for *BCL2* and *CBFA2T3* breaks was performed on all cases with IGH@ breaks (5 cases), since an *IGH@* break is required for the translocation. No positive cases were observed among those with *IGH@* breaks; NA, not available.

TABLE 4

Comparison of Clinicopathologic Features of Nodal PFL, PNMZL, and UFL

Diagnosis	Median Age	M:F	Head/Neck predilection	CD10+ (%)	BCL2+ (%)	PD-1	Starry sky	PTGC
UFL	24	1:2.2	No	91 [*]	81 [*]	increased	No	No
PFL, nodal	14	All M	Yes	100	20	decreased	Yes	No
PNMZL	17	9:1	Yes	0	NA	increased	Yes	Yes

^{*} When restricted to Grade 1–2 UFL, 100% CD10+, 83% Bcl-2+

TABLE 5

Comparison of Clinical Features of PFL and UFL Patients

PFL				
Histologic grade	Site	#Cases	Median Age (range) (year old)	M:F
NA	Tonsil	8	9 (3–18)	4:4
	Testis	4	5.5 (3–10)	All M
	LN	22	15 (5–25) (15 cases≤18 7 cases>18)	All M
UFL				
Grade 1–2	LN	24	24 (18–29)	9:16
	Extranodal	1		
Grade 3	LN	4	26.5 (21–28)	All F

TABLE 6
Immunophenotypic Features and FISH Results of MUM1+ PFL Cases

Case #	Age/Sex	Site	PCR/IG	IHC			FISH (breaks)	
				CD10	Bcl-6	MUM1	Light chain	IGH@ IRF4
1	5/M	W	+	+	+	+	-	+
2	5/F	W	+	-	+	+	-	+
5	18/M	W*	-	+	+	+	-	+
6	18/F	W	+	-	+	+	-	-
7	15/F	W	+	+	+	+	Kappa	NA
8	3/M	W	+	-	+	+	NA	-
19	14/M	LN	+	+	+	+	Lambda	NA

W: Waldeyer's ring; LN: lymph node;

* PFL with DLBCL