**Essentials in Ophthalmology**
*Series Editor:* Arun D. Singh

Arun D. Singh *Editor*

# Ocular and Adnexal Lymphoma

Springer

Essentials in Ophthalmology

Arun D. Singh
Editor

Arun D. Singh
Series Editor

# Ocular and Adnexal Lymphoma

Springer

*Editor*
Arun D. Singh
Department of Ophthalmic Oncology
Cole Eye Institute
Cleveland Clinic Foundation
Cleveland, OH
USA

*Series Editor*
Arun D. Singh
Department of Ophthalmic Oncology
Cole Eye Institute
Cleveland Clinic Foundation
Cleveland, OH
USA

ISBN 978-3-642-38498-1 ISBN 978-3-642-38499-8 (eBook)
DOI 10.1007/978-3-642-38499-8
Springer Heidelberg New York Dordrecht London

Library of Congress Control Number: 2013948129



Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

# Preface

Ocular and adnexal lymphomas are rare and diverse, hence their diagnosis and treatment usually requires special expertise. Increasingly, the care of such a patient is provided by a multidisciplinary team comprising of ocular oncologists, general oncologists, pathologists, radiation oncologists, and other specialists. The field of lymphoma is advancing rapidly because of accelerating progress in tumor biology, pharmacology, and the advent of targeted therapies. For all these reasons, we felt that there was scope for a monograph offering a comprehensive source of authoritative information on the subject of ocular and adnexal lymphoma.

This monograph, a conjoint effort of ocular oncologists, general oncologists, and pathologists, comprises of ten chapters covering classification, histology, molecular pathology, and epidemiology followed by clinical features and biopsy techniques. The description of staging procedures and treatment methods for primary vitreoretinal lymphoma and adnexal lymphoma are also included. We have also included chapters on rare variants such as T cell lymphoma and reactive lymphoid hyperplasia of the ocular adnexa.

It is our sincere hope that readers will find as much pleasure reading this textbook as we had writing and editing it.

Cleveland, OH, USA — Arun D. Singh

# Acknowledgements

To my parents who educated me beyond their means, my wife Annapurna, and my children, Nakul and Rahul, who make all my efforts worthwhile.

# Contents

# Lymphoid Neoplasms: Classification Systems

1

Sarah E. Coupland

## 1.1 Historical Background

Classification systems in pathology ideally should contain diseases that are clearly defined, clinically distinctive and nonoverlapping, and comprise all known entities. Classification systems, however, are continually evolving, as we better understand the histogenesis of tumors with improving technologies. While there has been generally long-term agreement with the classification of most nonlymphoid malignancies, the history of lymphoma classifications has been rather tumultuous with resolution and consensus having only been achieved in the two last decades.

The first clinical description of what we now recognize as lymphoma is generally attributed to Thomas Hodgkin in 1832 (Hodgkin 1832). The first use of the term "lymphosarcoma" is attributed to Rudolf Virchow in 1863 (see review by Trumper et al (2004)). In 1898 and 1902, Carl Sternberg and Dorothy Reed independently described the characteristic binucleate and multinucleate giant cells that came to be called the Reed-Sternberg or Sternberg-Reed cell of HL (Dawson 1999). Interestingly there was disagreement between the two as to whether these cells were reactive or neoplastic. The term "reticulum cell sarcoma" is attributed to Ewing, Oberling, and Roulet, who each described tumors of large cells, which were thought to be related to the supporting fibrous reticulum of lymphoid tissues (Trumper et al. 2004). In 1925, Brill and colleagues described patients with enlarged lymph nodes and spleen, characterized by a proliferation of lymphoid follicles (Brill et al. 1925); further cases were reported by Symmers, who also described progression of this disease entity, follicular lymphoma (FL), to a large-cell neoplasm (Symmers 1927, 1938). In 1941, Drs. Edward Gall and Tracy Mallory, pathologists at Massachusetts General Hospital, proposed a morphological classification, based on review of their own collection of 618 patients for whom they had clinical data available (Gall and Mallory 1942). They recognized FL as being a distinct entity and essentially subdivided the lymphomas into follicular and non-follicular subtypes. This was the first widely used "classification" of lymphomas in the USA; this classification included Hodgkin's disease as a separate type of lymphoma, but further subdivisions of Hodgkin's disease were subsequently proposed by Jackson and Parker (1944).

The first lymphoma classification of the "modern era" was that proposed by Henry Rappaport, originally published in 1956 (Rappaport et al. 1956) and later revised (Rappaport 1966; Sheehan and Rappaport 1970): despite initial success, it was a morphological classification with few categories and based on incorrect histogenetic concepts, i.e., some of the neoplasms affecting the lymph node were considered to be

S.E. Coupland
Department of Molecular and Clinical Cancer Medicine, University of Liverpool, 6th Floor Duncan Building, Daulby Street, Liverpool L69 3GA, UK
e-mail: s.e.coupland@liverpool.ac.uk

A.D. Singh (ed.), *Ocular and Adnexal Lymphoma*, Essentials in Ophthalmology,
DOI 10.1007/978-3-642-38499-8_1, © Springer-Verlag Berlin Heidelberg 2014

**Table 1.1** Rappaport's classification

| Nodular | Diffuse |
|---|---|
| Lymphocytic, well differentiated | Lymphocytic, well differentiated |
| Lymphocytic, poorly differentiated | Lymphocytic, poorly differentiated |
| Mixed, lymphocytic, and histiocytic | Mixed, lymphocytic, and histiocytic |
| Histiocytic | Histiocytic |
| | Undifferentiated Burkitt's |
| | Non-Burkitt's |

**Table 1.2** Lukes and Collins classification

| Undefined cell | |
|---|---|
| T cell | B cell |
| Small lymphocyte | Small lymphocyte |
| Convoluted lymphocyte | Plasmacytoid lymphocyte |
| Cerebriform cells of Sézary's and mycosis fungoides | Follicular center cell (FCC) |
| Lymphoepithelioid cell | Small cleaved |
| Immunoblastic sarcoma | Large cleaved |
| | Small noncleaved |
| | Large noncleaved |
| | FCC subtypes: follicular, diffuse follicular, and diffuse |
| | Immunoblastic sarcoma |
| Histiocytic | |
| Cell of uncertain classification | |
| Unclassifiable | |

derived from histiocytes (histiocytic lymphoma) and some from a mixture of lymphocytes and histiocytes (mixed lymphohistiocytic lymphoma) (Table 1.1). The early 1970s saw the emergence and publication of two functional lymphoma classifications of merit, which tried to address the limitations of the Rappaport classification. These attempted to relate malignant lymphomas to the cells of the normal immune system: the Lukes-Collins classification from the USA (Table 1.2) (Lukes and Collins 1974, 1975) and the Kiel classification proposed by the European Lymphoma Club led by Professor Karl Lennert (Table 1.3) (Lennert 1975). In the Lukes-Collins classification, there was for the first time the distinction of B- and T-cell-derived lymphomas. The Kiel classification was based on a clinically well-defined cohort of lymphoma samples from Kiel, which had been examined with developing techniques of the time, including electron microscopy and immunology. It was the first classification system that tried to link the neoplastic entity with the normal counterpart and that attempted to provide a grade of malignancy by assessing the number of blasts and mitotic frequency. While the Lukes-Collins classification encountered considerable resistance in the USA (mainly from those supportive of the Rappaport system), the Kiel classification became quite popular in Europe and was adopted by many leading institutions as well as cooperative groups, including the German Lymphoma Program, which rapidly enrolled numerous cases. The lymphoma classifications at this time, therefore, were discordant and competing, resulting in an "Atlantic divide" with respect to clinical and scientific collaborations. This prompted a couple of comments brimming with sarcasm to be published on both sides of the Atlantic Ocean (Kay 1974; Higby 1979). Despite this, the Kiel lymphoma classification underwent further revisions, incorporating further developments, particularly in immunohistochemistry (Table 1.3) (Lennert et al. 1975; Stansfeld et al. 1988; Lennert and Feller 1992).

With this discord abounding, the WHO attempted a revised lymphoma classification, which did not receive much approval (Chelloul et al. 1976; Jaffe et al. 1998), and following this, the International Working Formulation was also thrown "into the foray" for good measure (Table 1.4) (Institute 1982). The latter was published in 1982 by the National Cancer Institute, following the examination of about 1,000 lymphoma cases by the principle authors of the main lymphoma classifications used at that time, when they were asked to examine and reclassify the cases based on their own and others' criteria. There were fundamental problems associated with this study, namely, the use of hematoxylin- and eosin-stained slides only, with very limited immunohistochemical stains and with lacking clinical information. The "Working Formation for Clinical Usage" (Table 1.4) was rejected by many pathologists, including Lukes and Lennert, but was popular among treating physicians.

**Table 1.3** Original Kiel classification

| **Original Kiel classification** | |
|---|---|
| *Low-grade malignant lymphomas* | *High-grade malignant lymphomas* |
| Lymphocytic | Centroblastic |
| B-CLL | Lymphoblastic |
| T-CLL | Burkitt's type |
| Hairy cell leukemia | Convoluted-cell type |
| Mycosis fungoides and Sézary's syndrome | Unclassified |
| Lymphoplasmacytic/-cytoid (immunocytoma) | Immunoblastic |
| Plasmacytic | |
| Centrocytic | |
| Centroblastic-centrocytic | |
| Follicular +/– diffuse | |
| Diffuse | |
| +/– Sclerosis | |
| **Updated Kiel classification** | |
| **B cell** | **T cell** |
| *Low-grade malignant lymphomas* | *Low-grade malignant lymphomas* |
| Lymphocytic | Lymphocytic |
| Chronic lymphocytic leukemia | Chronic lymphocytic leukemia |
| Prolymphocytic leukemia | Prolymphocytic leukemia |
| Hairy cell leukemia | |
| Lymphoplasmacytic/-cytoid (immunocytoma) | |
| Plasmacytic | Small cell, cerebriform |
| Centrocytic | Mycosis fungoides, Sézary's syndrome |
| Centroblastic-centrocytic | Lymphoepithelioid (Lennert's Ly) |
| Follicular +/– diffuse | Angioimmunoblastic (AILD, LgX) |
| Diffuse | T-zone lymphoma |
| Centrocytic (mantle cell) | Pleomorphic, small cell (HTLV-$1^{+/-}$) |
| Monocytoid, including marginal zone cell | |
| High-grade malignant lymphomas | High-grade malignant lymphomas |
| Centroblastic | Pleomorphic med and large ($HTLV^{+/-}$) |
| Immunoblastic | Immunoblastic (HTLV-$1^{+/-}$) |
| Burkitt's lymphoma | Large-cell anaplastic (Ki-$1^{+}$) |
| Large-cell anaplastic (Ki-$1^{+}$) | |
| Lymphoblastic | Lymphoblastic |
| Rare types | Rare types |

In 1991, 18 hematopathologists from Europe and the USA met in London at the Royal College of Pathologists and established the ILSG. The aim of this group was to compare criteria used to establish diagnoses in routine cases. It became apparent that there were no major conceptual differences across the Atlantic Ocean and, however, that there were differences in the descriptive language used and the diagnoses issued. Subsequent meetings in Berlin and Boston resulted in the ILSG publishing the REAL classification in 1994 in *Blood* (Table 1.5) (Harris et al. 1994). The REAL classification represented a paradigm in the classification of lymphoid neoplasms, focusing on the identification of distinct ("real") disease entities, instead of classifying lymphomas according to their growth pattern, the cell type, or the morphology of tumor cells, as had been used in the Working Formulation and the Kiel and Lukes-Collins classification systems. It was the first worldwide consensus classification for hematological malignancies. The REAL classification was received by a mixed response and required a validation

exercise comparing the REAL with the Working Formulation and the Kiel classification (Project TN-HsLC 1997). The study encompassed about 3 years, and the results demonstrated the superiority of the REAL classification in comparison with the other 2 approaches (Project TN-HsLC 1997). It was shortly followed by preparations for the modern WHO classification, which was published in 2001 (third edition) (Table 1.6) and then later revised in 2008 (fourth edition) (Table 1.7). The task for developing the WHO classifications was undertaken as a cooperative project by the Society for Hematopathology and the European Association of Hematopathology, with a goal to maintain consensus and avoid the political divisions of the past. A steering committee was appointed by the two societies, which in turn established 10 individual committees to deal with different disease groups within hematopoietic and lymphoid malignancies. The fourth

**Table 1.4** The Working Formulation for clinical usage

| |
|---|
| *Low grade* |
| A. Small lymphocytic (consisted with CLL, plasmacytoid) |
| B. Follicular (predominantly small cleaved cell, diffuse areas, sclerosis) |
| C. Follicular (small cleaved and large cell, diffuse areas, sclerosis) |
| *Intermediate grade* |
| D. Follicular (predominantly large cells) |
| E. Diffuse (small cleaved cell, diffuse areas, sclerosis) |
| F. Diffuse (mixed, small and large cell, sclerosis, epithelioid component) |
| G. Diffuse (large cell, cleaved and noncleaved) |
| *High grade* |
| H. Large cell (immunoblastic: plasmacytoid, clear cell, polymorphous, epithelioid component) |
| I. Lymphoblastic (convoluted, nonconvoluted) |
| J. Small noncleaved cell (Burkitt's, follicular areas) |

**Table 1.5** The REAL classification

| |
|---|
| *B-cell neoplasms* |
| I. *Precursor* B-cell neoplasm: precursor B-lymphoblastic leukemia/lymphoma |
| II. *Peripheral* B-cell lymphoma |
| 1. B-cell chronic lymphocytic leukemia/ prolymphocytic leukemia/small lymphocytic lymphoma |
| 2. Lymphoplasmacytoid lymphoma/immunocytoma |
| 3. Mantle cell lymphoma |
| 4. Follicle center lymphoma, follicular |
| Provisional cytologic grades: I (small cells), II (mixed small and large cells), III (large cell) |
| Provisional subtype: diffuse, predominantly small cell type |
| 5. Marginal zone B-cell lymphoma |
| Extranodal (MALT type +/– monocytoid B cell) |
| Provisional subtype: nodal (+/– monocytoid B cell) |
| 6. Provisional entity: splenic marginal zone lymphoma (+/– villous lymphocytes) |
| 7. Hairy cell leukemia |
| 8. Plasmacytoma/plasma cell myeloma |
| 9. Diffuse large B-cell lymphoma[a] |
| Subtype: primary mediastinal (thymic) large B-cell lymphoma |
| 10. Burkitt's lymphoma |
| 11. Provisional entity: high-grade B-cell lymphoma, Burkitt's like[a] |
| *T-cell and putative NK-cell neoplasms* |
| I. *Precursor* B-cell neoplasm: precursor T-lymphoblastic leukemia/lymphoma |
| II. *Peripheral* T-cell lymphoma and NK-cell neoplasms |
| 1. T-cell chronic lymphocytic leukemia/ prolymphocytic leukemia |
| 2. Large granular lymphocytic leukemia: T-cell type and NK-cell type |
| 3. Mycosis fungoides/Sézary's syndrome |
| 4. Peripheral T-cell lymphoma, unspecified[a] |
| Provisional categories: medium-sized cells, mixed medium and large cells, large, lymphoepithelioid cells |
| Provisional subtypes: hepatosplenic γδ T-cell lymphoma |
| Provisional subtypes: subcutaneous panniculitic T-cell lymphoma |
| 5. Angioimmunoblastic T-cell lymphoma (AILD) |
| 6. Angiocentric lymphoma |
| 7. Intestinal T-cell lymphoma (+/– enteropathy associated) |
| 8. Adult T-cell lymphoma/leukemia |
| 9. Anaplastic T-cell lymphoma (ALCL), $CD30^+$, T- and null cell types |
| 10. Provisional entity: anaplastic T-cell lymphoma Hodgkin's like |
| *Hodgkin's disease* |
| I. Lymphocyte predominant |
| II. Nodular sclerosis |
| III. Mixed cellularity |
| IV. Lymphocyte depletion |
| V. Provisional entity: lymphocyte-rich classical HD |

[a]These categories are likely to include more than one disease entity

**Table 1.6** WHO classification 2001

| |
|---|
| *B-cell neoplasms* |
| Precursor B-cell neoplasms |
| Precursor B-lymphoblastic leukemia/lymphoma |
| Mature B-cell neoplasms |
| Chronic lymphocytic leukemia/small lymphocytic lymphoma |
| B-prolymphocytic leukemia |
| Lymphoplasmacytic lymphoma/Waldeström macroglobulinemia |
| Splenic marginal zone lymphoma |
| Hairy cell leukemia |
| Plasma cell myeloma |
| Solitary plasmacytoma of bone |
| Extraosseous plasmacytoma |
| Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) |
| Nodal marginal zone lymphoma |
| Follicular lymphoma |
| Mantle cell lymphoma |
| Diffuse large B-cell lymphoma |
| Mediastinal (thymic) large B-cell lymphoma |
| Intravascular large B-cell lymphoma |
| Primary effusion lymphoma |
| Burkitt's lymphoma/leukemia |
| B-cell proliferations of uncertain malignant potential |
| Lymphomatoid granulomatosis |
| Posttransplant lymphoproliferative disorder, polymorphic |
| *T-cell neoplasms* |
| Precursor T-cell neoplasm |
| Precursor B-lymphoblastic leukemia/lymphoma |
| Blastic NK-cell lymphoma |
| Mature T-cell and NK-cell neoplasm |
| T-cell prolymphocytic leukemia |
| T-cell large granular lymphocytic leukemia |
| Aggressive NK-cell leukemia |
| Adult T-cell leukemia/lymphoma |
| Extranodal NK/T-cell lymphoma nasal type |
| Enteropathy-type T-cell lymphoma |
| Hepatosplenic T-cell lymphoma |
| Subcutaneous panniculitis-like T-cell lymphoma |
| Mycosis fungoides |
| Sézary's syndrome |
| Primary cutaneous CD30-positive T-cell lymphoproliferative disorders |
| Primary cutaneous anaplastic T-cell lymphoma |
| Peripheral T-cell lymphoma, unspecified |
| Angioimmunoblastic T-cell lymphoma |
| Anaplastic T-cell lymphoma |
| B-cell proliferations of uncertain malignant potential |
| Lymphomatoid papulosis |
| *Hodgkin's disease* |
| Nodular lymphocyte-predominant Hodgkin's lymphoma |
| Classical Hodgkin's lymphoma |
| Nodular sclerosis classical Hodgkin's lymphoma |
| Mixed cellularity classical Hodgkin's lymphoma |
| Lymphocyte-rich classical Hodgkin's lymphoma |
| Lymphocyte-dep leted classical Hodgkin's lymphoma |

**Table 1.7** WHO classification 2008

| |
|---|
| *Precursor B- and T-cell neoplasms* |
| Precursor B-lymphoblastic leukemia/lymphoma |
| B-lymphoblastic leukemia/lymphoma, NOS |
| B-lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities |
| B-lymphoblastic leukemia/lymphoma with t(9;22)(q34;q11.2; BCR-ABL1) |
| B-lymphoblastic leukemia/lymphoma with t(v;11Q23); MLL rearranged |
| B-lymphoblastic leukemia/lymphoma with t(12;21)(p13;22); TEL_AML1,(ETV 6-RUNX1) |
| B-lymphoblastic leukemia/lymphoma with hyperdiploidy |
| B-lymphoblastic leukemia/lymphoma with hypodiploidy (hypodiploid ALL) |
| B-lymphoblastic leukemia/lymphoma with t(5;14)(q31;q32); IL-3-IGH |
| B-lymphoblastic leukemia/lymphoma with t(1;19)q23;p13.3;E2A-PBX1 (TCF3-PBX1) |
| Precursor T-lymphoblastic leukemia/lymphoma |
| *Mature B-cell neoplasms* |
| Chronic lymphocytic leukemia/small lymphocytic lymphoma |
| B-prolymphocytic leukemia |
| Splenic marginal zone lymphoma |
| Hairy cell leukemia |
| Splenic B-cell lymphoma/leukemia, unclassifiable |
| Splenic diffuse red pulp small B-cell lymphoma |
| Hairy cell leukemia |
| Lymphoplasmacytic lymphoma/Waldeström |
| macroglobulinemia |
| Heavy chain disease |
| α Heavy chain disease |
| γ Heavy chain disease |
| μ Heavy chain disease |
| Plasma cell myeloma |
| Solitary plasmacytoma of bone |

(continued)

**Table 1.7** (continued)

| |
|---|
| Extraosseous plasmacytoma |
| Extranodal marginal zone lymphoma (MALT lymphoma) |
| Nodal marginal zone lymphoma |
| Pediatric nodal marginal zone lymphoma |
| Follicular lymphoma |
| Pediatric follicular lymphoma |
| Mantle cell lymphoma |
| DLBCL, NOS |
| T-cell/histiocyte-rich large B-cell lymphoma |
| Primary DLBCL of CNS |
| Primary cutaneous DLBCL, leg type |
| DLBCL associated with chronic inflammation |
| Lymphomatoid granulomatosis |
| Mediastinal (thymic) large B-cell lymphoma |
| Intravascular large B-cell lymphoma |
| ALK-positive large B-cell lymphoma |
| Plasmablastic lymphoma |
| Large B-cell lymphoma arising in HHV8-associated multicentric Castleman's disease |
| Primary effusion lymphoma |
| Burkitt's lymphoma |
| B-cell lymphoma, unclassifiable, with features intermediate between diffuse and large B-cell lymphoma and Burkitt's lymphoma |
| B-cell lymphoma, unclassifiable, with features intermediate between diffuse and large B-cell lymphoma and classical Hodgkin's lymphoma |
| *Mature T-cell and NK-cell neoplasms* |
| T-cell prolymphocytic leukemia |
| T-cell large granular lymphocytic leukemia |
| Chronic lymphoproliferative disorder of NK cells |
| Aggressive NK-cell leukemia |
| Systemic EBV$^+$ T-cell lymphoproliferative disease of childhood |
| Hydroa vacciniforme-like lymphoma |
| Adult T-cell leukemia/lymphoma |
| Extranodal NK/T-cell lymphoma nasal type |
| Enteropathy-type T-cell lymphoma |
| Hepatosplenic T-cell lymphoma |
| Subcutaneous panniculitis-like T-cell lymphoma |
| Mycosis fungoides |
| Sézary's syndrome |
| Primary cutaneous CD30$^+$ T-cell lymphoproliferative disorders |
| Primary cutaneous anaplastic T-cell lymphoma (ALCL) |
| Lymphomatoid papulosis |
| Primary cutaneous γδ T-cell lymphoma |
| Primary cutaneous CD8$^+$ aggressive epidermotropic cytotoxic T-cell lymphoma |
| Primary cutaneous CD4 positive aggressive small to medium-sized T-cell lymphoma |
| Peripheral T-cell lymphoma, NOS |
| Angioimmunoblastic T-cell lymphoma |
| Anaplastic large-cell lymphoma, ALK$^+$ |
| Anaplastic large-cell lymphoma, ALK$^-$ |
| *Hodgkin's disease* |
| Nodular lymphocyte-predominant Hodgkin's lymphoma |
| Classical Hodgkin's lymphoma |
| Nodular sclerosis classical Hodgkin's lymphoma |
| Mixed cellularity classical Hodgkin's lymphoma |
| Lymphocyte-rich classical Hodgkin's lymphoma |
| Lymphocyte-depleted classical Hodgkin's lymphoma |
| *Posttransplant lymphoproliferative disorders (PTLD)* |
| Early lesions |
| Plasmacytic hyperplasia |
| Infectious mononucleosis-like PTLD |
| Polymorphic PTLD |
| Monomorphic PTLD (B- and T/NK-cell types) |
| Classical Hodgkin's lymphoma-type PTLD |

edition WHO lymphoma classification system, which involved the efforts of 138 authors with expertise in their respective areas of pathology, biology, and clinical treatment, is now used in all oncology and pathology centers and forms the basis of all clinical trials.

## 1.2 Principles of the REAL and WHO Lymphoma Classifications

The basic underlying principles of both the third and fourth editions of the WHO lymphoma classifications are essentially unchanged from those of the REAL classification. In the REAL classification, nosological distinct entities were defined using a constellation of features: morphology, immunophenotype, genetic features, and clinical presentation and course. Therefore, the REAL classification was the first lymphoma classification based on a multiparameter approach. Each of these elements played a part, and no one feature takes precedence over the others consistently. For some diseases, morphology alone is highly characteristic,

allowing one to confidently make the diagnosis without undertaking any further ancillary studies. Most cases of chronic lymphocytic leukemia (CLL) or FL presenting in lymph nodes would fall into this category. For other diseases, knowledge of the underlying genetics may be essential, such as in the diagnosis of anaplastic lymphoma kinase-positive anaplastic large-cell lymphoma (ALCL). The relative importance of each of these features varies among the lymphomas, depending on the state of current knowledge, and there is *no* one "gold standard" by which all diseases are defined. Despite this, lineage is a defining feature and forms the basis for the WHO classification's structure, recognizing B-cell, T-cell, and natural killer (NK)-cell neoplasms. Additionally, the underlying basic premise is the distinction between precursor lymphoid neoplasms and those derived from mature lymphoid cells.

