Unifocal and Multifocal Reactive Lymphoid Hyperplasia vs Follicular Lymphoma of the Ocular Adnexa

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• PURPOSE: **To characterize the differentiating histopathologic and immunophenotypic features of reactive lymphoid hyperplasia (RLH) and follicular lymphoma of the ocular adnexa.**

• DESIGN: **Retrospective case study with clinical follow-up and review of the literature.**

• METHODS: **Clinical records of 9 cases of RLH and 6 cases of follicular lymphoma from 2 institutions were reviewed. Light microscopic evaluation and immunohistochemical stains including CD20, CD3, CD5, CD21, CD23, BCL-2, BCL-6, CD10, kappa, lambda, and Ki67 were used to distinguish the 2 categories.**

• RESULTS: **RLH preferentially involved the conjunctiva, whereas follicular lymphoma had a propensity to involve the lacrimal gland. Microscopic analysis with immunohistochemical staining distinguished RLH from follicular lymphoma. BCL-2 was positive in follicular centers of follicular lymphoma but not in RLH. CD10 identified follicular center cells and Ki67 quantified cells in S-phase. CD21 and CD23 detected dendritic cell scaffoldings of indistinct germinal centers. None of the patients with RLH developed lymphoma during their clinical courses (up to 18 years). However, 3 patients with orbital, but not conjunctival, RLH developed immunohistochemically proven multifocal nonophthalmic supradiaphragmatic adnexal RLH (sites included lung, parotid, axillary nodes, and uvea). All 6 patients with follicular lymphoma had disseminated disease.**

• CONCLUSIONS: **A correct diagnosis of RLH vs follicular lymphoma can be reliably established employing immunohistochemical methods. A heretofore undescribed "multifocal RLH" syndrome must be distinguished from follicular lymphoma. Conjunctival RLH can usually be managed surgically without radiotherapy, but "multifocal RLH" required systemic treatment in 2 of 3 patients. Follicular lymphoma requires systemic chemotherapy if discovered beyond stage 1E. (Am J Ophthalmol 2010;150:412–426. © 2010 by Elsevier Inc. All rights reserved.)**

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BEFORE THE ROUTINE APPLICATION OF IMMUNOHISTOCHEMICAL techniques to evaluate benign and malignant ocular adnexal lymphoid tumors (OALTs), the unaided histomorphologic distinction between reactive lymphoid hyperplasia (RLH) and follicular lymphoma was difficult and often erroneous.[1] Creation of an additional category of atypical lymphoid hyperplasia (ALH)[2] did not remedy the problem, but rather led to more confusing results concerning the risk for coexistent or eventual systemic lymphoma in the various categories.[3,4] ALH was defined histomorphologically by the presence of scattered large atypical cells ("immunoblasts" discerned in diffuse areas), increased interfollicular mitotic activity, and/or the participation of small cells with irregular nuclear outlines.[2]

RLH has been regarded as a benign, yet autonomous, unicentric polyclonal lymphoproliferation that exhibits a follicular architecture (which always signals the presence of B lymphocytes), but may also appear diffuse and sheet-like.[2,3] In contrast, follicular lymphoma is a monoclonal B cell neoplasm that consistently exhibits a follicular pattern. In the ocular adnexa, follicular lymphomas are generally second or third to extranodal marginal zone lymphomas in frequency, constituting about 15% to 20% of OALTs.[3,5–7] When separately enumerated, RLHs have accounted for 7% of 175[8] and 20%[9] of 122 OALTs in the 2 largest series of biopsied orbital lesions of all types. Follicular lymphoma typically presents in older patients about 60 years old, and the disease course is indolent though usually not curable.[10] Among OALTs, it constituted 23% of the largest series of 353 cases.[5]

With the development of immunohistochemical markers, one can more easily distinguish between RLH and follicular lymphoma[3,11,12] which has obviated the need to invoke ALH. However, a detailed comparison of the light microscopic and immunohistochemical profiles of RLH and follicular lymphoma, coupled with clinical findings including follow-up information, has been lacking.[6,7,13–15] More recent studies have utilized some immunohistochemical markers but not the full panoply currently available for dissecting the immunophenotypic composition of OALTs. The largest investigation of OALTs employing a wide range of immunohistochemical markers excluded RLH and focused only on lymphomas.[5] Our aim in this study is to demonstrate t he diagnostic value and clinical relevancy of preserving