In the twentieth century the field of immunology shed light on the functional and immunophenotypic complexity of the immune system. Traditional morphological approaches were recognized as being insufficient to differentiate between the many benign and malignant cellular components of lymphoid malignancies. Monoclonal antibody technology provided an array of immunophenotypic markers that could delineate the various cell types, and technological advances soon permitted the immunohistochemical detection of most relevant antigens in routinely processed formalin-fixed paraffin-embedded (FFPE) sections. Significant contributors to the developments in the field of immunohistochemistry are too numerous to list here but include Kohler and Milstein, who created the hybridoma technology that led to the development of monoclonal antibodies (Kohler and Milstein 1975); McMichael et al. who created the first monoclonal antibody against a human lymphocyte differentiation antigen, generated against an antigen expressed on normal thymocytes (later called CD1a) (McMichael et al. 1979); as well as David Mason and others, who adapted the immunohistochemistry techniques to routine FFPE sections (Taylor and Mason 1974; Mason et al. 1982).

Many lymphoid malignancies have characteristic immunophenotypic profiles (Swerdlow et al. 2008) (see also Coupland "Histology" Chap 2), and these together with the morphological features enable pathologists to reach an unequivocal diagnosis. However, even among what are thought to be very homogenous entities, immunophenotypic variation may be seen, "catching out" the most experienced hematopathologist. For example, not all cases of CLL are CD5+ and CD23+; not all FLs are BCL-2+ or CD10+. CD5+ may be expressed in otherwise classic FL. Expression of ALK is essential for the diagnosis of ALK+ ALCL but is also expressed in ALK+ large B-cell lymphoma and a variety of nonlymphoid tumors (Tartari et al. 2011). Thus knowledge of the immunophenotype is a highly effective tool, but one that always must be used in context of morphological analysis.

There has been equally dramatic progress in the understanding of the genetics of lymphoid malignancies. Recurrent cytogenetic abnormalities have been identified for many leukemia and lymphoma subtypes. The first to be recognized were the t(14;18)(q32;q21) of FL and the t(8;14)(q24;q32) of Burkitt's lymphoma (BL) (Zech et al. 1976; Yunis et al. 1982). Subsequent studies led to the cloning of genes involved in both of these translocations. The laboratories of Philip Leder and Carlo Croce in 1982 both identified *MYC* as the gene that was translocated into the immunoglobulin genes in human BL (Dalla-Favera et al. 1982; Taub et al. 1982); other similar discoveries rapidly followed, such as BCL2/IGH@ in FL (Tsujimoto et al. 1985) and CCND1/IGH@ in mantle cell lymphoma (Tsujimoto et al. 1984). The translocations involving the immunoglobulin heavy chain gene, IGH@ at 14q24, usually result in a cellular proto-oncogene coming under the influence of the IGH@ promoter. There are also less frequent but parallel alterations involving the T-cell receptor genes in T-cell malignancies.

The process of rearrangement of the immunoglobulin and T-cell receptor genes during normal lymphoid cell development was discovered, and the technology exploited by using rearrangement

of the antigen receptor genes was used as markers of both lineage and clonality in lymphoid neoplasms (Arnold et al. 1983). Clonality analyses are part of the normal repertoire of any hematopathology center, in the routine "workup" of both B- and T-cell neoplasms, and have been improved to enable them to be applied using polymerase chain reaction (PCR) against both the B- and T-cell receptors in DNA extracted from FFPE material (Deane and Norton 1990).

The REAL classification recognized the importance of genetic abnormalities in defining disease entities. However, it has become clear that a purely genetic approach to defining disease is neither feasible nor appropriate. For example, although the *MYC* translocation is universally present in BLs, *MYC* involving the immunoglobulin genes are found as either secondary or less commonly as primary genetic abnormalities in other lymphoid malignancies, such as diffuse large B-cell lymphoma (DLBCL), plasmablastic malignancies, and some cases of B-lymphoblastic lymphoma/leukemia (Jaffe and Pittaluga 2011). Similarly, BCL-2/IGH@ is found in only 85–90 % of FLs and is present in up to 25–30 % of de novo DLBCLs with no prior evidence of FL (Tomita 2011).

Finally, the inclusion of *clinical* criteria was one of the novel aspects of the ILSG approach to lymphoma classification. The REAL classification distinguished for the first time between nodal and extranodal forms of lymphoma and their differences (Table 1.5). As a consequence, the site of involvement had clearly to be stated in the diagnostic reports. The REAL classification recognized that the site of presentation is often a signpost for biological distinctions, such as in extranodal marginal zone B-cell lymphomas arising in mucosa-associated lymphoid tissue (MALT) (Isaacson and Du 2004), primary mediastinal large B-cell lymphomas (Rodriguez et al. 1994), and many types of T/NK-cell lymphomas (Jaffe et al. 1999). The ILSG appreciated that accurate diagnosis requires knowledge of the clinical history, including blood profiles, because biologically distinct entities may appear cytologically similar. Integration of the clinical features is an essential aspect in the definition of disease entities and in accurate diagnoses in daily practice. The pathologist must be provided with relevant clinical details on receipt of the specimen to arrive at the correct diagnosis. Ideally, clinicopathological conferences (or "tumor boards") should occur at least weekly, in order to arrive at a final integrated report, particularly in difficult cases, where clinical and pathological diagnoses may be discordant.

It is also evident that clinical features are important prognostic indicators, and in many instances the treatment approach chosen is based on the clinical setting in conjunction with the pathologic diagnosis. For instance, some patients with FL can be followed with a "watch-and-wait" approach (e.g., conjunctival lymphomas, stage IE, removed by complete surgical excision), whereas in others a large and extensive tumor burden at diagnosis obviously dictates immediate therapy. Response to therapy is influenced not only by underlying clinical features but also by biologic and prognostic factors. Cytological grade varies in many disease entities, e.g., FL, with grades 1–3 being given according to the number of centroblasts present per 10 high-power fields. Other prognostic factors are based on tumor cell biology, such as ZAP-70 expression in CLL (Pekarsky et al. 2010), or host factors, such as the tumor microenvironment (Coupland 2011). For this reason it is not possible to stratify lymphoma subtypes in a "pigeon-hole"-like manner according to their aggressiveness.

## Conclusions

Disease definitions are not static and new disease entities or variants continue to be recognized. For example, recent studies have drawn attention to the biologic overlap between CHL and DLBCL. Similarly, there is a greater appreciation of the borders between BL and DLBCL. Strategies for the management of these borderline lesions are proposed. Additionally, age-specific and site-specific factors play an important role in the definition of new entities, which also have biologic underpinnings. The 2001 (third edition) WHO classification was rapidly adopted for clinical trials and successfully served as a common

language for scientists comparing genetic and functional data. The modifications made in the 2008 (fourth edition) WHO lymphoma classification are the result of this successful partnership among pathologists, clinicians, and biologists, but they provide only a stepping-stone to the future. From a practical point, training of oncologists, hemato-oncologists, general pathologists, and specialized ocular pathologists in the principles and practical application of current and future classifications is crucial to ensure broad acceptance of the classification and to facilitate communication.

## References


Arnold A, Cossman J, Bakhshi A, Jaffe ES, Waldmann TA, Korsmeyer SJ (1983) Immunoglobulin-gene rearrangements as unique clonal markers in human lymphoid neoplasms. N Engl J Med 309(26):1593–1599

Brill NE, Baehr G, Rosenthal N (1925) Generalized giant lymph follicle hyperplasia of lymph nodes and spleen: a hitherto undescribed type. J Am Med Assoc 84:668–671

Chelloul N, Diebold J, Flandrin G, Gerard-Marchant R, Nezelof C (1976) [Comments on the WHO Reference Center classification of non-Hodgkin hematosarcomas]. Ann Anat Pathol (Paris) 21(3):301–304

Coupland SE (2011) The challenge of the microenvironment in B-cell lymphomas. Histopathology 58(1):69–80

Dalla-Favera R, Bregni M, Erikson J, Patterson D, Gallo RC, Croce CM (1982) Human c-myc onc gene is located on the region of chromosome 8 that is translocated in Burkitt lymphoma cells. Proc Natl Acad Sci U S A 79(24):7824–7827

Dawson PJ (1999) The original illustrations of Hodgkin's disease. Ann Diagn Pathol 3:386–393

Deane M, Norton JD (1990) Detection of immunoglobulin gene rearrangement in B lymphoid malignancies by polymerase chain reaction gene amplification. Br J Haematol 74(3):251–256

Gall EA, Mallory TB (1942) Malignant lymphoma: a clinico-pathologic survey of 618 cases. Am J Pathol 18:381–429

Harris NL, Jaffe ES, Stein H et al (1994) A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. Blood 84(5):1361–1392

Higby DJ (1979) A practical classification of lymphomas. N Engl J Med 300(22):1283

Hodgkin T (1832) On some morbid experiences of the absorbent glands and spleen. Med Chir Trans 17:68–97

Institute NC (1982) Sponsored study of classifications of non-Hodgkin's lymphomas: summary and description of a working formulation for clinical usage. The Non-Hodgkin's Lymphoma Pathologic Classification Project. Cancer 49(10):2112–2135

Isaacson PG, Du MQ (2004) MALT lymphoma: from morphology to molecules. Nat Rev Cancer 4(8):644–653

Jackson H, Parker F (1944) Hodgkin's disease, II: pathology. N Engl J Med 231:35–44

Jaffe ES, Pittaluga S (2011) Aggressive B-cell lymphomas: a review of new and old entities in the WHO classification. Hematology Am Soc Hematol Educ Program 2011:506–514

Jaffe ES, Harris NL, Diebold J, Müller-Hermelink HK (1998) World Health Organization classification of lymphomas: a work in progress. Ann Oncol 9(Suppl 5):S25–S30. Review

Jaffe ES, Krenacs L, Kumar S, Kingma DW, Raffeld M (1999) Extranodal peripheral T-cell and NK-cell neoplasms. Am J Clin Pathol 111(1 Suppl 1): S46–S55

Kay HE (1974) Letter: classification of non-Hodgkin's lymphomas. Lancet 2(7880):586

Kohler G, Milstein C (1975) Continuous cultures of fused cells secreting antibody of predefined specificity. Nature 256(5517):495–497

Lennert K (1975) Morphology and classification of malignant lymphomas and so-called reticuloses. Acta Neuropathol Suppl Suppl 6:1–16

Lennert K, Feller A (1992) Histopathology of non-Hodgkin's lymphomas, 2nd edn. Springer-Verlag, New York

Lennert K, Stein H, Kaiserling E (1975) Cytological and functional criteria for the classification of malignant lymphomata. Br J Cancer 2:29–43

Lukes RJ, Collins RD (1974) Immunologic characterization of human malignant lymphomas. Cancer 34(4 Suppl):suppl:1488–1503

Lukes RJ, Collins RD (1975) New approaches to the classification of the lymphomata. Br J Cancer Suppl 2:1–28

Mason DY, Cordell JL, Abdulaziz Z, Naiem M, Bordenave G (1982) Preparation of peroxidase: antiperoxidase (PAP) complexes for immunohistological labeling of monoclonal antibodies. J Histochem Cytochem 30:1114–1122

McMichael AJ, Pilch JR, Galfre G, Mason DY, Fabre JW, Milstein C (1979) A human thymocyte antigen defined by a hybrid myeloma monoclonal antibody. Eur J Immunol 9(3):205–210

Pekarsky Y, Zanesi N, Croce CM (2010) Molecular basis of CLL. Semin Cancer Biol 20(6):370–376

Project TN-HsLC (1997) A clinical evaluation of the International Lymphoma Study Group classification of non-Hodgkin's lymphoma. Blood 89:3909–3918

Rappaport H (1966) Tumors of the hematopoietic system, vol Fasc. 8. Armed Forces Institute of Pathology, Washington, DC

Rappaport H, Winter W, Hicks E (1956) Follicular lymphoma: a re-evaluation of its position in the scheme of malignant lymphoma, based on a survey of 253 cases. Cancer 9:792–821

Rodriguez J, Pugh WC, Romaguera JE, Cabanillas F (1994) Primary mediastinal large cell lymphoma. Hematol Oncol 12(4):175–184

Sheehan WW, Rappaport H (1970) Morphological criteria in the classification of the malignant lymphomas. Proc Natl Cancer Conf 6:59–71

Stansfeld AG, Diebold J, Noel H et al (1988) Updated Kiel classification for lymphomas. Lancet 1(8580):292–293

Swerdlow SH, Campo E, Harris NL et al (2008) WHO classification of tumours of haematopoietic and lymphoid tissues, 4th edn. IARC Press, Lyon

Symmers D (1927) Follicular lymphadenopathy with splenomegaly: a newly recognized disease of the lymphatic system. Arch Pathol 3:816–820

Symmers D (1938) Giant follicular lymphadenopathy with or without splenomegaly: its transformation into polymorphous cell sarcoma of the lymph follicles and its association with Hodgkin's disease, lymphatic leukemia and an apparently unique disease of the lymph nodes and spleen—a disease entity believed heretofore undescribed. Arch Pathol 26: 603–647

Tartari CJ, Scapozza L, Gambacorti-Passerini C (2011) The ALK gene, an attractive target for inhibitor development. Curr Top Med Chem 11(11):1406–1419

Taub R, Kirsch I, Morton C et al (1982) Translocation of the c-myc gene into the immunoglobulin heavy chain locus in human Burkitt lymphoma and murine plasmacytoma cells. Proc Natl Acad Sci U S A 79(24): 7837–7841

Taylor CR, Mason DY (1974) The immunohistological detection of intracellular immunoglobulin in formalin-paraffin sections from multiple myeloma and related conditions using the immunoperoxidase technique. Clin Exp Immunol 18:417–429

Tomita N (2011) BCL2 and MYC dual-hit lymphoma/leukemia. J Clin Exp Hematop 51(1):7–12

Trumper LH, Brittinger G, Diehl V, Harris NL (2004) Non-Hodgkin's lymphoma: a history of classification and clinical observations. Lippincott, Williams and Wilkins, Philadelphia

Tsujimoto Y, Yunis J, Onorato-Showe L, Erikson J, Nowell PC, Croce CM (1984) Molecular cloning of the chromosomal breakpoint of B-cell lymphomas and leukemias with the t(11;14) chromosome translocation. Science 224(4656):1403–1406

Tsujimoto Y, Gorham J, Cossman J, Jaffe E, Croce CM (1985) The t(14;18) chromosome translocations involved in B-cell neoplasms result from mistakes in VDJ joining. Science 229(4720):1390–1393

Yunis JJ, Oken MM, Kaplan ME, Ensrud KM, Howe RR, Theologides A (1982) Distinctive chromosomal abnormalities in histologic subtypes of non-Hodgkin's lymphoma. N Engl J Med 307(20):1231–1236

Zech L, Haglund U, Nilsson K, Klein G (1976) Characteristic chromosomal abnormalities in biopsies and lymphoid-cell lines from patients with Burkitt and non-Burkitt lymphomas. Int J Cancer 17(1):47–56

## Suggested Reading

Amon U, Hartmann K, Horny HP, Nowak A (2010) Mastocytosis – an update. J Dtsch Dermatol Ges 8(9):695–711, quiz 712

Czader M, Orazi A (2012) World Health Organization classification of myelodysplastic syndromes. Curr Pharm Des 18(22):3149–3162

Kairouz S, Hashash J, Kabbara W, McHayleh W, Tabbara IA (2007) Dendritic cell neoplasms: an overview. Am J Hematol 82(10):924–928

Mathe G, Belpomme D, Dantchev D (1976) [Non-Hodgkin hematosarcomas. Classification of the Reference Center of the World Health Organization (WHO)]. Ann Anat Pathol (Paris) 21(3):285–300

Vardiman J, Hyjek E (2011) World Health Organization classification, evaluation, and genetics of the myeloproliferative neoplasm variants. Hematology Am Soc Hematol Educ Program 2011:250–256

# Ocular and Adnexal Lymphoma: Histopathology

2

Sarah E. Coupland

## 2.1 Introduction

*Lymphomas of the ocular adnexa* (i.e., the orbit, eyelids, conjunctiva, lacrimal gland, and lacrimal sac) are relatively uncommon, accounting for approximately 8 % of all extranodal malignant lymphomas (Freeman et al. 1972). Most are primary tumors and are usually NHL of B-cell type: The most common primary lymphoma subtype occurring in the ocular adnexa is the low-grade malignant extranodal marginal zone B-cell lymphoma (EMZL), accounting for approximately two-thirds of all ocular adnexal lymphomas (White et al. 1995; Coupland et al. 1998; Cho et al. 2003; Ferry et al. 2007). They are often termed "MALT" lymphomas when involving an overlying epithelium – e.g., the conjunctiva or acini of the lacrimal gland.

In the following chapter, the histological and immunophenotypical features of the *intraocular lymphomas* and *ocular adnexal lymphomas* will be reviewed. The molecular genetic features of each lymphoma subtype will be addressed in the next chapter and, however, will also be briefly summarized in an associated table.

S.E. Coupland
Department of Molecular and Clinical Cancer Medicine, University of Liverpool, 6th Floor Duncan Building, Daulby Street, Liverpool L69 3GA, UK
e-mail: s.e.coupland@liverpool.ac.uk

## 2.2 Intraocular Lymphoma

### 2.2.1 Vitreoretinal Lymphoma

As mentioned above, VRL is a lymphoma of high-grade malignancy, which is often associated with cerebral disease. The central nervous system (CNS) lymphoma may occur prior to, concurrently, or subsequent to the ocular disease. The clinical features and treatment of VRL will not be discussed here: The reader is referred to the accompanying chapters in this book, as well as to other recent reviews (Davis 2013; Chan et al. 2010).

Histologically, VRL can be subtyped in most cases as a diffuse large B-cell lymphoma (DLBCL) (Coupland 2013) according to the latest WHO lymphoma classification (Swerdlow et al. 2008). Rare subtypes include T-cell-rich B-cell lymphoma and T-cell lymphoma (Cummings et al. 2005). VRL are characterized by a subretinal or perivascular retinal infiltration of pleomorphic medium- to large-sized cells with minimal basophilic cytoplasm, indented or folded nuclei, and prominent, often multiple, nucleoli (Fig. 2.1a). Atypical mitotic figures can be seen. Necrosis and apoptosis, with background scavenging macrophages, are frequent characteristics of these tumors, making the diagnosis of lymphoma more difficult in some cases (Fig. 2.1b) (Coupland 2012; Mudhar and Sheard 2013).

Immunohistochemically, VRL are characterized by the following expression profile: positivity for B-cell antigens (CD79a, CD20,

A.D. Singh (ed.), *Ocular and Adnexal Lymphoma*, Essentials in Ophthalmology,
DOI 10.1007/978-3-642-38499-8_2, © Springer-Verlag Berlin Heidelberg 2014

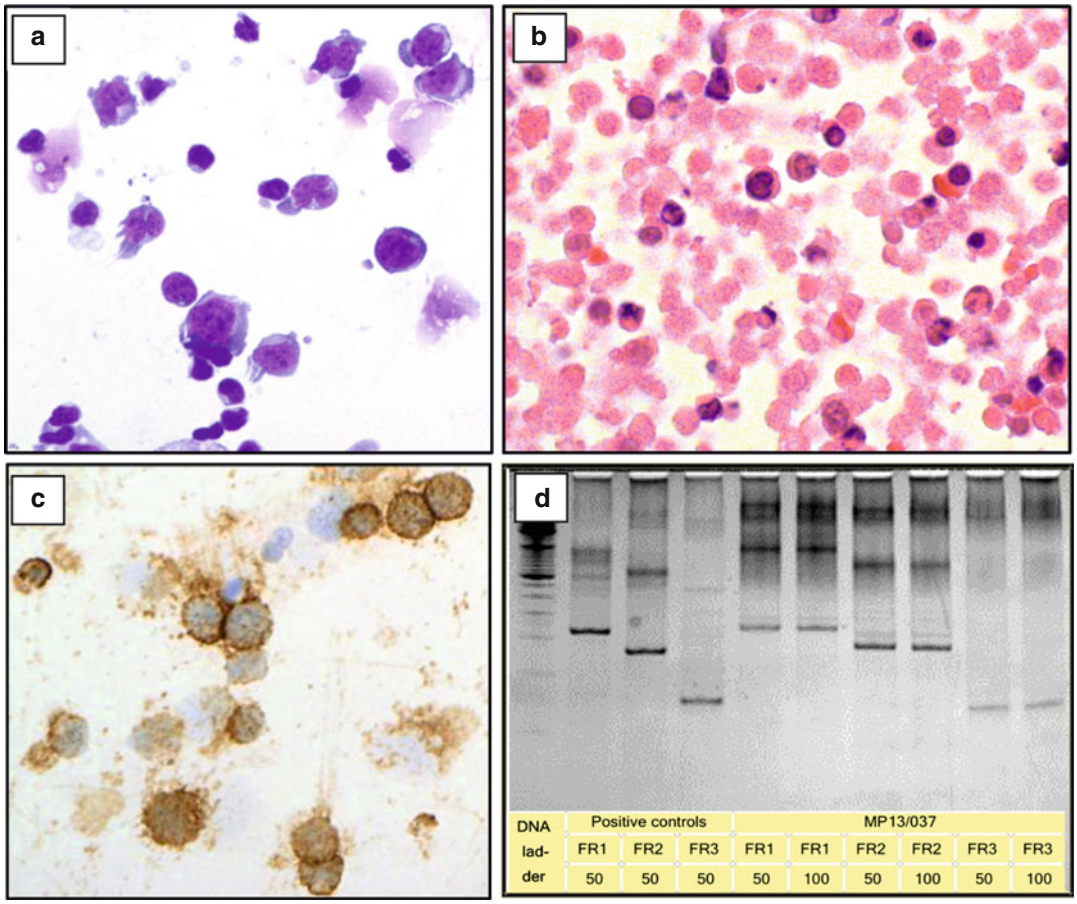


**Fig. 2.1** (**a**) Cytology spin of a vitreoretinal lymphoma (VRL) demonstrating large pleomorphic cells with bizarre sometimes clover leaf-like nuclei with prominent multiple nucleoli, consistent with a diffuse large B-cell lymphoma. (**b**) Histological section of a near-to-completely necrotic vitreoretinal lymphoma with ghost lytic tumor cells in the background. (**c**) Membranous positivity of the neoplastic B-cells for the B-cell antigen, CD20. (**d**) A case of VRL with clear monoclonality using BioMed2 primers for framework region (FR) FR1, FR2, and FR3 of the variable region of the immunoglobulin heavy chain gene (lanes 4 and 5, 6 and 7, 8 and 9, respectively)

PAX5) (Fig. 2.1c) as well as for BCL-2, MUM1/IRF4, OCT2, BOB.1, BCL-6+/−, CD10−/+, and Pu.1−/+. They are usually monotypical for IgM (Coupland and Damato 2008). Staining with Ki-67 shows that the tumor cell growth fraction is very high (i.e., about 80–90 %). Clonality assessment can also be performed on DNA extracted from VRL using polymerase chain reaction (PCR) directed against the immunoglobulin heavy and light chains in B-cell lymphomas (Fig. 2.1d) and against the T-cell receptor in the case of rare T-cell lymphomas (Coupland and Damato 2008).

With regard to molecular genetic alterations in VRL, it has been demonstrated that the tumor cells carry an intermediate to large number of hypermutated immunoglobulin variable region (IgV) genes with no convincing evidence of antigen selection or significant intraclonal heterogeneity (Coupland et al. 2005; Malumbres et al. 2007). Interestingly, a similar high mutation IgV frequency has been reported in CNSL (Montesinos-Rongen et al. 1999; Pels et al. 2005). The findings of a large somatic mutation load in the IgV genes of VRL, together with the tumor cell immunophenotype (MUM1/IRF4+,

BCL-6+/−, CD10), suggest that it is a DLBCL of activated B-cell-like (ABC) subtype (Alizadeh et al. 2000), i.e., they are derived from mature B-cells, which have undergone a prolonged interaction in the microenvironment of the GC and are either at the late GC stage of differentiation or are early post-GC B-cells (Coupland et al. 2005; Malumbres et al. 2007).

To date, the only reported chromosomal translocation in VRL is t(14;18), which involves the *bcl-2* gene, with rearrangements being reported in up to 67 % of cases (Chan 2003b). The presence of this mutation and consequent overexpression of the BCL-2 protein in some VRL could, therefore, suggest that some VRL are "germinal center B-cell-like" (GCB) DLBCL, which are proposed to be derived from centroblasts (Alizadeh et al. 2000). Further studies, particularly gene expression profiling (GEP), however are needed to confirm whether there are indeed differing molecular genetic subtypes of VRL, which may reflect the clinical behavior.

### 2.2.2 Uveal Lymphoma

Lymphoid proliferations of the uvea can be divided into two main groups: (a) primary uveal tumors and (b) secondary intraocular manifestations of systemic lymphoma (Coupland and Damato 2008). Although the exact frequency of the latter is unknown, they are not particularly unusual, especially in leukemia where intraocular (mainly choroidal) involvement may occur in as many as 80–90 % of patients during the late course of the disease (Augsburger and Greatrex 1989). Primary uveal lymphoma is rare and can be further divided according to location: i.e., primary choroidal lymphoma, primary ciliary body lymphoma, and primary iridal lymphoma, with the former being the most common.

#### 2.2.2.1 Primary Choroidal Lymphoma

Primary choroidal lymphoma was first described in 1920 (Treibenstein 1920) and occurs as a unilateral tumor in the absence of systemic disease at diagnosis (Fig. 2.2a). Due to the usual low-grade nature of these tumors, they were erroneously termed "reactive lymphoid hyperplasia" (Ryan et al. 1972) or "uveal pseudotumors" previously (Gass 1967; Ryan et al. 1971). Subsequent investigations, using more advanced diagnostic methods, such as improved immunohistology following antigen retrieval and a wider panel of antibodies, and clonality analysis using PCR have provided evidence that the majority of these tumors represent low-grade B-cell lymphoma (Ben-Ezra et al. 1989; Cockerham et al. 2000; Coupland et al. 2002; Grossniklaus et al. 1998). Most primary choroidal lymphomas can be subtyped as "extranodal marginal zone B-cell lymphomas" (EMZL) of MALT type, according to the latest WHO lymphoma classification since these tumors demonstrate clinical, morphological, immunophenotypical, and genetic features similar to EMZL in other locations (Coupland et al. 2002).

In most cases of primary uveal EMZL reported, the eyes were ultimately enucleated either due to difficulties in determining the nature of the uveal mass clinically or to pain as a result of secondary glaucoma. Some authors performed biopsies of the episcleral tumor nodules (Holz et al. 1999) and either aspirates or biopsies of the choroidal swelling (Cheung et al. 1994), in order to establish a definitive diagnosis of lymphoma.

In cytological specimens, the cells of primary choroidal EMZL are small, with a relatively monomorphous appearance and with a narrow cytoplasmic rim and indistinct nucleoli. Histological biopsies of primary choroidal EMZL demonstrate an expansion of centrocyte-like, monocytoid and plasmacytoid tumor cells with occasional blasts in the marginal zone surrounding reactive follicles, which are frequently present in larger biopsies. The degree of plasmacellular differentiation can be large with numerous tumor cells possessing intranuclear collections of immunoglobulin (Dutcher bodies) (Fig. 2.2b). Consistent with the indolent nature of these tumors, there are usually only a few mitoses. When arising in tissues associated with mucosa or epithelium, nests of EMZL tumor cells may infiltrate neighboring structures forming "lymphoepithelial lesions." (Coupland and Damato 2008) The malignant nature of the tumor

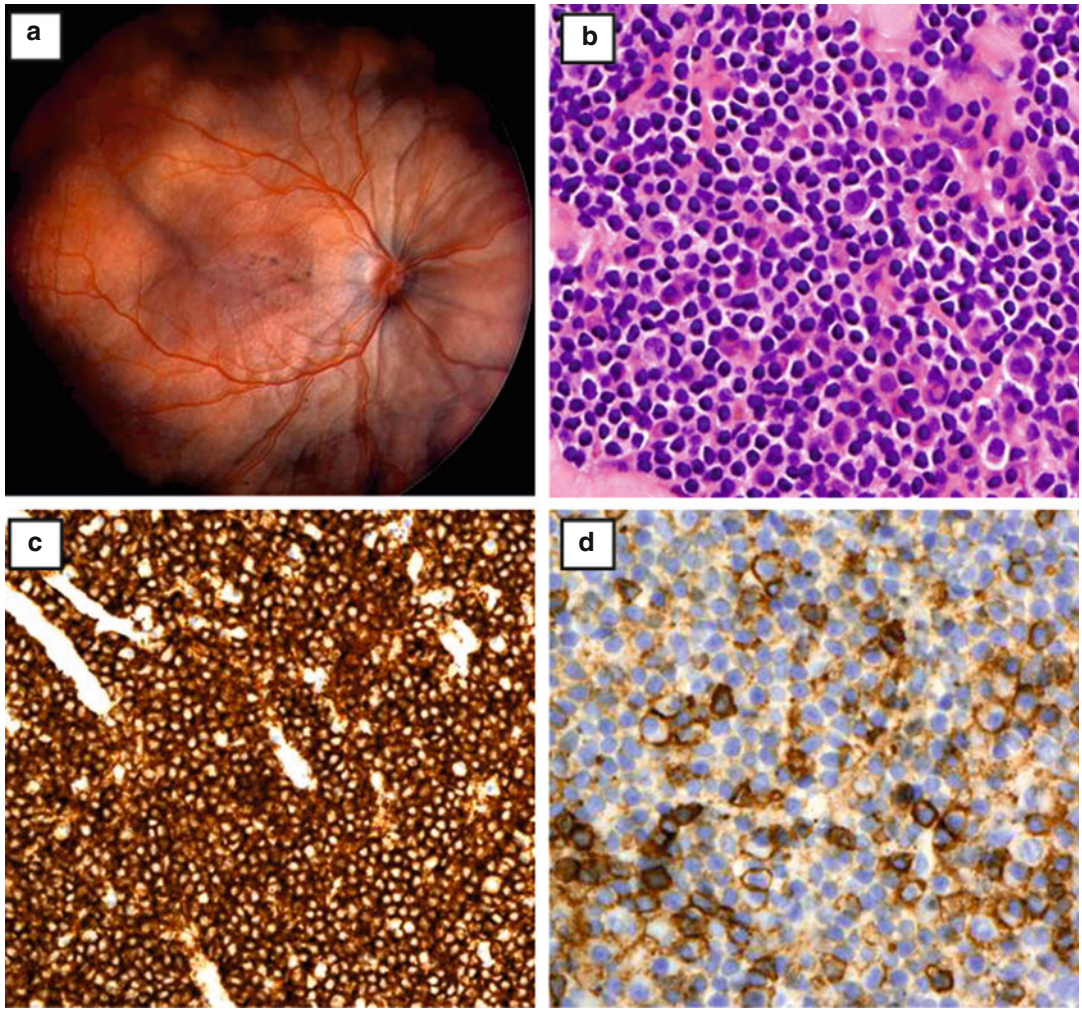


**Fig. 2.2** (**a**) Fundoscopy of a patient with choroidal lymphoma (courtesy of Prof. Bertil Damato). (**b**) Histological section of an intraocular biopsy taken demonstrates that the lymphomatous infiltrate is composed of small centrocyte-like cells with some plasmacellular differentiation and with very rare mitoses, consistent with an extranodal marginal zone B-cell lymphoma. (**c**). CD20 positivity of the tumor cells. (**d**) Membranous staining of some of the neoplastic B-cells also for CD138, confirming their plasmacellular differentiation

can be supported morphologically by the "uveal equivalent" of lymphoepithelial lesions (i.e., tumor cells infiltrating Bruch's membrane and the retinal epithelium) and the presence of extrascleral extension (Coupland and Damato 2008).

Immunohistochemical studies demonstrate a dominance of B-lymphocytes positive for CD79a, CD20, and PAX5 (Fig. 2.2c). The tumor cells can display an aberrant expression of T-cell antigens, e.g., CD43 (Coupland and Damato 2008) as well as monotypical expression of an immunoglobulin light and/or heavy chain (Fig. 2.2d). Primary choroidal lymphoma cells are usually negative for CD23, CD5, CD10, BCL-6, and cyclin D1, excluding therefore a secondary choroidal infiltration by other small cell B-cell lymphoproliferative disorders, such as B-cell lymphocytic leukemia (CD5+, CD23+, CD10−, BCL-6−, cyclin D1−), mantle cell lymphoma (CD5+, CD23−, CD10−, BCL-6−, cyclin D1+), and follicular lymphoma (CD5−, CD23−, CD10+, BCL-6+, cyclin D1−) (see Table 2.1). The Ki-67 stain is usually low (about 5–15 %) in choroidal EMZL, reflecting their indolent nature. Little is known about the chromosomal translocations in choroidal EMZL (*cf.* Sect. 2.3 – see below).

**Table 2.1** Morphological, immunophenotypic, and molecular characteristics of the four ocular lymphoma subtypes presented

| Lymphoma subtype | Morphology | Tumor cell immune profile | Molecular biological changes[a] | Cell of origin |
|---|---|---|---|---|
| EMZL lymphoma | Expansive growth in the marginal zone<br>Heterogeneous cell population: centrocyte-like cells, monocytoid B-cells, plasmacytoid cells, occasional blasts<br>Possibly "lymphoepithelial lesions"<br>Often multifocal growth | CD79a+, CD20+, CD43+/−, BCL-2+/−<br>IgM+, IgD-<br>CD10-, CD23-, CD5-, cyclin D1-<br>Presence of FDC's in reactive secondary follicles<br>Monotypic cytoplasmic Ig in 10 % | Clonal IgH and IgL rearrangements<br>Mutations in V-region of IgHgene<br>t(11;18)(q21;q21)in 15–40 %<br>t(14;18)(q32;q21) in 10 %<br>t(1;14)(p22;q32) in 5 %<br>t(3;14)(p14.1;q32)<br>Trisomy 3, trisomy 18 | "Memory" B-cell |
| DLBCL | Diffuse growth pattern<br>Morphological variants: centroblastic, immunoblastic, centroimmunoblastic, anaplastic, T-cell rich | CD79a+, CD20+<br>BCL-6+ (ca. 70 % of cases)<br>CD10+ (ca. 25–50 %)<br>IgM>IgG>IgA in 50–75 % of cases<br>CD30+ in lymphoma with anaplastic morphology<br>Rarely CD5+ or CD23+<br>No FDC-MW<br>Ki-67 nearly always >50 % | Clonal IgH and IgL rearrangements[b]<br>Numerous mutations in IgV<br>*Bcl-6* gene rearrangements in <40 %; *Bcl-2* gene rearrangements in 20 %–30 %<br>*C-myc* gene rearrangements extremely rare<br>REL gene amplification in 20 % mainly extranodal lymphoma | Dependent on whether GCB-like or ABC profile |
| FL | Usually follicular growth pattern with occasional diffuse areas; rarely purely diffuse<br>Mixture of centrocytes and centroblasts with dominance of former<br>Monomorphic GCs with loss of zonation<br>Minimal or no apoptosis in GC<br>Usually no macrophages with tingible bodies<br>Thin or even absence of the follicle mantle | CD20+, CD10+, BCL-2+ (90 %), BCL-6+, IgM (50 %), IgG (50 %)<br>CD43− (95 %), CD23−, CD5−<br>Dense follicular FDC-MW<br>Reduction in growth fraction in neoplastic GCs versus reactive GCs, particularly in BCL-2+ cases<br>Often CD10+ B-cells in the interfollicular region<br>Dense well-defined FDC meshworks in neoplastic germinal centers (demonstrated with CD21) | Clonal IgH and IgL rearrangements[b]<br>Numerous mutations in V-region of IgH gene with "ongoing" mutations (intraclonal diversity)<br>t(14;18)(q32;q21) in 70–95 %, resulting in the expression of BCL-2 in neoplastic germinal centers<br>t(2;18)(p12;q21) – rare<br>p53 gene mutations and *c-myc*-rearrangement in high-grade cases | Germinal center B-cell |

(continued)

**Table 2.1** (continued)

| Lymphoma subtype | Morphology | Tumor cell immune profile | Molecular biological changes[a] | Cell of origin |
|---|---|---|---|---|
| MCL | Diffuse vaguely nodular pattern<br>Small- to medium-sized lymphoid cells with irregular nuclear contours, similar to centrocytes<br>Hyalinized small vessels present<br>Scattered epithelioid histiocytes present<br>Variants recognized including the blastoid and pleomorphic variants | CD20+, CD5+, cyclin D1+, SOX11+<br>"Moth-eaten" follicular FDC-MW<br>Often CD10+ B-cells in the interfollicular region<br>Dense well-defined FDC meshworks in neoplastic germinal centers (demonstrated with CD21) | Clonal IgH and IgL rearrangements[b]<br>t(11;14)(q13;q32) in almost all cases, resulting in CCND1 (cyclin D1) overexpression<br>p53 gene mutations and *c-myc* rearrangement in high grade | Peripheral B-cell of inner mantle zone, naive pre-germinal center type |

*Key*: *MALT* mucosa-associated lymphoid tissue lymphoma, *DLBCL* diffuse large cell B-cell lymphoma+, *FL* follicular lymphoma, *NHL* non-Hodgkin's lymphoma, *FDC-MW* follicular dendritic cell meshworks, *IgL* immunoglobulin light chain, *IgH* immunoglobulin heavy chain

[a]These results arise from investigations of NHL in other locations.

[b]Rearrangements demonstrable only in 50–70 % of cases due to presence of somatic mutations.

#### 2.2.2.2 Primary Iridal Lymphoma

Primary iridal lymphomas are very rare, with only a dozen cases having been reported in the literature (Cooper and Riker 1951; Raju and Green 1982; Goldey et al. 1989; Jensen et al. 1994; Coupland et al. 1999a; Velez et al. 2000; Lobo et al. 2003; Yahalom et al. 2002; Yamada et al. 2003). All described primary iridal lymphomas consisted of sheets of large atypical lymphoid cells with pleomorphic cytology and numerous mitoses and apoptotic bodies. On immunohistological examination, the tumor cells demonstrated a dominance of either a B- or T-cell population with aberrant immunophenotypes. The Ki-67 growth fraction of the neoplastic cells was large, approximately 70–90 %. The prognosis of primary iridal lymphoma varies: Most patients develop either systemic or cerebral involvement (Lobo et al. 2003). In general, those patients who developed a cerebral or visceral manifestation did most poorly, compared to those patients whose tumors disseminated to lymph nodes or the skin.

#### 2.2.2.3 Primary Ciliary Body Lymphoma

Primary ciliary body lymphomas are exceptionally rare, with only one case of a low-grade EMZL having been published to date (Coupland and Damato 2008). Two further cases of ciliary body EMZL seen in elderly patients at the Liverpool Ocular Oncology Centre have been submitted for publication. All cases presented as localized ciliary body masses with low acoustic reflectivity, which through histological analysis of the intraocular biopsies, could be distinguished from melanomas. External beam radiation therapy induced rapid and complete regression and a good outcome.

## 2.3 Ocular Adnexal Lymphomas

Ocular adnexal lymphomas constitute 8 % of all extranodal lymphomas and are the most common malignant tumors of the orbit (Freeman et al. 1972). The conjunctiva is the second most commonly affected location (Coupland et al. 1998; Ferry et al. 2007; Auw-Haedrich et al. 2001; Sjo 2009).

As mentioned above, the most common lymphoma subtype of the ocular adnexal region is the EMZL (60–66 %) followed by follicular lymphoma (FL) (10–15 %) and diffuse large B-cell lymphoma (DLBCL) (8–13 %) (Coupland et al. 1998; Ferry et al. 2007; Sjo 2009). Of the rarer subtypes is mantle cell lymphoma (MCL) (1–5 %), a small cell lymphoma with high-grade potential (Rasmussen et al. 2009). While EMZL and FL are associated with low morbidity and mortality, patients with ocular adnexal DLBCL and ML have poor prognoses. Even with new improved treatments, the 5-year overall survival is approximately 60 % for patients with DLBCL (Rasmussen et al. 2013) and as low as 40 % in MCL patients (Rasmussen et al. 2009).

### 2.3.1 Extranodal Marginal Zone B-cell Lymphoma (EMZL)

EMZL were first described in the stomach by Isaacson and Wright in 1984 (Isaacson and Wright 1984). They are often termed "MALT" lymphomas when involving an overlying epithelium – e.g., the gastric mucosa, conjunctiva, or acini of the lacrimal gland. This term is not appropriate, however, for those lesions located deep in the orbit, where no epithelium is present; in such lesions, the overarching "EMZL" should be applied.

EMZL typically arise in conditions of chronic antigenic stimulation, as evidenced by the association of *H. pylori*, *C. jejuni*, *B. burgdorferi*, and hepatitis C virus with EMZLs that arise in the stomach, small intestine, skin, and spleen, respectively (Du 2011). The significance of *C. psittaci* with respect to the EMZL of the ocular adnexa remains unclear: There appears to be substantial geographic variation in its association (Chanudet et al. 2006). There may be a relationship with autoantigens produced, for example, in autoimmune diseases and the development of ocular adnexal EMZL, as evidenced by somatic mutation analyses of these tumors, suggesting an antigen selection process (Coupland et al. 1999b).

The morphological features of EMZL have been described above in the section addressing the primary choroidal lymphomas (Table 2.1). In the conjunctiva the neoplastic B-cells may extend into the overlying conjunctival epithelium, creating "lymphoepithelial lesions" and a "Swiss cheese" appearance when viewing the conjunctival specimens in the pancytokeratin stain. Consistent with the indolent nature of these tumors, there are usually few mitoses, with an increased mitotic count only being seen in EMZL that may be transforming to high-grade lymphomas. The *immunoprofile* of ocular adnexal EMZL is summarized in Table 2.1.

EMZL are genetically characterized by several recurrent, but mutually exclusive, chromosome translocations (Table 2.1). To date, it has been shown that at least the oncogenic products of t(11;18)(q21;q21)/API2-MALT1, t(1;14) (p22;q32)/BCL10-IGH, t(14;18)(q32;q21)/IGH-MALT1, and t(3;14)(p13;q32)/FOXP1- IGH.1 are present in some EMZL. Both BCL10 and MALT1 are critical components linking the antigen receptor signaling to the canonical nuclear factor (NF)-κB activation pathway. Expression of BCL10, MALT1, or API2-MALT1 both in vitro and in vivo causes NF-κB activation (Du 2011). The above translocations occur frequently in EMZL of the stomach and lung but *rarely* in those of the ocular adnexa, salivary glands, and thyroid (Du 2011). By genomic profiling of "translocation negative" ocular adnexal EMZL, it was demonstrated that the A20 gene, an essential global NF-κB inhibitor, was found to be inactivated either by somatic deletion and/or mutation in ocular adnexal EMZL (Chanudet et al. 2010). The A20 deletion is most commonly heterozygous and is mutually exclusive from the above-described MALT1 and IGH translocations. Further, it was shown that the A20 mutation/deletion is significantly associated with an increased expression of NF-κB target genes. These findings appear to be of clinical relevance: Complete A20 inactivation is associated with poor lymphoma-free survival, and the patients with A20 mutation/deletion required significantly higher radiation dosages than those without the A20 abnormalities to achieve complete remission (Bi et al. 2012).

### 2.3.2 Follicular Lymphoma

In European studies, FL is the second most common occurring primary B-NHL in the ocular adnexa. FL is a malignant neoplasm of follicle center cells (centrocytes and centroblasts), which has at least a partially follicular pattern (Fig. 2.3a). Areas of diffuse or even pure diffuse growth patterns can occur (Table 2.1). These tumors are graded according to the proportion of centrocytes and centroblasts present, with Grade 1 being centroblasts <5 % (Fig. 2.3b), Grade 2 being centroblasts being between 5 and 15 %, and Grade 3 being centroblasts >15 %. Grade 3 can be further subdivided according the presence of centrocytes (3A) or their absence (3B) (Swerdlow et al. 2008). The immunophenotype of FL is summarized in Table 2.1, but importantly there is a positivity of the neoplastic germinal center cells for BCL-2 protein in most cases (Fig. 2.3c, d). This is as a result of the translocation t(14;18)(q32;q21), involving the rearrangement of the *BCL-2* gene (70–95 % of cases). Consequently, there is an absence of apoptosis in the neoplastic FL germinal centers and a loss of the so-called light and dark zones, which can be highlighted in the Ki-67 stain. The meshworks of follicular dendritic cells (FDCs) within the neoplastic germinal centers are expanded. The Ki-67 growth rate of the tumor cells varies according to the grade of the FL (Fig. 2.3e). Rare FL have a t(2;18)(p12;q21), which places the *BCL-2* gene adjacent to the light chain on chromosome 2. Most FL have additional breaks, most commonly involving chromosomes 1, 2, 4, 5, 13, and 17, or additions of X, 7, 12, or 18 (Table 2.1) (Swerdlow et al. 2008).

### 2.3.3 Diffuse Large B-cell Lymphoma

Ocular adnexal DLBCL are histomorphologically characterized by diffuse infiltrates of B-lymphocytes of medium to large size with conspicuous nucleoli and basophilic cytoplasm as well as numerous mitoses including atypical mitotic figures (Fig. 2.4a). The aggressiveness

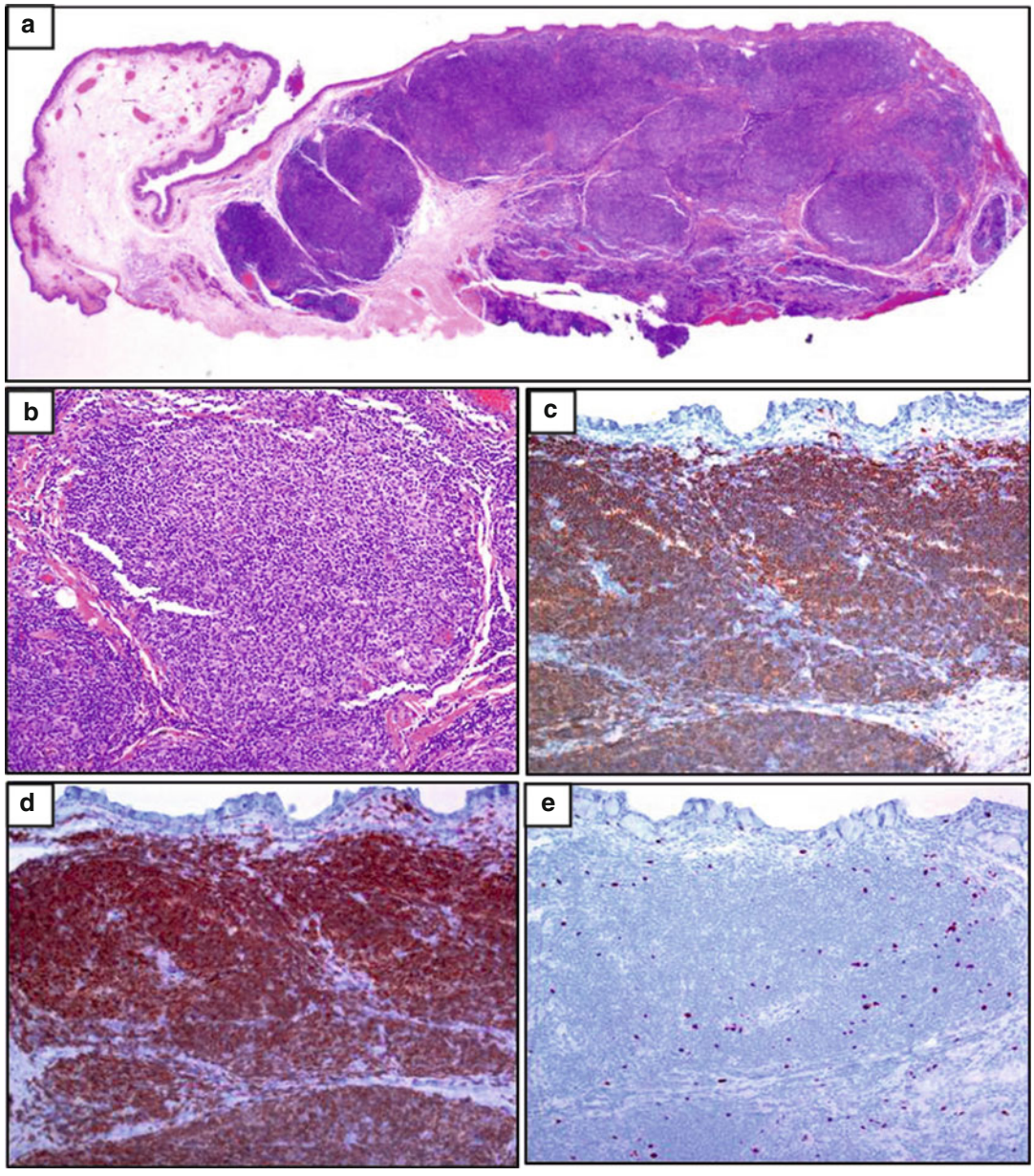


**Fig. 2.3** (**a**) Low-power magnification of a conjunctival biopsy with nodular lymphocytic infiltrates. (**b**) Higher power magnification demonstrates that the germinal centers are abnormal, lacking the usual zoning into the light and dark zones, as well as any evidence of apoptosis, consistent with a follicular lymphoma. (**c**) The CD79a immunostain shows that there is a clear dominance of the neoplastic B-cell population. (**d**) The neoplastic germinal centers aberrantly express BCL-2 protein by the centrocytes and centroblasts, indicating that the translocation t(14:18) has taken place in these cells. (**e**) The Ki-67 growth fraction is low, consistent with a Grade I follicular lymphoma

of these tumors is reflected by their infiltration and destruction of surrounding tissues such as the lacrimal gland parenchyma and the bony walls of the orbital cavity. The immunophenotype of ocular adnexal DLBCL varies according to whether they are (i) GC B-cell-like (GCB) DLBCL, which are derived from centroblasts, (ii) or activated B-cell-like (ABC) DLBCL, which resembles features of plasmablastic B-cells committed to terminal B-cell differentiation

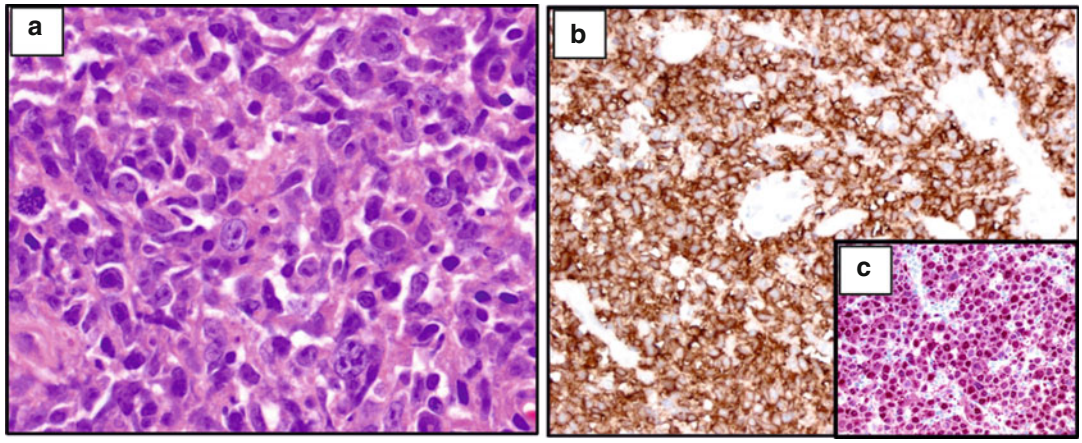


**Fig. 2.4** (**a**) High power magnification of an orbital high-grade lymphoma, comprised of medium-to-large pleomorphic cells with eccentrically placed prominent nuclei and scattered mitoses, consistent with a diffuse large B-cell lymphoma. (**b**) The neoplastic B-cells demonstrate clear positivity for CD20 and (**c**) co-express MUM1/IRF4

(Alizadeh et al. 2000). Essentially, however, most ocular adnexal DLBCL have the following immunophenotype, CD20+, BCL-2+, BCL-6+, CD10+/–, and MUM1+/–, and the Ki-67 growth fractions are high, varying between 50 and 80 % (Table 2.1) (Fig. 2.4b, c) (Coupland et al. 1998).