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TABLE 1. Immunophenotypic Markers Employed in This Study for Analysis of Ocular Adnexal Reactive Lymphoid Hyperplasias and Follicular Lymphomas

Antibody	Cell Stained	Protein Location or Function	Staining Pattern	Source	Dilution
CD20	B lymphocytes	Calcium channel membrane protein	Membranes	Mouse monoclonal[a,c]	Prediluted/1:50
CD5	T lymphocytes	Transmembrane glycoprotein	Cell membrane	Rabbit monoclonal,[a] mouse monoclonal[d]	Prediluted/1:10
CD3	T lymphocytes	T-cell receptor complex	Cell membrane	Rabbit polyclonal, mouse monocloncal[a]	Prediluted
CD21	Follicular dendritic cells	Membrane protein	Cell membranes/ processes	Mouse monoclonal[a,c]	Prediluted/1:10
CD23	Follicular dendritic cells	Membrane protein	Cell membranes/ processes	Mouse monoclonal[a,e]	Prediluted/1:20
BCL-2	B lymphocytes	Anti-apoptotic protein	Cytoplasmic	Mouse monoclonal[a,f]	Prediluted
BCL-6	B lymphocytes	Transcription factor	Nuclear	Rabbit polyclonal,[b] mouse monocloncal[c]	1:100/1:5
CD10	T lymphocytes and germinal center B cells	Membrane endopeptidase	Cell membrane	Mouse monoclonal[a,d]	Prediluted/1:5
kappa	Plasma cells	Ig light chain	Cytoplasmic	Rabbit polyclonal[c]	1:2000/1:32 000
lambda	Plasma cells	Ig light chain	Cytoplasmic	Rabbit polyclonal[c]	1:1400/1:64 000
Ki-67	Cells in proliferating state	Cell proliferation	Nuclear	Rabbit monoclonal[a]	Prediluted

[a]Ventana Medical Systems, Oro Valley, Arizona, USA.
[b]Leica Microsystems, Bannockburn, Illinois, USA.
[c]DAKO Corporation, Carpinteria, California, USA.
[d]Novocastra Laboratories Ltd., Newcastle upon Tyne, United Kingdom.
[e]Binding Site Inc, San Diego, California, USA.
[f]Cell Marque Corporation, Rocklin, California, USA.

the category of RLH particularly when refined by supplemental immunohistochemical analysis. We furthermore characterize a subset of patients reflecting a new entity of multifocal ophthalmic and nonophthalmic RLH with a more favorable prognosis than follicular lymphoma.

METHODS

BETWEEN JANUARY 1, 2005 AND DECEMBER 31, 2009, 9 cases of RLH and 6 cases of follicular lymphoma were found among a total of 85 biopsied OALTs at the Massachusetts Eye and Ear Infirmary and Cleveland Clinic. Tissue was biopsied from a conjunctivectomy (6 RLH, 2 follicular lymphoma) or orbitotomy (3 RLH, 4 follicular lymphoma). Four of the orbitotomies were lateral and 3 were anterior. In all cases the surgeons regarded the biopsy as representative with adequate surgical exposure. Tissue sections were embedded in paraffin and stained with hematoxylin-eosin. Immunohistochemical markers CD20, CD3, CD5, CD21, CD23, BCL-2, BCL-6, CD10, kappa, lambda, and Ki67 (Table 1) were used, following standard staining protocols as described elsewhere[16] on Ventana Benchmark automated immunostainers (Ventana Medical Systems, Oro Valley, Arizona, USA) at the Massachusetts General Hospital or the Cleveland Clinic. In situ hybridization of kappa and lambda was also performed in some tissues; these probes were also supplied by Ventana Medical Systems.

All patients' clinical histories, symptoms upon presentation, and subsequent clinical courses after ophthalmic presentation were obtained by reviewing the treating ophthalmologists' records or those of the primary care physicians and treating oncologists when necessary and available. Patients were generally followed after their diagnoses through various means including blood tests such as complete blood counts (CBC) and immunoglobulin (IgG) levels; magnetic resonance imaging (MRI) of the brain and orbits; computed tomography (CT) scans of the chest, abdomen, and pelvis; positron emission tomography (PET) scans; and bone marrow biopsies.