### 2.3.4 Mantle Cell Lymphoma

Ocular adnexal MCL possess a vaguely nodular growth pattern at lower power and consist of diffusely arranged small- to medium-sized cells with pale cytoplasm, inconspicuous nucleoli, and irregular or "cleaved" nuclei (Fig. 2.5a, b). Scattered epithelioid histiocytes are present (Fig. 2.5b). The meshwork of FDCs is prominent and disorganized ("moth-eaten appearance") as demonstrated by CD21 and CD23 stains. The tumor cells are characterized by positivity for CD45, CD20, CD5, cyclin D1 protein, and SOX11 (Fig. 2.5c, d), together with monoclonality for IgD/IgM and for lambda (more often than kappa). The Ki-67 growth fraction is usually about 20 % but can increase in the blastoid variant to above 35 % (Table 2.1) (Swerdlow et al. 2008; Auw-Haedrich et al. 2001; Rasmussen et al. 2009).

**Conclusions**

There has been a great deal of progress made in the understanding of the histogenesis of lymphoma in general. The WHO lymphoma classification has improved our ability to subtype the malignant lymphomas into particular entities, which are characterized by particular morphological, immunophenotypical, and molecular biological features. Further "fine-tuning" of our understanding of pathogenesis has been recently achieved with the application of the new technologies. A large amount remains to be learned about the pathogenesis of the lymphomas affecting ocular and ocular adnexal tissues: The new methodologies must be applied in these tumors in multicenter studies with the hope of optimizing patient treatment.

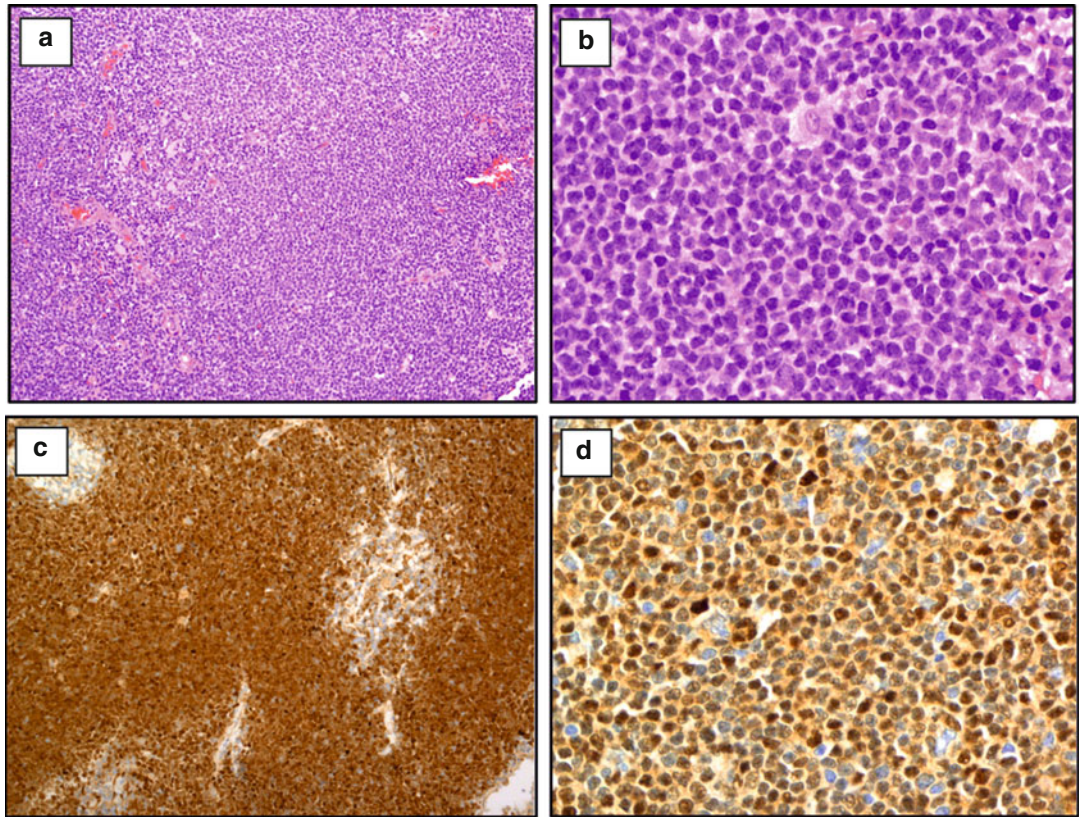


**Fig. 2.5** (**a**) A conjunctival mantle cell lymphoma shows a vaguely nodular pattern at low power. (**b**) It is comprised of small- to medium-sized cells with "coffee bean"-like shape and scattered in the background are larger so-called epithelioid histiocytes with pale pink cytoplasm. (**c**) Mantle cell lymphomas are characteristic in their immune profile, i.e., CD5+, cyclinD1+ (illustrated in c), SOX-11+ (in **d**), and monotypical for IgD, in the absence of CD23 and CD10 (in most cases)

## References

Alizadeh AA, Eisen MB, Davis RE et al (2000) Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature 403(6769):503–511

Augsburger JJ, Greatrex KV (1989) Intraocular lymphoma: clinical presentations, differential diagnosis and treatment. Trans Pa Acad Ophthalmol Otolaryngol 41:796–808

Auw-Haedrich C, Coupland SE, Kapp A, Schmitt-Graff A, Buchen R, Witschel H (2001) Long term outcome of ocular adnexal lymphoma subtyped according to the REAL classification. Revised European and American Lymphoma. Br J Ophthalmol 85(1):63–69

Ben-Ezra D, Sahel JA, Harris NL, Hemo I, Albert DM (1989) Uveal lymphoid infiltrates: immunohistochemical evidence for a lymphoid neoplasia. Br J Ophthalmol 73(10):846–851

Bi Y, Zeng N, Chanudet E et al (2012) A20 inactivation in ocular adnexal MALT lymphoma. Haematologica 97(6):926–930

Chan CC (2003b) Molecular pathology of primary intraocular lymphoma. Trans Am Ophthalmol Soc 101:275–292

Chan CC, Rubenstein JL, Coupland SE et al (2010) Primary vitreoretinal lymphoma: a report from an International Primary Central Nervous System Lymphoma Collaborative Group symposium. Oncologist 16(11):1589–1599

Chanudet E, Zhou Y, Bacon CM et al (2006) Chlamydia psittaci is variably associated with ocular adnexal MALT lymphoma in different geographical regions. J Pathol 209(3):344–351

Chanudet E, Huang Y, Ichimura K et al (2010) A20 is targeted by promoter methylation, deletion and inactivating mutation in MALT lymphoma. Leukemia 24(2):483–487

Cheung MK, Martin DF, Chan CC, Callanan DG, Cowan CL, Nussenblatt RB (1994) Diagnosis of reactive lymphoid hyperplasia by chorioretinal biopsy. Am J Ophthalmol 118(4):457–462

Cho EY, Han JJ, Ree HJ et al (2003) Clinicopathologic analysis of ocular adnexal lymphomas: extranodal marginal zone b-cell lymphoma constitutes the vast majority of ocular lymphomas among Koreans and affects younger patients. Am J Hematol 73(2):87–96

Cockerham GC, Hidayat AA, Bijwaard KE, Sheng ZM (2000) Re-evaluation of "reactive lymphoid hyperplasia of the uvea": an immunohistochemical and molecular analysis of 10 cases. Ophthalmology 107(1):151–158

Cooper EL, Riker JL (1951) Malignant lymphoma of the uveal tract. Am J Ophthalmol 34(8):1153–1158

Coupland SE (2012) Vitreous biopsy: specimen preparation and interpretation. Monogr Clin Cytol 21:61–71

Coupland SE (2013) Molecular pathology of lymphoma. Eye (Lond) 27(2):180–189

Coupland SE, Damato B (2008) Understanding intraocular lymphomas. Clin Experiment Ophthalmol 36(6): 564–578

Coupland SE, Krause L, Delecluse HJ et al (1998) Lymphoproliferative lesions of the ocular adnexa. Analysis of 112 cases. Ophthalmology 105(8): 1430–1441

Coupland SE, Foss HD, Assaf C et al (1999a) T-cell and T/natural killer-cell lymphomas involving ocular and ocular adnexal tissues: a clinicopathologic, immunohistochemical, and molecular study of seven cases. Ophthalmology 106(11):2109–2120

Coupland SE, Foss HD, Anagnostopoulos I, Hummel M, Stein H (1999b) Immunoglobulin VH gene expression among extranodal marginal zone B-cell lymphomas of the ocular adnexa. Invest Ophthalmol Vis Sci 40(3):555–562

Coupland SE, Foss HD, Hidayat AA, Cockerham GC, Hummel M, Stein H (2002) Extranodal marginal zone B cell lymphomas of the uvea: an analysis of 13 cases. J Pathol 197(3):333–340

Coupland SE, Hummel M, Muller HH, Stein H (2005) Molecular analysis of immunoglobulin genes in primary intraocular lymphoma. Invest Ophthalmol Vis Sci 46(10):3507–3514

Cummings TJ, Stenzel TT, Klintworth G, Jaffe GJ (2005) Primary intraocular T-cell-rich large B-cell lymphoma. Arch Pathol Lab Med 129(8):1050–1053

Davis JL (2013) Intraocular lymphoma: a clinical perspective. Eye (Lond) 27(2):153–162

Du MQ (2011) MALT lymphoma: many roads lead to nuclear factor-kappab activation. Histopathology 58(1): 26–38

Ferry JA, Fung CY, Zukerberg L et al (2007) Lymphoma of the ocular adnexa: a study of 353 cases. Am J Surg Pathol 31(2):170–184

Freeman C, Berg JW, Cutler SJ (1972) Occurrence and prognosis of extranodal lymphomas. Cancer 29: 252–260

Gass JD (1967) Retinal detachment and narrow-angle glaucoma secondary to inflammatory pseudotumor of the uveal tract. Am J Ophthalmol;64(3):Suppl:612–621

Goldey SH, Stern GA, Oblon DJ, Mendenhall NP, Smith LJ, Duque RE (1989) Immunophenotypic characterization of an unusual T-cell lymphoma presenting as anterior uveitis. A clinicopathologic case report. Arch Ophthalmol 107(9):1349–1353

Grossniklaus HE, Martin DF, Avery R et al (1998) Uveal lymphoid infiltration. Report of four cases and clinicopathologic review. Ophthalmology 105(7):1265–1273

Holz FG, Boehmer HV, Mechtersheimer G, Ott G, Volcker HE (1999) Uveal non-Hodgkin's lymphoma with epibulbar extension simulating choroidal effusion syndrome. Retina 19(4):343–346

Isaacson P, Wright DH (1984) Extranodal malignant lymphoma arising from mucosa-associated lymphoid tissue. Cancer 53(11):2515–2524

Jensen OA, Johansen S, Kiss K (1994) Intraocular T-cell lymphoma mimicking a ring melanoma. First manifestation of systemic disease. Report of a case and survey of the literature. Graefes Arch Clin Exp Ophthalmol 232(3):148–152

Lobo A, Larkin G, Clark BJ, Towler HM, Lightman S (2003) Pseudo-hypopyon as the presenting feature in B-cell and T-cell intraocular lymphoma. Clin Experiment Ophthalmol 31(2):155–158

Malumbres R, Davis J, Ruiz P, Lossos IS (2007) Somatically mutated immunoglobulin IGHV@ genes without intraclonal heterogeneity indicate a postgerminal centre origin of primary intraocular diffuse large B-cell lymphomas. Br J Haematol 138(6):749–755

Montesinos-Rongen M, Kuppers R, Schluter D et al (1999) Primary central nervous system lymphomas are derived from germinal-center B cells and show a preferential usage of the V4-34 gene segment. Am J Pathol 155(6):2077–2086

Mudhar HS, Sheard R (2013) Diagnostic cellular yield is superior with full pars plana vitrectomy compared with core vitreous biopsy. Eye (Lond) 27(1):50–55

Pels H, Montesinos-Rongen M, Schaller C et al (2005) VH gene analysis of primary CNS lymphomas. J Neurol Sci 228(2):143–147

Raju VK, Green WR (1982) Reticulum cell sarcoma of the uvea. Ann Ophthalmol 14(6):555–560

Rasmussen P, Sjo LD, Prause JU, Ralfkiaer E, Heegaard S (2009) Mantle cell lymphoma in the orbital and adnexal region. Br J Ophthalmol 93(8):1047–1051

Rasmussen PK, Ralfkiaer E, Prause JU et al (2013) Diffuse large B-cell lymphoma of the ocular adnexal region: a nation-based study. Acta Ophthalmol 91(2): 163–169. doi: 10.1111/j.1755-3768.2011.02337.x

Ryan SJ Jr, Frank RN, Green WR (1971) Bilateral inflammatory pseudotumors of the ciliary body. Am J Ophthalmol 72(3):586–591

Ryan SJ, Zimmerman LE, King FM (1972) Reactive lymphoid hyperplasia. An unusual form of intraocular pseudotumor. Trans Am Acad Ophthalmol Otolaryngol 76(3):652–671

Sjo LD (2009) Ophthalmic lymphoma: epidemiology and pathogenesis. Acta Ophthalmol;87 Thesis 1:1–20

Swerdlow SH, Campo E, Harris NL et al (2008) WHO classification of tumours of haematopoietic and lymphoid tissues, 4th edn. IARC Press, Lyon

Treibenstein O (1920) Ein Beitrag zur Frage der aleukaemischen Augenveraenderungen. Klin Monatsbl Augenheilkd 64:825–836

Velez G, de Smet MD, Whitcup SM, Robinson M, Nussenblatt RB, Chan CC (2000) Iris involvement in primary intraocular lymphoma: report of two cases and review of the literature. Surv Ophthalmol 44(6):518–526

White WL, Ferry JA, Harris NL, Grove AS Jr (1995) Ocular adnexal lymphoma. A clinicopathologic study with identification of lymphomas of mucosa-associated lymphoid tissue type. Ophthalmology 102(12): 1994–2006

Yahalom C, Cohen Y, Averbukh E, Anteby I, Amir G, Pe'er J (2002) Bilateral iridociliary T-cell lymphoma. Arch Ophthalmol 120(2):204–207

Yamada K, Hirata A, Kimura A, Tanihara H (2003) A case of primary B-cell type non-Hodgkin lymphoma originating in the iris. Am J Ophthalmol 136(2): 380–382

## Suggested Reading

Chan CC (2003a) Primary intraocular lymphoma: clinical features, diagnosis, and treatment. Clin Lymphoma 4(1):30–31

# Ocular and Adnexal Lymphoma: Molecular Pathology

3

Alia Rashid and Hans E. Grossniklaus

## 3.1 Introduction

Lymphomas of the ocular adnexa represent between 10 and 15 % of tumors arising within the orbit, eyelids, conjunctiva, and lacrimal sac (Spraul and Grossniklaus 1997). Intraocular lymphomas are rare, representing <1 % of all intraocular tumors (Bardenstein 1998). The incidence of ocular lymphomas has increased significantly in the west. One study by Moslehi et al looking at the increasing incidence of ocular lymphoma between 1975 and 2001 found that there was rapid and steady increase of about 6.5 % annually among white Americans of ocular NHL (Moslehi et al. 2006). Recently there has been much research showing a connection between infection by certain agents such as Chlamydophila psittaci (Cp) and ocular adnexal extranodal marginal zone lymphomas (EMZL) (Ferreri et al. 2004; Ponzoni et al. 2008). EMZL are the commonest ocular lymphomas, constituting between 50 and 80 % of ocular lymphomas. It is therefore a very likely possibility that increased prevalence of infection by such an agent Cp will lead to an increase in the incidence of ocular lymphoma (particularly EMZL) (Moslehi et al. 2006; Ponzoni et al. 2011). A new study published by Ferreri et al has evaluated the prevalence of Cp DNA in patients with newly diagnosed stage 1 EMZL, finding that 39/47 (89 %) had Cp DNA detected on biopsy, and subsequently found that treatment of the Cp infection with doxycycline positively affected the prognosis and response of the EMZL to lymphoma treatment (Ferreri et al. 2012). This has lead to the realization that accurate diagnosis of lymphoma subtype, through genetic evaluation as well as immunohistochemistry, may enable future developments for lymphoma treatment that target causative pathogens as well as the lymphoma itself with more accuracy. Furthermore, cytogenetic analysis may reveal further connections with either pathogens or genetic aberrations that can be targeted with alternative therapies and concomitantly allow more accurate prognostic evaluation to take place (Dagklis et al. 2012).

Attempting to diagnose these lesions accurately using traditional histopathology is often a challenge as the biopsy tissue specimens are typically very small. Furthermore, cytologic detail may not provide enough evidence to distinguish between lesions that are reactive or neoplastic (Chap. 9). Clinically many ocular lymphomas have an indolent course with symptoms that mimic more relatively benign processes. Due to these unavoidable shortcomings, diagnosis of ocular lymphomas can be a challenge; however, the use of ancillary tests using immunohistochemistry and molecular genetic studies certainly helps to pinpoint the correct diagnosis with considerable accuracy.

A. Rashid
L.F. Montgomery Laboratory, Emory Eye Center, Emory University, Atlanta, Georgia

H.E. Grossniklaus, MD (✉)
BT 428, L.F. Montgomery Laboratory, Emory University School of Medicine, 1365 Clifton Road NE, Atlanta, GA 30322, USA
e-mail: ophtheg@emory.edu

A.D. Singh (ed.), *Ocular and Adnexal Lymphoma*, Essentials in Ophthalmology,
DOI 10.1007/978-3-642-38499-8_3, © Springer-Verlag Berlin Heidelberg 2014

## 3.2 Ocular Adnexal Lymphoma

Primary lymphoma of the ocular adnexa may involve the lacrimal glands, eyelids, or conjunctiva in the anterior orbital compartment and can also occur in the retrobulbar compartment. Lymphomas of the ocular adnexa represent between 10 and 15 % of tumors arising within the orbit, eyelids, conjunctiva, and lacrimal sac (Spraul and Grossniklaus 1997), making them a far more common location for this entity than an intraocular location. However, ocular lymphomas only account for about 5–10 % of all extranodal lymphomas (Freeman et al. 1972). In western countries, the incidence of non-Hodgkin's lymphomas, which are the predominant type of lymphoma found in the ocular region, has increased markedly in the last few decades (Groves et al. 2000; Clarke and Glaser 2002).

A study by Fung et al reported that the subtypes of ocular adnexal lymphoma varied depending on whether they were primary or secondary neoplasms (Fung et al. 2003). For primary lymphomas, in order of decreasing incidence, mucosa-associated lymphoid tissue (MALT) was most common (57 %), followed by follicular (18 %), DLBCL (11 %), low-grade lymphomas not specified (7 %), and mantle cell lymphoma (4 %), with Burkitt's lymphoma, chronic lymphocytic leukemia, and others making up the remaining 3 %. For secondary neoplasms, follicular was most common (36 %), followed closely by mantle cell lymphoma (29 %) and then chronic lymphocytic leukemia and low-grade lymphoma not specified (both 14 %) and then DLBCL (7 %). Interestingly, no MALT lymphomas were found in the secondary neoplasms category.

## 3.3 Extranodal Marginal Zone Lymphoma

MALT lymphomas are the most common subtype of ocular adnexal lymphoma and are also the most common cause of primary choroidal lymphoma (Coupland and Damato 2008). Although MALT lymphoma suggests that the lymphoma arises in an epithelium tissue, this cannot be said for this subtype when it arises in the retrobulbar compartment. As such, the term extranodal marginal zone lymphoma (EMZL) is more accurate; however, the majority of research studies involving this subtype in the orbital compartment use the term MALT (in particular due to the fact the MALT1 gene is often involved). Here it will be referred to as EMZL.

Elsewhere in the body, EMZL have been found to arise in the presence of chronic antigenic stimulation through infection by various pathogens (Wotherspoon et al. 1991; Cerroni et al. 1997; Lecuit et al. 2004). In the eye, an association between Chlamydophila psittaci (Cp) and EMZL has been made (Ferreri et al. 2004; Collina et al. 2012), but the strength of this relationship has been questioned due to variability from studies undertaken in different geographic locations (Chanudet et al. 2006; Decaudin et al. 2008; Carugi et al. 2010). In concordance with studies of treatment of antigenic stimuli in EMZL from other body locations, a study by Ferreri et al has found that treating Cp+ patients with EMZL for the infection improved outcome (Ferreri et al. 2012).

EMZL characteristically reveal an expanded marginal zone, which contains small B-cells made of monocytoid- and centrocyte-like cells, small lymphocytes, immunoblasts, and few mitotic figures (Chap. 2). Immunohistochemistry profiling reveals CD20+, CD43+/−, CD79+, and BCL2+ with monotypical proliferations of Ig heavy/light chains (most commonly IgM or IgK). CD5, CD10, CD23, BCL6, and cyclin-D1 are typically negative and that Ki67 shows a low growth fraction (Fig. 3.1) (Coupland 2013).

## 3.4 Follicular Lymphoma

Follicular lymphoma (FL) is a large-cell lymphoma of the orbit. It represents approximately 30 % of all B-cell NHL (Leich et al. 2009); however, in the orbit it is extremely rare, comprising about 1 % of OAL. Although initially indolent, this lymphoma tends to recur and after some time becomes refractory to treatment, in some cases (10–70 %, with a risk of 3 % per year)

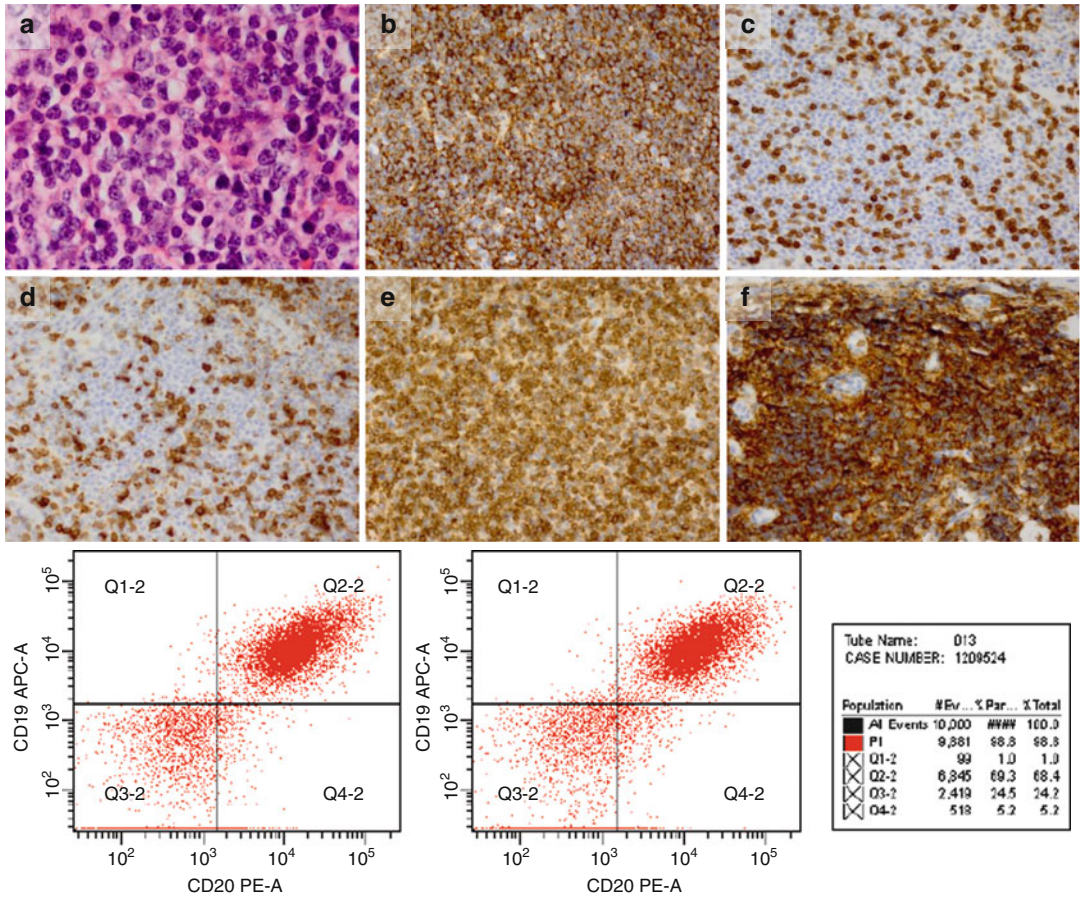


**Fig. 3.1** Extranodal marginal zone lymphoma (MALT type) is composed of small round lymphocytes and larger lymphocytes with open chromatin and prominent nucleoli (**a**, H&E at 250×). Immunostains are positive for CD20 in many lymphocytes (**b**, 100×), for CD5 in scattered lymphocytes (**c**), for CD3 in scattered lymphocytes (**d**, 100×), for BCL2 in many lymphocytes (**e**, 100×), and CD45 (LCA, 100×) for numerous lymphocytes (**f**, 100×)

transforming to DLBCL, thus making it a very aggressive lymphoproliferative disease (Freedman 2005; Montoto and Fitzgibbon 2011). Follicular lymphoma has been categorized into grades I–III by the WHO classification, based on the number of centroblasts counted per high-power field in the lesion (Swerdlow et al. 2008a). Grades I–IIIa have both centrocytes and centroblasts, whereas grade IIIb consists only of sheets of centroblasts and is considered a much more aggressive form of FL (Ott et al. 2002). Follicular lymphoma is considered to be a germinal-center-derived neoplasm due to its atypical follicular structures that are characteristic of this entity typically expressing CD10, CD19, CD20, BCL-2, BCL-6, and immunoglobulin light chain while being negative for CD5 and CD23 (Fig. 3.2). In 80–100 % of cases in North America and Europe, the molecular defect in FL has been found to be t(14;18)(q32;q21) (Biagi and Seymour 2002), where the BCL-2 gene is apposed to the IgH gene, resulting in the enhancement and dysregulation of BCL-2. This leads to high levels of functional BCL-2, which subsequently leads to inhibition of apoptosis. Over 90 % of indolent FL have been found to express high levels of BCL-2 (Zha et al. 2004). FL can be subdivided into grades I–IIIb. Studies have reported that t(14;18) is present in grades I, II, and IIIa FL but often lacking in grade IIIb. Those grade IIIb cases of FL negative for t(14;18) have been found to have a unique 3q27 aberration instead (Bosga-Bouwer

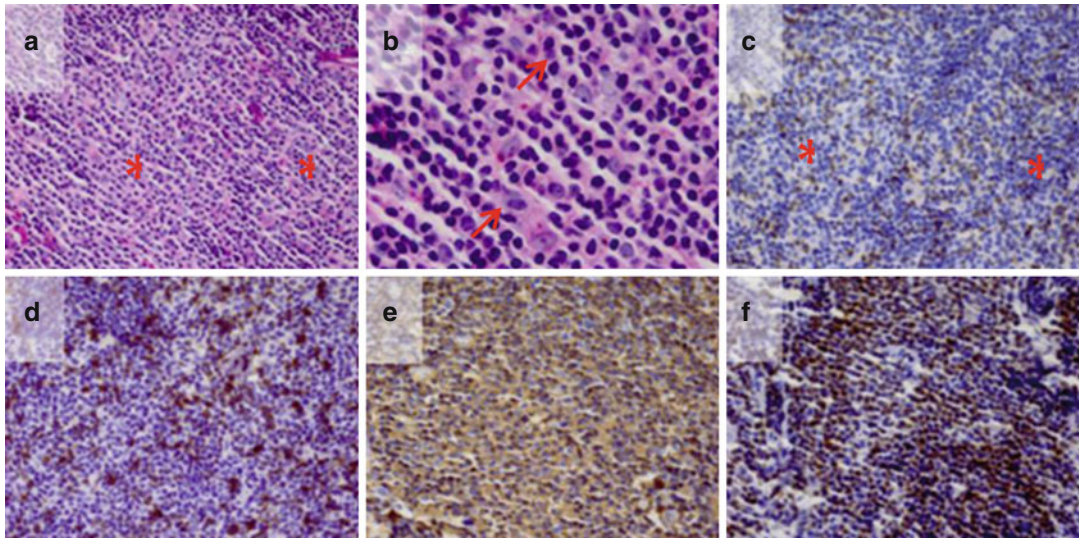


**Fig. 3.2** Follicular lymphoma is composed of poorly formed follicles (*) surrounded by small round lymphocytes (**a**, H&E, 100×). The follicular center lymphoma cells are large with large nuclei and prominent nucleoli (**b**, *arrows* H&E, 250×). Occasional small round lymphocytes stain for CD3 and are interspersed in between follicular centers (* **c**, 100×). Occasional CD5-positive lymphocytes are present (**d**, 100×). CD20 is positive in numerous lymphocytes (**e**, 100×). BCL2 is positive in numerous lymphocytes (**f**, 100×)

et al. 2003). Furthermore, FL has been found to occasionally have a rearranged BCL-6 instead of BCL-2 (Jardin et al. 2002). Several studies found that when immunostaining was positive for BCL-2 and CD10, a FISH analysis would reveal a t(14;18) aberration in virtually every case (Godon et al. 2003; Sekiguchi et al. 2005) (Fig. 3.3).

## 3.5 Mantle Cell Lymphoma

Mantle cell lymphoma (MCL) typically shows multiple lymph node and bone marrow involvement and has a clear male predominance (Banks et al. 1992). It is characterized by a poor prognosis and short survival time. MCL is hard to distinguish from the more indolent lymphomas such as MALT and follicular lymphoma, using histopathology and immunophenotyping alone (Coupland et al. 2004b; Ferry et al. 2007). The use of genetic testing to elucidate the exact nature of the lymphoma is therefore extremely important in risk management and prognostication. Vaandrager et al used FISH to determine that MCL showed a t(11;14)(q13;q32) translocation in over 95 % of cases (Vaandrager et al. 1996). As a result of this translocation, the IGH gene promoter on 14q32 apposes with the cyclin-D1 gene on 11q13, leading to marked overexpression of cyclin-D1. Li et al found that FISH was far superior to both cytogenetic testing and PCR in the detection of this translocation (Li et al. 1999).

Mantle cell lymphoma characteristically reveals monomorphic small-to-medium-sized cells with a CD5+, CD10−, and CD23− phenotype, with cyclin-D1 rearrangement and its nuclear expression considered the hallmark for MCL diagnosis (Swerdlow et al. 2008b). Looi et al assessed at MCL in the ocular adnexal region and found that 30 % of the tumors failed to co-express CD5, a finding supported by other studies (Looi et al. 2005; Rasmussen et al. 2009). This is in contrast to MALT lymphomas which typically do not express CD5 and have an indolent course. Several studies and reports have postulated that CD5 expression in MALT lymphomas is a marker for both earlier dissemination, most commonly to bone marrow, and aggressive disease course (Ferry et al. 1996;

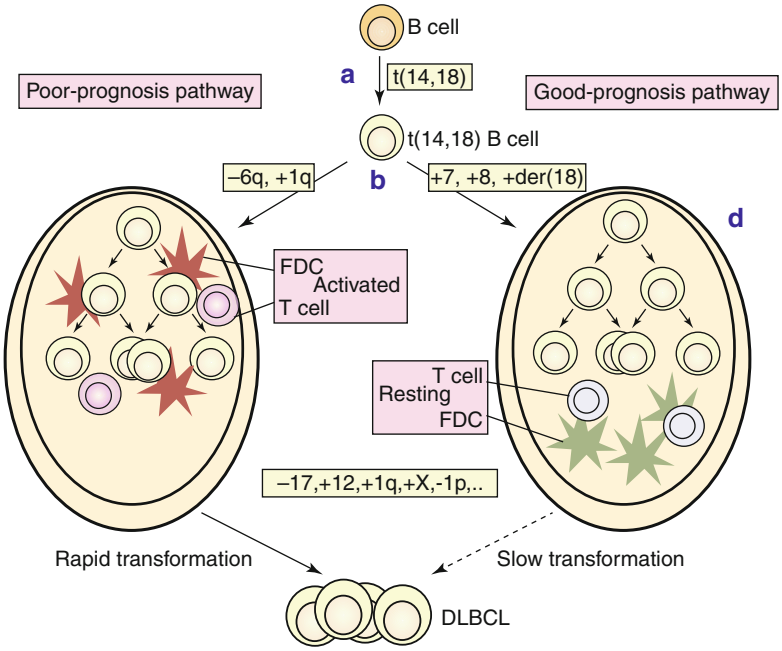


**Fig. 3.3** Follicular lymphoma: dual prognosis pathway. In the initial event, the presence of t(14;18), or similarly biologic genomic events, results in resistance of the B-cell to apoptosis in the absence of antigen (*A*). A prognostic dichotomy occurs at the second key juncture, where the development of secondary genomic alterations occurs (*B*). The poor prognosis pathway incurs genomic alterations which induce activation of the immune system (activated follicular dendritic cells and activated T-cells playing dominant roles) and also further infer genomic instability of the cell, resulting in rapid transformation from follicular lymphoma to DLBCL (*C*). The good prognosis pathway involves secondary genomic alterations which not only confer a more stable genomic state but also maintain follicular dendritic cells and T-cells in a resting state (*D*). As a result, genomic alterations accumulate at a slower rate making the follicular lymphoma cells relatively resistant to transformation to DLBCL, thus affording a good prognosis. Modified with permission from: de Jong (2005)

Wenzel et al. 2001). In MCL where CD5 is much more commonly found (>70 % of cases), its presence may confirm the suspicion that CD5 positivity leads to a more aggressive lymphomatous entity. Rasmussen et al followed 21 patients with orbital and adnexal MCL and found that those treated with anti-CD20 therapy (Rituximab) had a significantly better overall survival at 5 years compared to those who did not have adjunct anti-CD20 chemotherapy (83 % vs. 8 %) (Rasmussen et al. 2009).

A recent review article by Seto looked at those cases of MCL which are cyclin-D1 negative (Seto 2013). Although cyclin-D1 negative MCL is rare, findings reviewed in the Seto article reveal that the presence of a cyclin-D2 rearrangement indicates a poor prognosis subtype that should be treated intensively (Figs. 3.4 and 3.5).

## 3.6 Diffuse Large B-Cell Lymphoma

Diffuse large B-cell lymphoma (DLBCL) is the most common form of NHL across all sites in the body, accounting for around 40 % of lymphomas (Coiffier 2001). DLBCL is very much a heterogeneous entity with considerable variation seen in the clinical features, morphology, and genetics between cases (Swerdlow et al. 2008a). As a consequence of this, the response to chemotherapy is variable and difficult to predict. Several studies have attempted to elucidate the features, either clinical, morphological, or genetic that may improve prognostication. The WHO had outlined a classification system for DLBCL in its 2001 report (Jaffe et al. 2001). This recommendation included the use of a standard panel of antibodies

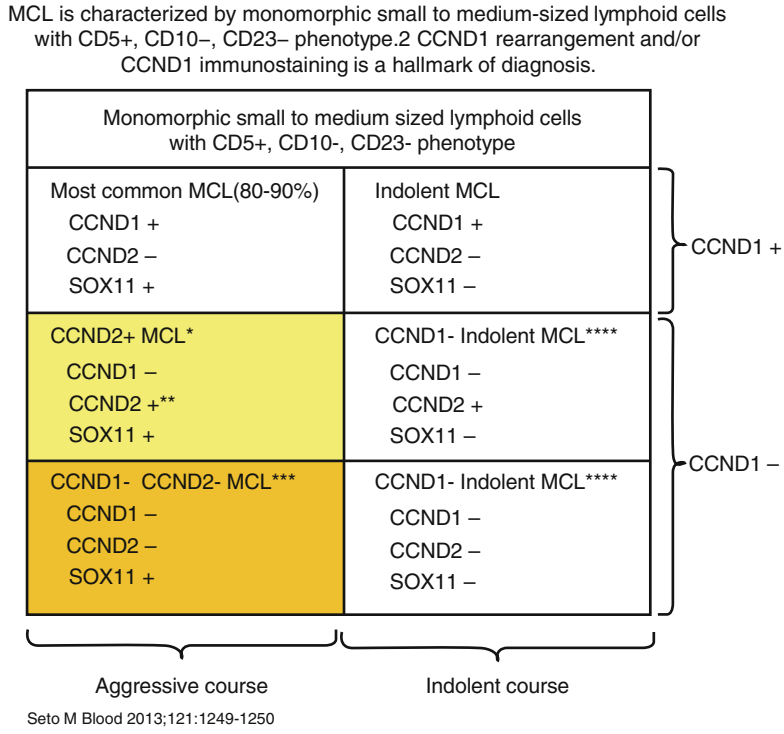


**Fig. 3.4** Clinical and pathologic subtypes of mantle cell lymphoma. Reproduced with permission from: Seto (2013)

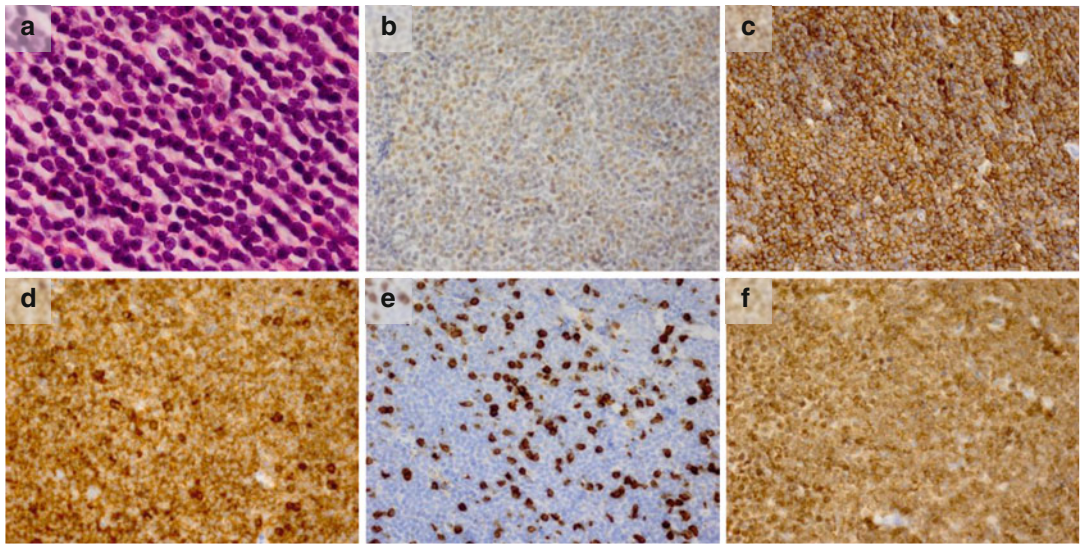


**Fig. 3.5** Mantle cell lymphoma is composed of sheets of round nuclei and a moderate amount of cytoplasm (**a**, H&E 250×). Few lymphocytes stain for CD3 (**b**, 100×). Many lymphocytes are positive for CD5 (**c**, 100×) and for CD20 (**d**, 100×). Occasional lymphocytes express Cyclin-D1 (**e**, 100×). Numerous lymphocytes may stain for BCL2 (**f**, 100×)

used in immunohistochemistry to confirm a diagnosis of DLBCL, i.e., CD3, CD5, CD20, CD21, CD23, CD79a, and cyclin-D1. More recently the use of genetic testing to try and stratify DLBCL into prognostically valid subgroups has come to the forefront of research into this entity. There has been some suggestion from various studies that some DLBCL may arise from a preexisting MALT lymphoma. The chromosomal aberrations most commonly associated with DLBCL appear to be t(11;18), t(3;14), and t(14;18) (Kramer et al. 1998; Alizadeh et al. 2000). Aneuploidy is a very common finding in DLBCL, with trisomy 18 and to some extent trisomy 3 being the most prevalent. Chromosomal instability has been shown to be an important factor in the development and aggressive nature of cancers that display aneuploidy, such as DLBCL (Carter et al. 2006; Weaver and Cleveland 2007). A paper by Bakhoum et al found that in patients with DLBCL, chromosomal instability demonstrated a poor prognosis (Bakhoum et al. 2011). They found that a twofold increase in the frequency of aneuploidy in DLBCL led to a 24 % decrease in overall survival and a massive 48 % decrease in relapse-free survival after treatment.

Studies that evaluated the mRNA profile of DLBCL found that those DLBCL with an mRNA profile analogous to that of germinal-center B-cells (GCB) tended to have a more indolent course than in tumors with an mRNA profile similar to that in activated B-lymphocytes (ABC) (Alizadeh et al. 2000; Rosenwald et al. 2002; Chang et al. 2004; Berglund et al. 2005; Poulsen et al. 2005). A study by Hans et al used immunohistochemical markers to separate DLBCL into either germinal-center B-cell (GCB) or non-germinal-center activated B-cell (ABC) subtypes (Hans et al. 2004). Using CD10, BCL-6, and MUM1, they were able to design an algorithm, which allowed discrimination of DLBCL into one of the two subtypes, and using this approach they found that GCB had a significantly better prognosis than ABC DLBCL (Fig. 3.6a). A similar approach was used by Muris et al who developed an algorithm for subgrouping of DLBCL using the immunohistochemical markers BCL-2, CD10, and MUM1 (Fig. 3.6b) (Muris et al. 2006).

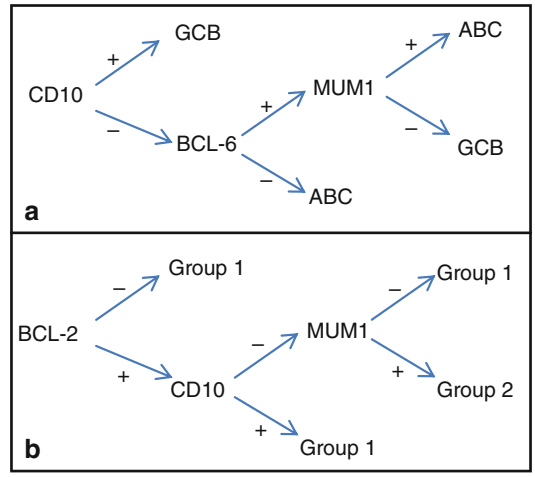


**Fig. 3.6** DLBCL subtype and group decision algorithm. (**a**) Modified with permission from: Hans et al. (2004) and (**b**) from Muris et al. (2006)

Muris et al felt this choice of immune markers provided a more accurate way to subtype the DLBCL in a prognostically significant way, finding that the difference between overall survivals of Group 1 and Group 2 DLBCL to be even higher than that found by Hans et al.

A study by Sjo et al used immunohistochemistry to try to profile prognostic subgroups of DLBCL (Sjo et al. 2007). One hundred and eight cases of DLBCL (not ocular) were evaluated using antibodies CD10, BCL-2, BCL-6, and MUM1, and the use of the two algorithms developed by Hans and Muris was employed. CD10 and BCL-6 were found to relate to more favorable prognosis, whereas BCL-2 appeared to be a marker for adverse prognostic outcome. Sjo et al found that Hans' algorithm which separated DLBCL into GCB-DLBCL or ABC-DLBCL showed a statistically significant difference in the overall 5 year survival (43 % for GCB vs. 26 % in ABC, $P=0.001$) that was greater than that found using the Muris stratification of Group 1 versus Group 2 (35 % vs. 21 %, $P=0.02$), revealing that the Hans algorithm did a slightly better job of distinguishing between low- and high-risk DLBCL. Furthermore, when Cox regression multivariate analysis was employed to establish which of the prognostic indicators including risk groups, antibody positivity, and Hans and Muris subtyping,

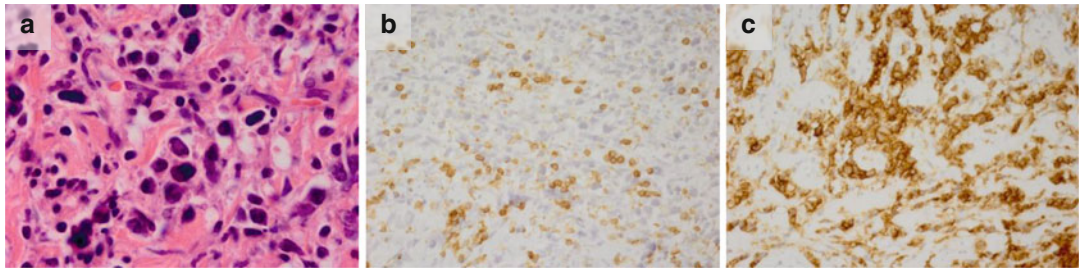


**Fig. 3.7** DLBCL is comprised of large, bizarre lymphocytes with high nuclear to cytoplasmic ratios and hyperchromatic nuclei (**a**, H&E 250×). Occasional CD3-positive reactive lymphocytes may be present in the lesion (**b**, 100×). The large lymphoma cells stain for CD20 (**c**, 100×)

only the expression of BCL-6 retained prognostic significance ($P=0.30$). A recently published study by Stacy et al, a retrospective multicenter case study of the clinical and immunohistochemical features of 20 patients with orbital DLBCL, found that the clinical stage was the most helpful predictor of outcome, whereas the immunohistopathologic features, or combinations of biomarkers, were not as useful for prognostication (Stacy et al. 2012). Immunohistochemistry using Bcl-6, CD5, CD10, CD20, FOXP1, GCET1, and MUM1 was utilized to differentiate between the ABC- and GCB-DLBCL subtypes; however, this categorization was found to have no correlation with outcome.

Chromosomal aberrations in DLBCL include both translocations and aneuploidy. Aneuploidy seems to be the more commonly found aberration, with trisomy of chromosomes 18 and/or 3 being most common in DLBCL as well as MALT lymphomas negative for the t(11;18) translocation. This has led to speculation that these numerical aberrations could be due to the transformation of a MALT lymphoma to DLBCL (Remstein et al. 2002). A Japanese study by Takada et al looked at 45 cases of lymphoproliferative disorders of the ocular adnexa (Takada et al. 2003). Of these cases, two were classified as DLBCL with MALT lymphoma and 12 of DLBCL only, the rest being MALT lymphomas or RLH. FISH and RT-PCR were used to identify the presence of t(11;18) as well as numerical aberrations of chromosomes 3, 7, 12, and 18. FISH found one of one tested case of DLBCL+MALT, and one of three cases tested of DLBCL only, to be positive for t(11;18). RT-PCR detected the t(11;18) aberration in 2 of 12 cases (17 %) of DLBCL without MALT lymphoma that were tested, one of which was the case detected by FISH. In total 2/14 cases (14 %) of DLBCL with/without MALT lymphoma were found to harbor a t(11;18) aberration. Aneuploidy was observed in all cases of DLBCL examined by FISH, with trisomy 3 and both trisomy 18 and tetrasomy 18 being discovered. As a result of this study, the group surmised that some DLBCL of the ocular adnexa may derive from MALT lymphomas (Fig. 3.7).

## 3.7 Intraocular Lymphoma

Primary intraocular lymphoma (PIOL) is a very rare type of neoplasm. It represents approximately 1 % of non-Hodgkin's lymphomas, about 1 % of intracranial tumors, and <1 % of all intraocular tumors (Bardenstein 1998). PIOL is a rare subtype of primary central nervous system lymphoma (PCNSL) (Pe'er et al. 2009). PIOL fall into four different groups: vitreoretinal/retinal lymphomas, choroidal lymphomas, iridial/ciliary body lymphomas, and secondary uveal lymphomas. The most common subtype of PIOL is diffuse large B-cell lymphoma (DLBCL), and rarely T-cell lymphomas can also occur (Hormigo et al. 2004). Bilateral involvement in PIOL occurs in about 80 % of cases (Levy-Clarke et al. 2005).

The causative factors or theories for the origin of PIOL have been postulated to range from chronic infection within the eye resulting in

continuous antigenic stimulation to extra-global transformation resulting in malignant cells being trapped within the immune privileged confines of the intraocular environment.

## 3.8 Vitreoretinal Lymphoma

Primary vitreoretinal lymphomas (PVRL) are generally high-grade B-cell malignancies and the most common presentation of PIOL. Diffuse large B-cell lymphoma (DLBCL) is the commonest subtype (Coupland and Damato 2008; Coupland et al. 2009), but a small number of both T-cell-rich B-cell lymphomas and T-cell lymphomas have also been described in the literature (Coupland et al. 1999, 2005; Cummings et al. 2005). PVRL is often diagnosed late as the condition masquerades as a chronic posterior uveitis, with the unfortunate result that in approximately 65–90 % of patients with these malignancies, there is the subsequent development of CNS lymphoma (Coupland et al. 2004a). Diagnosis of primary vitreoretinal lymphomas is achieved by using a combination of histologic and immunohistochemical analyses (Fig. 3.8). Histopathological analysis can be difficult due to the often scanty hauls of lymphoma cells found in vitreous aspirates or retinal biopsies. Immunohistochemistry to indicate the presence of monoclonal populations is often more valuable. In addition to these two methods of diagnosis, the evaluation of interleukin levels may be used as an adjunct to aid diagnosis. Several studies have shown that an IL-10:IL-6 ratio >1 or an elevation of IL-10 levels in the intraocular fluid is highly suggestive of B-cell PVRL (Chan et al. 1995; Wolf et al. 2003; Cassoux et al. 2007).

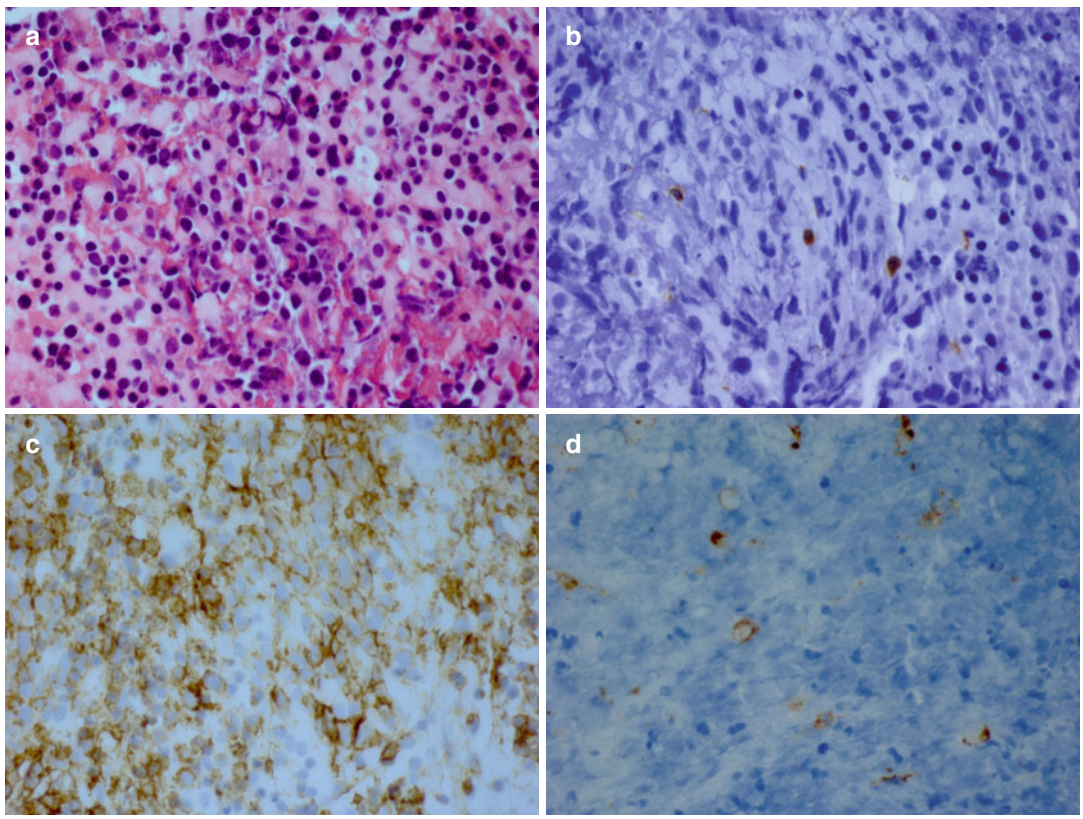


**Fig. 3.8** Primary vitreoretinal lymphoma. There are large lymphoma cells present and scattered small round lymphocytes (**a**, H&E 150×). The small round lymphocytes stain for CD3 (**b**, 150×). The large lymphoma cells stain for CD20 (**c**, 150×). Occasional macrophages are positive for CD68 (**d**, 150×)

Molecular testing using microdissection followed by PCR is used to determine the presence or absence of gene rearrangements, with a focus on the IgH gene rearrangement in B-cell lymphomas and T-cell receptor (TCR) gene rearrangement in T-cell lymphomas (Shen et al. 1998).

## 3.9 Choroidal Lymphoma

Choroidal lymphomas can either be of primary or secondary origin; both are exceedingly rare. If there is no systemic disease evident at the time of diagnosis, the lesion is considered to be of primary choroidal origin. Primary choroidal lymphomas are typically low-grade B-cell lymphomas and fewer than a 100 cases have been reported in the literature (Coupland et al. 2002). Using the WHO lymphoma classification, primary choroidal lymphomas are typically classed as EMZL. The EMZL in the uvea are usually low grade with an indolent nature, and this initially led to the belief that these were not malignant neoplasms, but in fact a form of reactive lymphoid hyperplasia. Using immunohistochemical analysis, several studies showed that this was not in fact the case, and these proliferations had monoclonal populations (Ben-Ezra et al. 1989; Cockerham et al. 2000; Coupland et al. 2002). The immunophenotype is therefore similar to that of ocular adnexal EMZL with CD20+, CD43+, CD79a+, BCL-2+, IgM+, and low Ki-67 <15 % (Coupland and Damato 2006). As the putative cell of origin is the memory B-cell in the post-germinal center, there are likely to be genotypic changes such as t(11;18) or aneuploidy of chromosomes 3 and/or 18 (Coupland and Damato 2008).

## 3.10 Iris Lymphoma

Primary iridial lymphomas are very rare malignancies and can be of either B- or T-cell origin. A review of the literature by Velez et al found 163 cases of NHL of the CNS affecting the eye, of which only five were found to have iris involvement and only one of those was suspected clinically, the rest being found on autopsy/enucleation-confirmed histopathology (Velez et al. 2000). The majority of primary iris lymphomas are high-grade malignancies with poor overall survival rates (Coupland and Damato 2008). A recent case series by Mashayeki et al reviewed 14 cases of iris lymphoma in 13 patients (Mashayekhi et al. 2013). Of these, 6/13 were found to only have iris involvement with no evidence of systemic lymphoma. The seven cases that had concomitant systemic involvement were all noted to have relatively aggressive forms of systemic lymphoma with large-cell lymphoma being the most common, a finding supported by several other studies (Duker et al. 1987; Goldey et al. 1989; Hykin et al. 1996; O'Keefe et al. 2002). The cases were all found to have B-cell lymphoma, apart from one case which revealed a T-cell iris lymphoma secondary to a systemic γδ-T-cell leukemia/lymphoma. A review of the literature found a few cases of iris T-cell lymphoma; some were primary in origin, and the secondary lymphomas were associated with various forms of systemic T-cell lymphoma, including mycosis fungoides (Saga et al. 1984; Jensen et al. 1994; Shimonagano et al. 2006; Ralli et al. 2009). In addition to DLBCL and various T-cell lymphomas as the main lymphoma type in iris-based lesions, mantle cell lymphoma has also been implicated in a case of secondary iris lymphoma (Economou et al. 2007). In view of these findings, the immunohistochemistry needs to be targeted towards the systemic disease if there is any present; otherwise, the focus should be on intraocular DLBCL versus T-cell lymphoma. To date there are not any available studies in the literature that have investigated the genotypic variations of primary iris lymphomas, although again, one would expect to find genotypic aberrations similar to those found in DLBCL or T-cell lymphoma.

## 3.11 Ciliary Body Lymphoma

Primary ciliary body lymphoma is extremely rare, with only one known report in the literature (Coupland and Damato 2008). The case showed a predominantly B-cell type lymphoma that was

CD43 positive, with low Ki-67 rate of <5 %, and immunohistochemistry revealed monoclonality of IgM. Due to the rarity of this entity, no genotypic information is available.

## 3.12 Rare Primary Intraocular Lymphoma

Primary intravascular lymphoma, previously known as malignant or neoplastic angioendotheliomatosis (NAE), is a very rare cause of PIOL. Characteristically, there are widespread intravascular proliferations of malignant cells of endothelial origin. Due to the pancorporeal nature of the disease, it has proved be rapidly fatal. An autopsy study by Elner et al looked at three cases of neoplastic angioendotheliomatosis, with evaluation of both the eyes and the central nervous system (Elner et al. 1986). All three cases revealed anaplastic cells in the intravascular space, and in two of the patients, massive extravascular involvement was evident. A review of the literature revealed that ophthalmic manifestations are common in NAE; however, it has been typically reported from the point of view of cutaneous and central nervous system manifestations. Elner reported on three cases of NAE, all were elderly Caucasian males who complained of blurred vision, amongst other body symptoms. All three men expired from pancorporeal involvement within 2 years of presentation. Several studies have shown that the tumor cells in NAE will stain positive with monoclonal antibodies to common leukocyte antigen (CLA) indicating that it is a type of extranodal lymphoma (Wrotnowski et al. 1985; Wick et al. 1986). Furthermore, the ABC immunoperoxidase technique has been shown to distinguish hematopoietic from non-hematopoietic neoplasms, using anti-CLA antibodies in FFPE specimens (Kurtin and Pinkus 1985).

Human T-cell lymphotropic virus type 1 (HTLV-1) is a single-stranded RNA retrovirus that has been established as a cause of a very rare ocular lymphoma – adult T-cell leukemia/lymphoma (ATL). HTLV-1 infection is endemic in the Caribbean Islands, Japan, South America, and Central Africa, with transmission through direct contact with bodily fluids (Kumar et al. 1994; Liu et al. 2010). ATL is a CD4+ T-lymphocytic aggressive malignancy. A case report by Levy-Clarke described a 40-year-old Jamaican female who had complained of blurred vision in her left eye and clinically appeared to have a necrotizing retinal vasculitis (Levy-Clarke et al. 2002). She was investigated for various collagen vascular diseases and treated with corticosteroids with no response. A retinal biopsy was eventually obtained and analyzed by PCR. The results revealed a TCRγ gene rearrangement consistent with a T-cell lymphoid malignancy as well as the presence of the HTLV-1 *pol* gene. Furthermore a complete blood count revealed a leukocytosis with elevated CD3+ and CD4+ T-cells and elevated T-cell-activated antigen cells. ATL is classified into four subgroups, of which lymphoma is one subtype. The lymphoma subtype is the only one that does not demonstrate the flower cells on microscopic examination, and furthermore, it is resistant to chemotherapy and therefore has a very poor prognosis. This case clearly demonstrates the importance of accurate diagnosis with the use of molecular analysis by microdissection and PCR as ATL has often been reported to masquerade as other conditions. Alternative analysis, using a Southern blot technique on HTLV-1 proviral DNA, can be useful when nontraditional tissues such as the retina and uvea are involved (Shibata et al. 1997). Vitreous biopsy can be used to evaluate the soluble IL-2 receptor alpha (sIL-2α) levels which may indicate the presence of ocular infiltration and aid prognostication (Sugita et al. 2006).

## 3.13 Secondary Intraocular Lymphoma

Systemic dissemination of lymphoma to the intraocular space is usually limited to the choroid (Fredrick et al. 1989; Verbraeken et al. 1997; Hochman et al. 2001). Secondary choroidal lymphomas are usually DLBCL, although other types such as EMZL and Burkitt's lymphoma can sometimes occur (Coupland and Damato 2008).

The immunophenotype and genotype would therefore depend on the nature of the systemic lymphoma infiltrating the uvea/choroid.

## 3.14 Laboratory Investigations

### 3.14.1 Immunohistochemistry

Immunohistochemistry (IHC) and flow cytometry (FC) are commonly used to determine the phenotype of a lymphoid proliferation using immunofluorescent antibody markers. Immunohistochemistry involves placing the FFPE biopsy specimen on a glass slide and staining it with a panel of antibody markers for B- and T-lymphocytes, kappa and lambda light chains, natural killer cells, and macrophages. Flow cytometry is useful when there is a small biopsy specimen with fewer cells available for analysis, e.g., vitreous aspirates. The cells in the biopsy specimen are firstly stained with the appropriate panel of antibody markers and then separated using a fluorescence-activated cell sorter.

The benefit of IHC is that it does not require specialized preparation of the tissue, and a formalin-fixed paraffin-embedded sample of the biopsy specimen can be used for analysis, while FC requires advance planning to supply a fresh unfixed tissue specimen. With immunophenotypic studies the size of the antibody panel should be tailored to the size of the specimen and the nature of the tissue and the differential diagnoses. A judicious choice of antibodies to test in the panel should take into consideration cost, as a larger panel would cost more and not necessarily add more information, but bearing in mind a too small or narrow panel may miss key immunophenotypic features as may be the case in atypical presentations. FFPE IHC studies are popular due to several reasons: firstly, no special processing is required and they can be obtained from archived FFPE specimens; secondly, the architecture of the tissue remains intact, allowing cytologic evaluation of neoplastic and normal cells; and finally, the broad range of antibodies available for FFPE tissue include some that may not be available for FC evaluation. There are a few drawbacks to using FFPE IHC such as "background" staining and difficulty with achieving satisfactory non-plasmacytic κ- or λ-staining. Quantification of cell populations is also subjective.

Flow cytometry is a very commonly used adjunctive diagnostic test and has over recent years become more accurate both in terms of the range of available antibodies and fluorochromes and the analysis software required to assess the sample (Swerdlow 2013). This is beneficial when there is a small biopsy specimen, as the cells can be labeled with multiple antibodies simultaneously in the same tube, allowing at the very least a basic phenotype to be recognized. The software now available for analysis of the graphs produced by the FC studies is able to assess multiple phenotypes within a sample simultaneously and also provides information on the size and complexity of the cell populations in the specimen. These parameters allow the clinician to infer the presence of mono- or polyclonal proliferations. FC is especially useful when trying to diagnose the presence of neoplastic T-cells on a background of normal T-cells. FC is also the best method for evaluating antigen intensity, which can have prognostic significance for some lymphomas. The main pitfalls of using FC are that architectural features cannot be assessed and occasionally the presence of an aberrant phenotype can be veiled by the normal cell population in the suspension diluting the neoplastic population. Nonspecific staining can be mistakenly interpreted as normal, whereby it masks specific staining, or conversely be judged as positive for a neoplastic proliferation when it is not the case.

### 3.14.2 Cytokine Analysis

Differential expression of cytokines in neoplastic versus inflammatory processes has been the basis of many recent studies revealing that the ratio of interleukin IL-10 to IL-6 in vitreous fluid can help determine between the two processes (Buggage et al. 1999a, b; Cassoux et al. 2007; Ohta et al. 2009; Sugita et al. 2009; Kimura et al. 2012). Malignant cells produce IL-10 in great quantities, whereas IL-6 predominates in inflammatory

processes (Coupland et al. 2004a; Levy-Clarke et al. 2005; Choi et al. 2006; Sen et al. 2009). One study found that a ratio of IL-10:IL-6 greater than 1 is strongly indicative of PIOL (Levy-Clarke et al. 2005); however, this should not be used as a diagnostic test but as an adjunct, providing supportive information to aid diagnosis. Utilizing cytokine analysis of a vitreous specimen has been shown to supportive evidence in the diagnosis of retinal PIOL (Chan et al. 1995; Cassoux et al. 2007). A high IL-10 to IL-6 ratio from both vitreous aspirate and cerebrospinal fluid samples has revealed a correlation consistent with large B-cell lymphoma (Whitcup et al. 1997). A study by Velez et al investigating iris involvement in PIOL actually found that use of cytokine analysis of aqueous humor found significant levels of the cytokines there and thus may provide another useful and less invasive way of aiding diagnosis of intraocular lymphoma (Velez et al. 2000).

### 3.14.3 Molecular Analysis

Polymerase chain reaction (PCR) is used to provide molecular analysis of suspected lymphoma tissue biopsies to obtain a diagnosis. In suspected B-cell lymphomas, the DNA is extracted from the B-lymphocytes and using PCR is amplified, using primers which are directed to the CD3 IgH variable region which detects any clonal rearrangements of the IgH gene. In B-cell lymphomas, rearrangement of the immunoglobulin heavy chain (IgH) gene is strong supportive evidence for diagnosis (Coupland et al. 2004a; Levy-Clarke et al. 2005; Choi et al. 2006; Sen et al. 2009). B-cell proliferations can be analyzed for monoclonality by assessing the FR2/FR3 gene loci (Sen et al. 2009). The benefit of this method is that it allows the monoclonality of neoplastic processes to be identified clearly, even when inflammatory cells are present (which usually reveal polyclonal amplification products). A study by Chan et al of 50 cases of PIOL found that the CDR3 of the IgH gene showed 100 % monoclonality (Chan 2003). If a T-cell lymphoma is suspected, the PCR utilizes primers targeted at the T-cell receptor gamma chain and TCR gene rearrangement studies are used to demonstrate monoclonality (Sen et al. 2009). Furthermore, PCR can be utilized to detect other chromosomal changes such as BCL-2 or BCL-6 gene rearrangements in the DNA of cells.

## 3.15 Molecular Genotyping

Molecular genetics of ocular lymphoma is a relatively new and rapidly advancing field. Over the last decade or so, the use of genetic profiling in ocular lymphomas has enabled the delineation of genetics of specific ocular lymphoma subtypes, revealing both favorable and unfavorable characteristics and allowing a greater understanding of the pathogenic mechanisms that result in the development of lymphoma within the ocular compartment. Of the ocular adnexal lymphomas, MALT lymphoma is by far the most common subtype, making up more than 50 % of lymphomas diagnosed in the ocular compartment (Cho et al. 2003; Ferry et al. 2007). The use of genome-wide expression profiling (GEP) has found several chromosomal aberrations associated with MALT lymphoma in the orbit: t(14;18)(q32;q21), t(11;18)(q21;q21), t(1;14)(p22;q32), and t(3;14)(p14;q32). Each translocation involves either the immunoglobulin heavy chain gene (IgH) or the MALT1 gene being juxtaposed with another gene, leading to dysregulation of proteins which all target the NF-κB signaling pathway (Lucas et al. 2001). (For the other ocular adnexal lymphoma subtypes, similar translocations have been found.) Over 80 % of cases of follicular lymphoma in North America and Europe are found to carry the molecular defect t(14;18)(q32;q21) (Biagi and Seymour 2002). In mantle cell lymphoma, a t(11;14)(q13;q32) translocation was found in over 95 % of cases (Vaandrager et al. 1996). DLBCL is most commonly associated with t(11;18), t(3;14), and t(14;18) as well as being found to be a heterogeneously aneuploid tumor revealing trisomy 3 and/or trisomy 18 (Kramer et al. 1998; Alizadeh et al. 2000).

Molecular studies using PCR techniques to look for B- or T-cell clonality have improved

significantly with the development of more specific primers. They do require knowledge of the technique and understanding of the significance of test results in order to be able to interpret correctly. Molecular diagnostics should always be interpreted in the context of the histologic/cytologic features and clinical information. A real risk is that the PCR results can be misleading, if a particular gene rearrangement is detected that may not necessarily be the one related to the neoplasm in question or if a clone is detected that may be masking another more important finding (Figs. 3.9 and 3.10).

### 3.15.1 t(11;18)(q21;q21)

This chromosomal aberration results in the API2 gene found on chromosome 11 at location q21 aligning with the MALT1 gene located on chromosome 18 at the q21 locus (Dierlamm et al. 1999). The resulting fusion proteins FUS1 and FUS2 derived from the API2-MALT1 molecule are able to activate the NF-κB pathway as well as inhibiting p53-mediated apoptosis, unlike the individual genes alone (Uren et al. 2000; Stoffel et al. 2004). In MALT lymphomas of the gastrointestinal and pulmonary tracts, t(11;18)

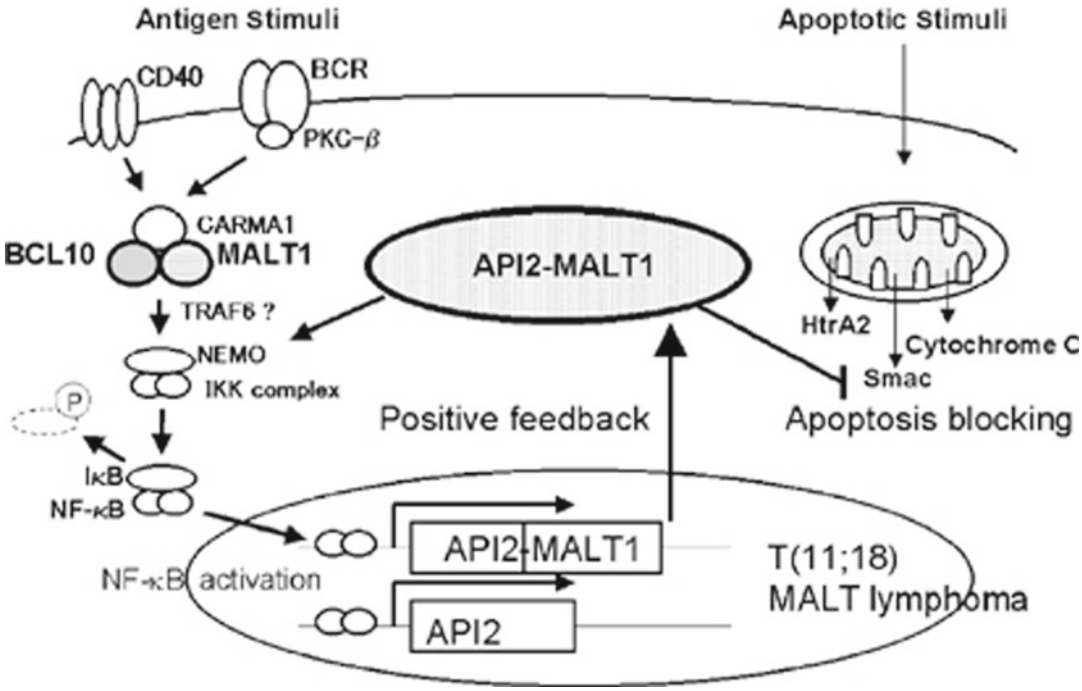


**Fig. 3.9** Molecular mechanisms underlying the pathogenesis of mucosa-associated lymphoid tissue (MALT) lymphomas with t(11;18), t(1;14), and t(14;18) translocations. Antigen stimulation and CD40 triggering synergize in nuclear factor kappa B (NF-**κ**B) activation through formation of CARMA1–BCL10–MALT1 ternary complex. BCL10 induces the oligomerization of MALT1, and activated MALT1 then induces the ubiquitination of NEMO, thereby ultimately leading to NF-B activation. It remains to be determined whether TRAF6 binds to the activated MALT1 and functions as a ubiquitin ligase for NEMO. In t(1;14) or t(14;18) MALT lymphoma cells, deregulated expression of BCL10 or MALT1 induces the ubiquitination of NEMO, resulting in NF-B activation. In t(11;18) MALT lymphoma cells, API2-MALT1 can bypass the normal BCL10–MALT1 signaling pathway, resulting in NF-B activation. Induced NF-B activation might contribute to expression of the target genes, including both API2 and API2-MALT1 fusion genes, resulting in the enhancement of antiapoptotic effect through a positive feedback-loop pathway. Conversely, API2-MALT1 can exert an antiapoptotic effect through its direct interaction with apoptotic regulators, including Smac. It can thus be hypothesized that the antiapoptotic effect by API2-MALT1 may be mediated through two signaling pathways: one mediated by its interaction with apoptotic regulators and the other mediated by the upregulation of apoptotic inhibitor genes. Reproduced with permission from: Nakagawa et al. (2006)

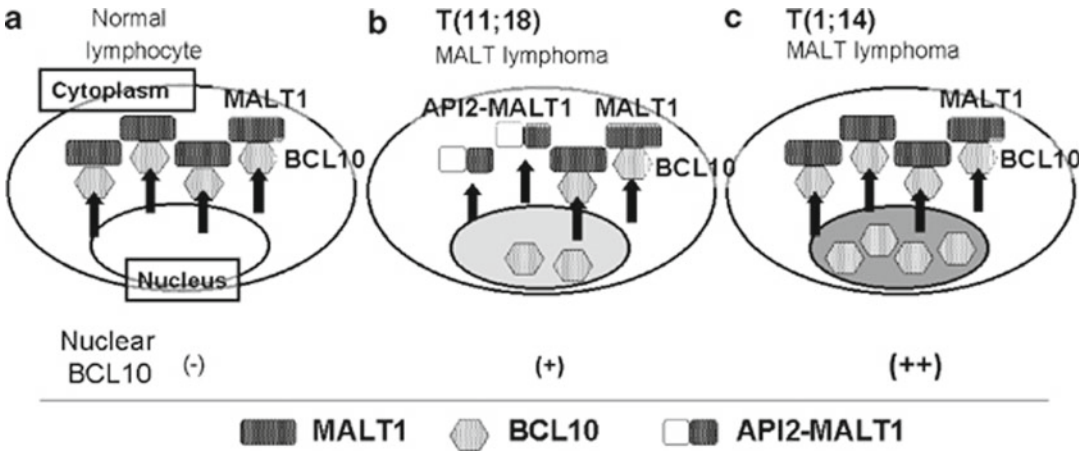


**Fig. 3.10** A model for subcellular localization of BCL10 under various conditions. (**a**) Normal lymphocyte: BCL10 is localized in the cytoplasm because nuclear BCL10 is exported by MALT1. (**b**) t(11;18) mucosa-associated lymphoid tissue (MALT) lymphoma: normal MALT1 products are reduced by half because the translocated allele generates API2-MALT1. As the API2-MALT1 cannot export nuclear BCL10, the reduction of normal MALT1 proteins will be less efficient in the process of BCL10 export, thus resulting in nuclear retention of BCL10. (**c**) t(1;14) MALT lymphoma: overexpressed BCL10 results in nuclear retention because of a relative shortage of MALT1. Reproduced with permission from: Nakagawa et al. (2006)

(q21;q21) is the most commonly found chromosomal translocation (Streubel et al. 2004). In ocular lymphomas it is rarely found; however, one study by Ye et al found that t(11;18)(q21;q21) occurred in 19 % of conjunctival MALT lymphomas and 14 % of orbital MALT lymphomas (Ye et al. 2003a). Streubel et al tested 37 ocular adnexal lymphomas with FISH, of which only 1 (3 %) was positive for t(11;18), and many other studies have found that this translocation has not been found in any of their ocular adnexal MALT lymphoma cases (Streubel et al. 2004; Remstein et al. 2006; Tanimoto et al. 2006).

One study by Remstein et al found that non-gastric MALT lymphomas with either t(11;18)+ status or aneuploidy were associated with an increased risk of recurrence (Remstein et al. 2002). This study looked at 133 primary MALT lymphomas, of which 24 were from the ocular adnexa, and using PCR and FISH investigated the translocation frequency of all four currently known translocations in MALT lymphomas. In concordance with other studies, they found that all the translocations were mutually exclusive, and aneuploidy was rarely found in t(11;18)+ MALT lymphomas.

The translocation t(11;18) is much more common in gastric MALT lymphomas; however, studies have shown that the translocation is associated with H. pylori-negative gastric MALT lymphoma (Ye et al. 2003b) and that the translocation is seen at a very high frequency (75 %) in gastric MALT lymphoma that is resistant to H. pylori eradication therapy (Liu et al. 2001a). It is therefore likely that the presence of this translocation in gastric MALT lymphomas marks out more aggressive disease, and it may be that with further research into this translocation in the ocular adnexal area, similar correlations could be made. An interesting study by Liu et al found that t(11;18) was significantly associated with more advanced gastric MALT lymphomas, as was increased nuclear BCL-10 expression (Liu et al. 2001b). The correlation between the clinical stage of the gastric MALT lymphomas and their nuclear expression of BCL-10 was very significant. Although t(11;18) results in the formation of fusion proteins from the linkage of API2-MALT1 and affects the NF-κB pathway in this manner, the possibility that a simultaneous nuclear BCL-10 overexpression causes dysregulation of apoptosis and the concomitant effect of these changes leads to a relatively more aggressive disease course has to be considered.

### 3.15.2 t(14;18)(q32;q21)

This translocation involves the IgH promoter on chromosome 14 at q32 being moved to the vicinity of the MALT1 gene found on chromosome 18 at the q21 locus. The juxtaposition of the IgH promoter with the MALT1 leads to dysregulation of MALT1 gene expression (Streubel et al. 2003). MALT1 works in conjunction with BCL-10 to augment the activation of the NF-κB pathway, leading to the development of lymphoma (Lucas et al. 2001).

Although it is the most frequently encountered translocation in ocular adnexal lymphomas, the frequency of t(14;18) varies in studies of ocular MALT lymphomas from 3 to 38 % (Streubel et al. 2003; Streubel et al. 2004; Tanimoto et al. 2006). The higher frequencies were detected in European and American studies, with the lowest frequencies coming from Japan. This considerable geographic variability in frequency of the t(14;18) translocation may reflect variability in genetics or geographic pathogens.

A 2005 study by Ye et al investigated whether there was any correlation between cytoplasmic expression of BCL-10 or MALT1 and the t(14;18) translocation (Ye et al. 2005). They examined 423 cases of MALT lymphoma, of which 73 were from the ocular adnexa. Of these, 7/73 were revealed to have t(11;18)+ and 5/73 were t(14;18)+. The study found that in all of the t(14:18)+ MALT lymphomas, the tumor cells showed a strong expression of cytoplasmic MALT1 and BCL-10 as tested by both immunohistochemistry and RT-PCR. They also found using RT-PCR that MALT1 mRNA expression was significantly higher in t(14;18)+ MALT lymphomas than in those with other translocations or those negative for any of the studied translocations. Although MALT1 expression is expected to be high in this translocation due to the effect of the IgH promoter on the MALT1 gene, the strong cytoplasmic expression of BCL-10 was not expected, and the authors hypothesized that this may be due to MALT1 interacting with BCL-10 and stabilizing it in the cytoplasm, thus leading to high levels of it accumulating in the cytoplasm of the tumor cells within t(14;18)+ MALT lymphomas. They concluded that as strong cytoplasmic expression of MALT1 and BCL-10 was characteristic of this translocation, immunohistochemistry for these two markers could be used as an initial screening tool for this genetic aberration in a MALT lymphoma, with the utilization of an interphase FISH test for confirmation.

### 3.15.3 t(1;14)(p22;q32)

The t(1;14)(p22;32) translocation affords the conjunction of the IgH promoter located at p22 on chromosome 1 with the BCL-10 gene found on chromosome 14 at the q32 locus. The result of this alignment is that the IgH promoter gene causes dysregulation of the BCL-10 gene, greatly increasing BCL-10 protein production (Lucas et al. 2001). A study by Wotherspoon et al looked at the molecular genetics of low-grade cases of MALT lymphoma with t(1;14)(p22;q32) and found that although these events were rare, they appeared to be recurrent (Wotherspoon et al. 1992). Further studies have found that the 1p22 abnormality associated with this translocation confers a more aggressive disease course than the typically indolent path of these lymphomas (Franco et al. 2006). The BCL-10 gene regulates apoptosis. A study by Willis et al investigated the involvement of BCL-10 in t(1;14)(p22;q32) of MALT lymphomas (Willis et al. 1999). They transfected 293 cells with BCL-10 and found that wild-type BCL-10 induced apoptosis but activated the NF-κB pathway. However, when they transfected the cells with the truncated BCL-10 gene (due to the t(1;14) translocation), they found that its NF-κB-activating properties remained intact; however, it did not exhibit any significant proapoptotic activity. This study concluded that the full-length BCL-10 protein was not vital for NF-κB activation but was absolutely necessary for BCL-10 to maintain its apoptotic function. Furthermore, the truncated BCL-10 was found to manifestly increase the number of transformed cell colonies and hasten their development.

### 3.15.4 t(3;14)(p14.1;q32)

Unlike the other translocations, t(3;14)(p14.1;q32) is found in DLBCL as well as MALT lymphomas

(Barrans et al. 2004; Brown et al. 2008). This translocation results in the IgH promoter gene exerting control over the forkhead box protein 1 (FOXP1) gene which is located at p14.1 on chromosome 3 (Streubel et al. 2005). The subsequent dysregulatory effect of IgH on the FOXP1 gene leads to overexpression of FOXP1. FOXP1 is considered to be an important component in B-cell development, thus overexpression of the protein inevitably leads to increased B-cell production, and several studies have linked high FOXP1 expression to a poor prognosis in both MALT and DLBCL (Sagaert et al. 2006; Han et al. 2009; Jiang et al. 2012). One study by Streubel et al looked at the prevalence of t(3;14) in MALT lymphomas from different sites (e.g., ocular adnexa, skin, thyroid) (Streubel et al. 2005). Of a total 91 cases of MALT lymphoma that had tested negative for the other three translocations, 9 (10 %) were found to harbor the t(3;14) abnormality. Of the 20 ocular adnexal cases, 4 (20 %) were found to have the translocation. The nine translocation positive cases were tested for the presence of other genetic aberrations with the results revealing 5/9 cases also had trisomy 3, one case having tetrasomy 18, and one case revealing trisomy 22. Furthermore, a further 38 MALT lymphomas that tested positive for one of the other translocations were all tested for the presence of t(3;14) and all found to be negative, indicating the mutually exclusive nature of the translocations.

### 3.15.5 Aneuploidy

The incidence of aneuploidy in MALT lymphomas is higher than the incidence of chromosomal translocations. The most commonly seen chromosomal aberrations in MALT lymphomas of the ocular adnexa involve chromosome 3 and/or 18. Typically these are trisomies, although tetrasomy of chromosome 18 has been noted. The incidence of trisomy 3 in MALT lymphomas from various tissue locations has been shown to have a high degree of variability, with incidence ranging from 11 to 85 % (Schiby et al. 2007). In studies specific to ocular adnexal lymphomas, the frequency of aneuploidy appears much lower – the incidence of trisomy 3 varying between 20 and 40 % and of trisomy 18 varying between 0 and 20 % (Matteucci et al. 2003). A review of 18 orbital lymphoid cell infiltrates by Schiby et al found that 68.7 % of cases exhibited trisomy 3 and 56.6 % had trisomy 18, and these numerical chromosomal aberrations were more common than translocations in the series (Schiby et al. 2007). They also found that trisomy 18 always occurred with trisomy 3 in their series, hypothesizing that trisomy 18 may be a secondary genetic aberration. Additionally, they found that the translocations t(11;18) and t(14;18) did not occur in the presence of the numerical chromosomal aberrations. This suggestion of possible mutual exclusivity contradicts findings from several other studies, which generally agree that concomitant chromosomal aberrations are rare with t(11;18) but are associated with t(14;18)+ MALT lymphomas (Schreuder et al. 2003; Streubel et al. 2003).

Tanimoto et al used FISH analysis to look at 34 cases of primary ocular adnexal lymphoma (Tanimoto et al. 2006). They found a similarly high percentage of cases of aneuploidy – trisomy 3 in 62 % of cases and trisomy 18 in 47 % of cases. This study also attempted to draw correlations between the clinical and histopathological findings and the genetic aberrations. They found that cases with trisomy 3 tended to occur in patients over the age of 50, whereas trisomy 18 tended to be found in younger patients. In addition trisomy 18 tended to occur in female patients, with the conjunctiva being the most frequently involved site. In terms of histopathology, trisomy 18 was significantly associated with a lower degree of nodularity of the tumor and an abundance of lymphoepithelial lesions. Furthermore, the cases in the study with trisomy 18 were found to have a significantly reduced recurrence-free survival. They did not find any associations between the presence of trisomy 3 in a tumor and its histopathological features.

## 3.16 Summary

In the diagnosis of PIOL and ocular and adnexal masses where a malignant neoplasm is suspected, histologic and cytologic evaluation is

considered the "gold standard." Not only does it allow evaluation of the cells and tissues but also aids further treatment decisions, e.g., the need for further biopsy/excision or risk of metastasis. Immunohistochemistry and flow cytometry are often readily available and can be helpful in delineating clonal populations. Although molecular genotyping has allowed some risk stratification and prognostication of ocular lymphomas, there is still a considerable amount of research to be undertaken in this area, particularly with regard to intraocular lymphomas.

In ocular adnexal lymphomas, the tissue biopsies usually provide adequate material for FFPE preparation; hence H&E and PAS for histologic and cytologic evaluation, coupled with immunostains judiciously chosen depending on the microscopic appearance of the tissue, should be the first line in diagnosis. If there is sufficient tissue, IHC/FC can also be very useful, particularly in more ambiguous cases, e.g., DLBCL/MALT. PCR for molecular analysis may be of benefit in some cases, for prognostic value as well as to aid treatment decisions, as shown by the previously mentioned study carried out by Tanimoto et al, where trisomy 18 showed a significantly reduced survival.

In PIOL, diagnostic vitrectomies often contain very limited amounts of cellular material. For this reason priority should be given to diagnostic histopathology and cytologic evaluation, with confirmatory adjunct studies such as IHC and flow cytometry utilized should there be adequate biopsy material. There are few studies of molecular analysis of intraocular lymphomas, so the relevance of this test may not be absolutely clear at this time. Cytokine analysis can be useful as an adjunct to other laboratory investigations; however, it is not considered a diagnostic test.

## References


Alizadeh AA, Eisen MB et al (2000) Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature 403(6769):503–511

Bakhoum SF, Danilova OV et al (2011) Chromosomal instability substantiates poor prognosis in patients with diffuse large B-cell lymphoma. Clin Cancer Res 17(24):7704–7711

Banks PM, Chan J et al (1992) Mantle cell lymphoma. A proposal for unification of morphologic, immunologic, and molecular data. Am J Surg Pathol 16(7):637–640

Bardenstein DS (1998) Intraocular lymphoma. Cancer Control 5(4):317–325

Barrans SL, Fenton JA et al (2004) Strong expression of FOXP1 identifies a distinct subset of diffuse large B-cell lymphoma (DLBCL) patients with poor outcome. Blood 104(9):2933–2935

Ben-Ezra D, Sahel JA et al (1989) Uveal lymphoid infiltrates: immunohistochemical evidence for a lymphoid neoplasia. Br J Ophthalmol 73(10):846–851

Berglund M, Thunberg U et al (2005) Evaluation of immunophenotype in diffuse large B-cell lymphoma and its impact on prognosis. Mod Pathol 18(8):1113–1120

Biagi JJ, Seymour JF (2002) Insights into the molecular pathogenesis of follicular lymphoma arising from analysis of geographic variation. Blood 99(12):4265–4275

Bosga-Bouwer AG, van Imhoff GW et al (2003) Follicular lymphoma grade 3B includes 3 cytogenetically defined subgroups with primary t(14;18), 3q27, or other translocations: t(14;18) and 3q27 are mutually exclusive. Blood 101(3):1149–1154

Brown PJ, Ashe SL et al (2008) Potentially oncogenic B-cell activation-induced smaller isoforms of FOXP1 are highly expressed in the activated B cell-like subtype of DLBCL. Blood 111(5):2816–2824

Buggage RR, Velez G et al (1999a) Primary intraocular lymphoma with a low interleukin 10 to interleukin 6 ratio and heterogeneous IgH gene rearrangement. Arch Ophthalmol 117(9):1239–1242

Buggage RR, Whitcup SM et al (1999b) Using interleukin 10 to interleukin 6 ratio to distinguish primary intraocular lymphoma and uveitis. Invest Ophthalmol Vis Sci 40(10):2462–2463

Carter SL, Eklund AC et al (2006) A signature of chromosomal instability inferred from gene expression profiles predicts clinical outcome in multiple human cancers. Nat Genet 38(9):1043–1048

Carugi A, Onnis A et al (2010) Geographic variation and environmental conditions as cofactors in Chlamydia psittaci association with ocular adnexal lymphomas: a comparison between Italian and African samples. Hematol Oncol 28(1):20–26

Cassoux N, Giron A et al (2007) IL-10 measurement in aqueous humor for screening patients with suspicion of primary intraocular lymphoma. Invest Ophthalmol Vis Sci 48(7):3253–3259

Cerroni L, Zochling N et al (1997) Infection by Borrelia burgdorferi and cutaneous B-cell lymphoma. J Cutan Pathol 24(8):457–461

Chan CC (2003) Molecular pathology of primary intraocular lymphoma. Trans Am Ophthalmol Soc 101: 275–292

Chan CC, Whitcup SM et al (1995) Interleukin-10 in the vitreous of patients with primary intraocular lymphoma. Am J Ophthalmol 120(5):671–673

Chang CC, McClintock S et al (2004) Immunohistochemical expression patterns of germinal center and activation B-cell markers correlate with prognosis

in diffuse large B-cell lymphoma. Am J Surg Pathol 28(4):464–470
Chanudet E, Zhou Y et al (2006) Chlamydia psittaci is variably associated with ocular adnexal MALT lymphoma in different geographical regions. J Pathol 209(3):344–351
Cho EY, Han JJ et al (2003) Clinicopathologic analysis of ocular adnexal lymphomas: extranodal marginal zone b-cell lymphoma constitutes the vast majority of ocular lymphomas among Koreans and affects younger patients. Am J Hematol 73(2):87–96
Choi JY, Kafkala C et al (2006) Primary intraocular lymphoma: a review. Semin Ophthalmol 21(3):125–133
Clarke CA, Glaser SL (2002) Changing incidence of non-Hodgkin lymphomas in the United States. Cancer 94(7):2015–2023
Cockerham GC, Hidayat AA et al (2000) Re-evaluation of "reactive lymphoid hyperplasia of the uvea": an immunohistochemical and molecular analysis of 10 cases. Ophthalmology 107(1):151–158
Coiffier B (2001) Diffuse large cell lymphoma. Curr Opin Oncol 13(5):325–334
Collina F, De Chiara A et al (2012) Chlamydia psittaci in ocular adnexa MALT lymphoma: a possible role in lymphomagenesis and a different geographical distribution. Infect Agent Cancer 7:8
Coupland SE (2013) Molecular pathology of lymphoma. Eye (Lond) 27:180–189
Coupland SE, Damato B (2006) Lymphomas involving the eye and the ocular adnexa. Curr Opin Ophthalmol 17(6):523–531
Coupland SE, Damato B (2008) Understanding intraocular lymphomas. Clin Experiment Ophthalmol 36(6):564–578
Coupland SE, Foss HD et al (1999) T-cell and T/natural killer-cell lymphomas involving ocular and ocular adnexal tissues: a clinicopathologic, immunohistochemical, and molecular study of seven cases. Ophthalmology 106(11):2109–2120
Coupland SE, Foss HD et al (2002) Extranodal marginal zone B cell lymphomas of the uvea: an analysis of 13 cases. J Pathol 197(3):333–340
Coupland SE, Heimann H et al (2004a) Primary intraocular lymphoma: a review of the clinical, histopathological and molecular biological features. Graefes Arch Clin Exp Ophthalmol 242(11):901–913
Coupland SE, Hellmich M et al (2004b) Prognostic value of cell-cycle markers in ocular adnexal lymphoma: an assessment of 230 cases. Graefes Arch Clin Exp Ophthalmol 242(2):130–145
Coupland SE, Anastassiou G et al (2005) Primary intraocular lymphoma of T-cell type: report of a case and review of the literature. Graefes Arch Clin Exp Ophthalmol 243(3):189–197
Coupland SE, Chan CC et al (2009) Pathophysiology of retinal lymphoma. Ocul Immunol Inflamm 17(4):227–237
Cummings TJ, Stenzel TT et al (2005) Primary intraocular T-cell-rich large B-cell lymphoma. Arch Pathol Lab Med 129(8):1050–1053
Dagklis A, Ponzoni M et al (2012) Immunoglobulin gene repertoire in ocular adnexal lymphomas: hints on the nature of the antigenic stimulation. Leukemia 26(4):814–821
de Jong D (2005) Molecular pathogenesis of follicular lymphoma: a cross talk of genetic and immunologic factors. J Clin Oncol 23(26):6358–6363
Decaudin D, Dolcetti R et al (2008) Variable association between Chlamydophila psittaci infection and ocular adnexal lymphomas: methodological biases or true geographical variations? Anticancer Drugs 19(8):761–765
Dierlamm J, Baens M et al (1999) The apoptosis inhibitor gene API2 and a novel 18q gene, MLT, are recurrently rearranged in the t(11;18)(q21;q21) associated with mucosa-associated lymphoid tissue lymphomas. Blood 93(11):3601–3609
Duker JS, Shields JA et al (1987) Intraocular large cell lymphoma presenting as massive thickening of the uveal tract. Retina 7(1):41–45
Economou MA, Kopp ED et al (2007) Mantle cell lymphoma of the iris. Acta Ophthalmol Scand 85(3):341–343
Elner VM, Hidayat AA et al (1986) Neoplastic angioendotheliomatosis. A variant of malignant lymphoma immunohistochemical and ultrastructural observations of three cases. Ophthalmology 93(9):1237–1245
Ferreri AJ, Guidoboni M et al (2004) Evidence for an association between Chlamydia psittaci and ocular adnexal lymphomas. J Natl Cancer Inst 96(8):586–594
Ferreri AJ, Govi S et al (2012) Chlamydophila psittaci eradication with doxycycline as first-line targeted therapy for ocular adnexae lymphoma: final results of an international phase II trial. J Clin Oncol 30(24):2988–2994
Ferry JA, Yang WI et al (1996) CD5+ extranodal marginal zone B-cell (MALT) lymphoma. A low grade neoplasm with a propensity for bone marrow involvement and relapse. Am J Clin Pathol 105(1):31–37
Ferry JA, Fung CY et al (2007) Lymphoma of the ocular adnexa: a study of 353 cases. Am J Surg Pathol 31(2):170–184
Franco R, Camacho FI et al (2006) Nuclear bcl10 expression characterizes a group of ocular adnexa MALT lymphomas with shorter failure-free survival. Mod Pathol 19(8):1055–1067
Fredrick DR, Char DH et al (1989) Solitary intraocular lymphoma as an initial presentation of widespread disease. Arch Ophthalmol 107(3):395–397
Freedman AS (2005) Biology and management of histologic transformation of indolent lymphoma. Hematology Am Soc Hematol Educ Program: 2005:314–320
Freeman C, Berg JW et al (1972) Occurrence and prognosis of extranodal lymphomas. Cancer 29(1):252–260
Fung CY, Tarbell NJ et al (2003) Ocular adnexal lymphoma: clinical behavior of distinct World Health Organization classification subtypes. Int J Radiat Oncol Biol Phys 57(5):1382–1391

Godon A, Moreau A et al (2003) Is t(14;18)(q32;q21) a constant finding in follicular lymphoma? An interphase FISH study on 63 patients. Leukemia 17(1):255–259

Goldey SH, Stern GA et al (1989) Immunophenotypic characterization of an unusual T-cell lymphoma presenting as anterior uveitis. A clinicopathologic case report. Arch Ophthalmol 107(9):1349–1353

Groves FD, Linet MS et al (2000) Cancer surveillance series: non-Hodgkin's lymphoma incidence by histologic subtype in the United States from 1978 through 1995. J Natl Cancer Inst 92(15):1240–1251

Han SL, Wu XL et al (2009) FOXP1 expression predicts polymorphic histology and poor prognosis in gastric mucosa-associated lymphoid tissue lymphomas. Dig Surg 26(2):156–162

Hans CP, Weisenburger DD et al (2004) Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood 103(1):275–282

Hochman J, Assaf N et al (2001) Entry routes of malignant lymphoma into the brain and eyes in a mouse model. Cancer Res 61(13):5242–5247

Hormigo A, Abrey L et al (2004) Ocular presentation of primary central nervous system lymphoma: diagnosis and treatment. Br J Haematol 126(2):202–208

Hykin PG, Shields JA et al (1996) Recurrent systemic B cell lymphoma of the iris. Br J Ophthalmol 80(10):929

Jaffe ES, Harris N, Stein H, Vardiman JW (2001) Pathology and genetics of tumours of haematopoietic and lymphoid tissues. IARC Press, Lyon

Jardin F, Gaulard P et al (2002) Follicular lymphoma without t(14;18) and with BCL-6 rearrangement: a lymphoma subtype with distinct pathological, molecular and clinical characteristics. Leukemia 16(11):2309–2317

Jensen OA, Johansen S et al (1994) Intraocular T-cell lymphoma mimicking a ring melanoma. First manifestation of systemic disease. Report of a case and survey of the literature. Graefes Arch Clin Exp Ophthalmol 232(3):148–152

Jiang W, Li L et al (2012) Expression of FOXP1 in mucosa-associated lymphoid tissue lymphoma suggests a large tumor cell transformation and predicts a poorer prognosis in the positive thyroid patients. Med Oncol 29(5):3352–3359

Kimura K, Usui Y et al (2012) Clinical features and diagnostic significance of the intraocular fluid of 217 patients with intraocular lymphoma. Jpn J Ophthalmol 56(4):383–389

Kramer MH, Hermans J et al (1998) Clinical relevance of BCL2, BCL6, and MYC rearrangements in diffuse large B-cell lymphoma. Blood 92(9):3152–3162

Kumar SR, Gill PS et al (1994) Human T-cell lymphotropic virus type I-associated retinal lymphoma. A clinicopathologic report. Arch Ophthalmol 112(7):954–959

Kurtin PJ, Pinkus GS (1985) Leukocyte common antigen–a diagnostic discriminant between hematopoietic and nonhematopoietic neoplasms in paraffin sections using monoclonal antibodies: correlation with immunologic studies and ultrastructural localization. Hum Pathol 16(4):353–365

Lecuit M, Abachin E et al (2004) Immunoproliferative small intestinal disease associated with Campylobacter jejuni. N Engl J Med 350(3):239–248

Leich E, Salaverria I et al (2009) Follicular lymphomas with and without translocation t(14;18) differ in gene expression profiles and genetic alterations. Blood 114(4):826–834

Levy-Clarke GA, Buggage RR et al (2002) Human T-cell lymphotropic virus type-1 associated t-cell leukemia/lymphoma masquerading as necrotizing retinal vasculitis. Ophthalmology 109(9): 1717–1722

Levy-Clarke GA, Chan CC et al (2005) Diagnosis and management of primary intraocular lymphoma. Hematol Oncol Clin North Am 19(4):739–749, viii

Li JY, Gaillard F et al (1999) Detection of translocation t(11;14)(q13;q32) in mantle cell lymphoma by fluorescence in situ hybridization. Am J Pathol 154(5):1449–1452

Liu H, Ruskon-Fourmestraux A et al (2001a) Resistance of t(11;18) positive gastric mucosa-associated lymphoid tissue lymphoma to Helicobacter pylori eradication therapy. Lancet 357(9249):39–40

Liu H, Ye H et al (2001b) T(11;18)(q21;q21) is associated with advanced mucosa-associated lymphoid tissue lymphoma that expresses nuclear BCL10. Blood 98(4):1182–1187

Liu MM, Furusato E et al (2010) Ocular manifestations and pathology of adult T-cell leukemia/lymphoma associated with human T-lymphotropic virus type 1. Rare Tumors 2(4):e63

Looi A, Gascoyne RD et al (2005) Mantle cell lymphoma in the ocular adnexal region. Ophthalmology 112(1):114–119

Lucas PC, Yonezumi M et al (2001) Bcl10 and MALT1, independent targets of chromosomal translocation in malt lymphoma, cooperate in a novel NF-kappa B signaling pathway. J Biol Chem 276(22): 19012–19019

Mashayekhi A, Shields CL et al (2013) Iris involvement by lymphoma: a review of 13 cases. Clin Experiment Ophthalmol 41:19–26

Matteucci C, Galieni P et al (2003) Typical genomic imbalances in primary MALT lymphoma of the orbit. J Pathol 200(5):656–660

Montoto S, Fitzgibbon J (2011) Transformation of indolent B-cell lymphomas. J Clin Oncol 29(14): 1827–1834

Moslehi R, Devesa SS et al (2006) Rapidly increasing incidence of ocular non-hodgkin lymphoma. J Natl Cancer Inst 98(13):936–939

Muris JJ, Meijer CJ et al (2006) Immunohistochemical profiling based on Bcl-2, CD10 and MUM1 expression improves risk stratification in patients with primary nodal diffuse large B cell lymphoma. J Pathol 208(5):714–723

Nakagawa M, Seto M et al (2006) Molecular pathogenesis of MALT lymphoma: two signaling pathways underlying the antiapoptotic effect of API2-MALT1 fusion protein. Leukemia 20(6):929–936

O'Keefe JS, Sippy BD et al (2002) Anterior chamber infiltrates associated with systemic lymphoma: report of two cases and review of the literature. Ophthalmology 109(2):253–257

Ohta K, Sano K et al (2009) Cytokine and molecular analyses of intraocular lymphoma. Ocul Immunol Inflamm 17(3):142–147

Ott G, Katzenberger T et al (2002) Cytomorphologic, immunohistochemical, and cytogenetic profiles of follicular lymphoma: 2 types of follicular lymphoma grade 3. Blood 99(10):3806–3812

Pe'er J, Hochberg FH et al (2009) Clinical review: treatment of vitreoretinal lymphoma. Ocul Immunol Inflamm 17(5):299–306

Ponzoni M, Ferreri AJ et al (2008) Chlamydia infection and lymphomas: association beyond ocular adnexal lymphomas highlighted by multiple detection methods. Clin Cancer Res 14(18):5794–5800

Ponzoni M, Bonetti F et al (2011) Central nervous system marginal zone B-cell lymphoma associated with Chlamydophila psittaci infection. Hum Pathol 42(5):738–742

Poulsen CB, Borup R et al (2005) Microarray-based classification of diffuse large B-cell lymphoma. Eur J Haematol 74(6):453–465

Ralli M, Goldman JW et al (2009) Intraocular involvement of mycosis fungoides. Arch Ophthalmol 127(3):343–345

Rasmussen P, Sjo LD et al (2009) Mantle cell lymphoma in the orbital and adnexal region. Br J Ophthalmol 93(8):1047–1051

Remstein ED, Kurtin PJ et al (2002) Mucosa-associated lymphoid tissue lymphomas with t(11;18)(q21;q21) and mucosa-associated lymphoid tissue lymphomas with aneuploidy develop along different pathogenetic pathways. Am J Pathol 161(1):63–71

Remstein ED, Dogan A et al (2006) The incidence and anatomic site specificity of chromosomal translocations in primary extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) in North America. Am J Surg Pathol 30(12):1546–1553

Rosenwald A, Wright G et al (2002) The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. N Engl J Med 346(25):1937–1947

Saga T, Ohno S et al (1984) Ocular involvement by a peripheral T-cell lymphoma. Arch Ophthalmol 102(3):399–402

Sagaert X, de Paepe P et al (2006) Forkhead box protein P1 expression in mucosa-associated lymphoid tissue lymphomas predicts poor prognosis and transformation to diffuse large B-cell lymphoma. J Clin Oncol 24(16):2490–2497

Schiby G, Polak-Charcon S et al (2007) Orbital marginal zone lymphomas: an immunohistochemical, polymerase chain reaction, and fluorescence in situ hybridization study. Hum Pathol 38(3):435–442

Schreuder MI, Hoeve MA et al (2003) Mutual exclusion of t(11;18)(q21;q21) and numerical chromosomal aberrations in the development of different types of primary gastric lymphomas. Br J Haematol 123(4):590–599

Sekiguchi N, Kobayashi Y et al (2005) Follicular lymphoma subgrouping by fluorescence in situ hybridization analysis. Cancer Sci 96(2):77–82

Sen HN, Bodaghi B et al (2009) Primary intraocular lymphoma: diagnosis and differential diagnosis. Ocul Immunol Inflamm 17(3):133–141

Seto M (2013) Cyclin D1-negative mantle cell lymphoma. Blood 121(8):1249–1250

Shen DF, Zhuang Z et al (1998) Utility of microdissection and polymerase chain reaction for the detection of immunoglobulin gene rearrangement and translocation in primary intraocular lymphoma. Ophthalmology 105(9):1664–1669

Shibata K, Shimamoto Y et al (1997) Ocular manifestations in adult T-cell leukemia/lymphoma. Ann Hematol 74(4):163–168

Shimonagano Y, Nakao K et al (2006) Iris involvement in natural killer/T-cell lymphoma. Jpn J Ophthalmol 50(6):557–558

Sjo LD, Poulsen CB et al (2007) Profiling of diffuse large B-cell lymphoma by immunohistochemistry: identification of prognostic subgroups. Eur J Haematol 79(6):501–507

Spraul CW, Grossniklaus HE (1997) Analysis of 24,444 surgical specimens accessioned over 55 years in an ophthalmic pathology laboratory. Int Ophthalmol 21(5):283–304

Stacy RC, Jakobiec FA et al (2012) Diffuse large B-cell lymphoma of the orbit: clinicopathologic, immunohistochemical, and prognostic features of 20 cases. Am J Ophthalmol 154(1):87–98 e81

Stoffel A, Chaurushiya M et al (2004) Activation of NF-kappaB and inhibition of p53-mediated apoptosis by API2/mucosa-associated lymphoid tissue 1 fusions promote oncogenesis. Proc Natl Acad Sci U S A 101(24):9079–9084

Streubel B, Lamprecht A et al (2003) T(14;18)(q32;q21) involving IGH and MALT1 is a frequent chromosomal aberration in MALT lymphoma. Blood 101(6):2335–2339

Streubel B, Simonitsch-Klupp I et al (2004) Variable frequencies of MALT lymphoma-associated genetic aberrations in MALT lymphomas of different sites. Leukemia 18(10):1722–1726

Streubel B, Vinatzer U et al (2005) T(3;14)(p14.1;q32) involving IGH and FOXP1 is a novel recurrent chromosomal aberration in MALT lymphoma. Leukemia 19(4):652–658

Sugita S, Takase H et al (2006) Intraocular soluble IL-2 receptor alpha in a patient with adult T cell leukaemia with intraocular invasion. Br J Ophthalmol 90(9):1204–1206

Sugita S, Takase H et al (2009) Diagnosis of intraocular lymphoma by polymerase chain reaction analysis and cytokine profiling of the vitreous fluid. Jpn J Ophthalmol 53(3):209–214

Swerdlow SH (2013) Lymphoma classification and the tools of our trade: an introduction to the 2012 USCAP long course. Mod Pathol 26(Suppl 1):S1–S14

Swerdlow SH, Campo E, Harris NL et al (eds) (2008a) WHO classification of tumours of haematopoietic and lymphoid tissues. IARC Press, Lyon

Swerdlow SH, Campo E, Seto M et al (2008b) Mantle cell lymphoma. In: Campo E, Swerdlow SH, Harris NL (eds) WHO classification of tumours of haematopoietic and lymphoid tissues. IARC Press, Lyon, pp 229–232

Takada S, Yoshino T et al (2003) Involvement of the chromosomal translocation t(11;18) in some mucosa-associated lymphoid tissue lymphomas and diffuse large B-cell lymphomas of the ocular adnexa: evidence from multiplex reverse transcriptase-polymerase chain reaction and fluorescence in situ hybridization on using formalin-fixed, paraffin-embedded specimens. Mod Pathol 16(5):445–452

Tanimoto K, Sekiguchi N et al (2006) Fluorescence in situ hybridization (FISH) analysis of primary ocular adnexal MALT lymphoma. BMC Cancer 6:249

Uren AG, O'Rourke K et al (2000) Identification of paracaspases and metacaspases: two ancient families of caspase-like proteins, one of which plays a key role in MALT lymphoma. Mol Cell 6(4):961–967

Vaandrager JW, Schuuring E et al (1996) Direct visualization of dispersed 11q13 chromosomal translocations in mantle cell lymphoma by multicolor DNA fiber fluorescence in situ hybridization. Blood 88(4):1177–1182

Velez G, de Smet MD et al (2000) Iris involvement in primary intraocular lymphoma: report of two cases and review of the literature. Surv Ophthalmol 44(6):518–526

Verbraeken HE, Hanssens M et al (1997) Ocular non-Hodgkin's lymphoma: a clinical study of nine cases. Br J Ophthalmol 81(1):31–36

Weaver BA, Cleveland DW (2007) Aneuploidy: instigator and inhibitor of tumorigenesis. Cancer Res 67(21):10103–10105

Wenzel C, Dieckmann K et al (2001) CD5 expression in a lymphoma of the mucosa-associated lymphoid tissue (MALT)-type as a marker for early dissemination and aggressive clinical behaviour. Leuk Lymphoma 42(4):823–829

Whitcup SM, Stark-Vancs V et al (1997) Association of interleukin 10 in the vitreous and cerebrospinal fluid and primary central nervous system lymphoma. Arch Ophthalmol 115(9):1157–1160

Wick MR, Mills SE et al (1986) Reassessment of malignant "angioendotheliomatosis". Evidence in favor of its reclassification as "intravascular lymphomatosis". Am J Surg Pathol 10(2):112–123

Willis TG, Jadayel DM et al (1999) Bcl10 is involved in t(1;14)(p22;q32) of MALT B cell lymphoma and mutated in multiple tumor types. Cell 96(1):35–45

Wolf LA, Reed GF et al (2003) Vitreous cytokine levels. Ophthalmology 110(8):1671–1672

Wotherspoon AC, Ortiz-Hidalgo C et al (1991) Helicobacter pylori-associated gastritis and primary B-cell gastric lymphoma. Lancet 338(8776):1175–1176

Wotherspoon AC, Doglioni C et al (1992) Low-grade gastric B-cell lymphoma of mucosa-associated lymphoid tissue (MALT): a multifocal disease. Histopathology 20(1):29–34

Wrotnowski U, Mills SE et al (1985) Malignant angioendotheliomatosis. An angiotropic lymphoma? Am J Clin Pathol 83(2):244–248

Ye H, Liu H et al (2003a) Variable frequencies of t(11;18)(q21;q21) in MALT lymphomas of different sites: significant association with CagA strains of H pylori in gastric MALT lymphoma. Blood 102(3): 1012–1018

Ye H, Liu H et al (2003b) High incidence of t(11;18)(q21;q21) in Helicobacter pylori-negative gastric MALT lymphoma. Blood 101(7):2547–2550

Ye H, Gong L et al (2005) MALT lymphoma with t(14;18)(q32;q21)/IGH-MALT1 is characterized by strong cytoplasmic MALT1 and BCL10 expression. J Pathol 205(3):293–301

Zha H, Raffeld M et al (2004) Similarities of prosurvival signals in Bcl-2-positive and Bcl-2-negative follicular lymphomas identified by reverse phase protein microarray. Lab Invest 84(2):235–244

# Ocular and Adnexal Lymphoma: Epidemiological Aspects

4

Jin Sook Yoon, Christopher Seungkyu Lee, and Sungchul Lee

## 4.1 Incidence of NHL and OAL

The incidence of non-Hodgkin's lymphoma (NHL) has doubled over the past two decades in the USA and most other Westernized countries (Groves et al. 2000; Howe et al. 2001; Clarke and Glaser 2002; Adamson et al. 2007). In the USA, the rates of NHL increased by 77 % in black males and by 53 % in white males and by 39 and 33 % among black and white females, respectively, from the early 1980s to the mid-1990s (Groves et al. 2000). Lymphoma has recently been estimated to be the fifth most common occurring cancer both in men and women in the USA, and there are approximately 19 new cases per 100,000 persons in the USA each year (Greenlee et al. 2001; Fisher and Fisher 2004). The incidence of systemic NHL is estimated to be 43,000 cases per year in the USA, rising 4 % per year, and about one half of cases are thought to be due to changes in classification as well as an increase in AIDS and better reporting tumor cases (Fisher and Fisher 2004; Bardenstein 2005). Similarly in European countries, the incidence of NHL was reported to be increased over 4 % annually between 1985 and 1992, with a higher rate of incidence in males than in females (Cartwright et al. 1999). More recently, trends of a consistent increase in the estimated incidence of NHL in both sexes were found, alongside a decrease in HL in 13 European countries for which the registry data are considered reliable over a sufficiently extended time period (Adamson et al. 2007). This increase has been also found in Asia including India, Japan, and Singapore and in South America including Brazil and Colombia (Devesa and Fears 1992).

The vast majority of lymphoproliferative lesions in the ocular adnexa are represented by the NHL group, whereas Hodgkin's lymphoma rarely involves this area. The majority of OALs are B-cell NHL, and these are predominantly extranodal marginal zone lymphoma (ENMZL) of mucosa-associated lymphoid tissue (MALT) type (Bardenstein 2005; McKelvie et al. 2001; Coupland et al. 2002; Coupland and Damato 2006; Jakobiec 2008; Stefanovic and Lossos 2009; Lauer 2000). Data on incidence of OAL is relatively sparse. OALs are a heterogenous group of lymphomas, accounting for 8 % of extranodal lymphoma (Freeman et al. 1972). Of the 314 primary orbital malignancies identified in the Florida Cancer Data System registry from 1981 to 1993, 55 % of malignant orbital tumors were lymphoma, and lymphoma showed the greatest rise in annual incidence (Margo and Mulla 1998). From 1975 to 2001, there was a rapid and steady increase in incidence of ocular adnexal NHL, with annual increase of 6.2 and 6.5 % among white males and females, respectively, with no evidence of peaking from an incidence data

J.S. Yoon, MD • C.S. Lee, MD • S. Lee, MD (✉)
Department of Ophthalmology, Institute of Vision Research, Yonsei University College of Medicine, Severance Hospital, 50 Yonsei-ro, Sodaemungu, Seoul 120-752, Korea
e-mail: sunglee@yuhs.ac

A.D. Singh (ed.), *Ocular and Adnexal Lymphoma*, Essentials in Ophthalmology,
DOI 10.1007/978-3-642-38499-8_4, © Springer-Verlag Berlin Heidelberg 2014

from 12 Surveillance, Epidemiology, and End Results (SEER) program (Moslehi et al. 2006). There was an increased incidence rate of OAL for whole Danish population from 1980 to 2005, corresponding to an annual average increase of 3.4 % (Sjo 2009).

Of the 965 orbital space-occupying lesions, biopsied by Danish pathological departments during the period 1974–1997, the most frequent orbital lesion in adults was malignant lymphoma, accounting for 14.9 % (Johansen et al. 2000). Among 1,264 consecutive patients referred to an ocular oncology service in Wills Eye Hospital due to space-occupying orbital lesions over a 30-year period, malignant lymphoma was the most common malignancy in older patients, representing 10 % of cases (Shields et al. 2004).

### 4.1.1 Primary and Secondary OAL

OAL is considered primary if the tumor involves the ocular adnexa alone and secondary if another site is involved by an identical type of lymphoma. While the most common primary OAL is the MALT-type lymphoma (Coupland et al. 1998), most of the secondary OALs are usually associated with more aggressive histologic subtypes (Esmaeli et al. 2002).

The majority of OALs are primary extranodal lymphomas, and secondary OALs with systemic lymphomas account for approximately 10–32 % in most series (McKelvie et al. 2001; Coupland et al. 1998; Knowles et al. 1990; Johnson et al. 1999; White et al. 1995; Jenkins et al. 2000; Fung et al. 2003; Meunier et al. 2004; Sullivan et al. 2005; Auw-Haedrich et al. 2001; Coupland et al. 2004; Cho et al. 2003; Ferry et al. 2007). In an autopsy series of 1,269 patients with systemic lymphoma, ocular adnexal involvement had developed in a total of 1.3 % (Rosenberg et al. 1961). Secondary ocular involvement occurs in approximately 2–5 % of patients with systemic NHL (Stefanovic and Lossos 2009; Bairey et al. 1994). The true incidence of secondary OAL may be underestimated and closely depends on the length of follow-up, as systemic NHL increases in incidence over time, with lymph nodes and bone marrow being the most common systemic locations.

### 4.1.2 Age and Gender

Between 1978 and 1995, the annual age-adjusted incidence rates of NHL for white and black males were 17.1 and 11.5 per 100,000, respectively (Fisher and Fisher 2004). For white and black women, the rates were 12.6 and 7.4 per 100,000 (Groves et al. 2000). Incidence of NHL rises exponentially with age. In persons over the age of 65, the incidence of disease rapidly increases to 68/100,000 (Fisher and Fisher 2004).

Patients presenting with OAL are mostly commonly seen in the fifth to seventh decade of life (median age, -65 years), with female predominance (male/female = 1:1.5–2) (Coupland et al. 2002; Sjo 2009; Ferry et al. 2007; Bernardini and Bazzan 2007). OAL is reported as the most common orbital malignancy in senior adults more than 60 years of age, with an incidence of 24 % in this group of patients (Demirci et al. 2002). In contrast, studies in Korean populations reveal a significantly younger age (median, -46 years) at time of diagnosis, with male rather than female predominance (Cho et al. 2003; Yoon et al. 2007; Oh and Kim 2007).

### 4.1.3 Pathologic Features

More than 95 % are of B-cell origin, and 80 % are low-grade lymphomas. Burkitt's and Hodgkin's lymphomas in the ocular adnexa are rare except in endemic regions. ENMZL of MALT type is the most common subtype of primary OAL, accounting for 35–80 % of cases, followed by follicular lymphoma (McKelvie et al. 2001; Coupland et al. 1998; Knowles et al. 1990; Johnson et al. 1999; White et al. 1995; Jenkins et al. 2000; Fung et al. 2003; Meunier et al. 2004; Sullivan et al. 2005; Auw-Haedrich et al. 2001; Coupland et al. 2004; Cho et al. 2003; Ferry et al. 2007). The proportion of MALT lymphoma among primary OAL is reported higher (80–90 %) in series from Japan and Korea and occurs

in younger patients (Cho et al. 2003; Yoon et al. 2007; Oh and Kim 2007; Tanimoto et al. 2007; Mannami et al. 2001). Follicular lymphoma and diffuse large B-cell lymphoma account for about 20 and 8 % of primary OAL in most Western series, with very small percentages of mantle cell lymphoma, small lymphocytic lymphoma, and lymphoplasmacytic lymphoma (McKelvie et al. 2001; Coupland et al. 1998; Knowles et al. 1990; Johnson et al. 1999; White et al. 1995; Jenkins et al. 2000; Fung et al. 2003; Meunier et al. 2004; Sullivan et al. 2005; Auw-Haedrich et al. 2001; Coupland et al. 2004). T-cell lymphoma affecting ocular adnexal involvement is rare. Woog et al. reported eight patients with natural killer/T-cell lymphomas of the orbit reporting from multicenters, and they found that ocular involvement was mostly secondary to invasion from adjacent nasal or paranasal involvement with mortality of 87.5 % (Woog et al. 2006).

### 4.1.4 Anatomic Sites

Anatomic sites that are usually affected by OAL include the orbit, the lacrimal sac, the conjunctiva, and/or the eyelids. OAL occurring in the orbit may affect the lacrimal gland, the extraocular muscles, and the orbital space diffusely. The frequency of involvement of periocular areas has been reported as 20–33 % in the conjunctiva, 46–74 % in the orbit, and 5–20 % in the eyelid (McKelvie et al. 2001; Coupland et al. 1998; Knowles et al. 1990).

### 4.1.5 Association with Immunodeficiency

Lymphoma occurring in immunodeficiency states is usually the high-grade B-cell type, involving extensive extranodal sites and associated with poor prognosis (Levine 1992). The incidence of lymphoma is found increased in frequency in patients with acquired immunodeficiency syndrome (AIDS). Orbital involvement of NHL has been reported in children and young male adults with AIDS, although rare in incidence (Nadal et al. 1994; Reifler et al. 1994; Antle et al. 1990).

### 4.1.6 Association with Microorganism Infection

Several reports have established the relationship between microorganism infection causing chronic antigen stimulation and lymphoma. Gastric MALT lymphomas were found to be associated with *Helicobacter pylori* infection in more than 90 % of cases (Wotherspoon et al. 1991). Treatment of antigenic instigation and gastritis with 1 week of triple antibiotic therapy seems to result in complete resolution of stage I lymphoma in more than 60 % of cases (Isaacson 1999). Using PCR amplification and southern blot hybridization, *Helicobacter pylori* DNA was found in 4 of the 5 conjunctival MALT lymphoma cells (Chan et al. 2004).

A strong correlation between OAL and chronic infection by *Chlamydia psittaci* (Cp) has been reported. Studies from Italy showed *Cp* DNA was detected by immunochemistry and PCR analysis in 87 % of the 40 specimens of MALT OAL, and complete or partial regression of the disease was demonstrated after Cp eradication in four of nine cases (Ferreri et al. 2004; 2005). Similar findings were found in Korea, where Cp DNA was detected in 26/33 samples (79 %) (Yoo et al. 2007). However, such an association was not found in cases of MALT OAL from South Florida and Rochester, New York, USA (Vargas et al. 2006; Rosado et al. 2006). In a report of PCR screening for infectious agents including Cp in 246 OAL of various subtypes from multiple countries, Cp was associated with MALT OAL, but that association was highly variable according to the geographical origin, with a frequency ranging from 11 % to nearly 50 % in different geographical areas examined (Chanudet et al. 2006). The prevalence of Cp was significantly higher in MALT lymphoma (22 %) than in non-lymphoproliferative disorder (10 %) and non-marginal zone lymphomas (9 %); however, the prevalence showed marked variation among the six geographical regions examined,

being most frequent in Germany (47 %) followed by the East Coast of the USA (35 %) and the Netherlands (29 %), but relatively low in Italy (13 %), the UK (12 %), and Southern China (11 %) (Chanudet et al. 2006). In a meta-analyses of the association between Cp and OAL and the response of OAL to antibiotics, of the 11 studies with 458 cases from ten different countries, overall Cp positivity was much low (23 %) and only 3 out of 11 studies showed 90 % of Cp positivity, suggesting a high variability in the association between Cp and OAL across geographic regions and even between studies from the same geographical regions (Husain et al. 2007).

A pathogenic link between hepatitis C (HCV) and MALT lymphomas has been suggested (Ascoli et al. 1998), but there is still a controversy. HCV seropositivity was detected in 13 % patients with OAL of MALT type, and HCV infection was significantly associated with more disseminated disease and aggressive behavior in OAL (Ferreri et al. 2006). However, in another report in southern Switzerland, HCV infection was not correlated with MALT-type histology (Zucca et al. 2000).

## 4.2 Primary Vitreoretinal Lymphoma

Primary vitreoretinal lymphoma (PVRL) is considered a variant of primary central nervous and has often been referred to as primary intraocular lymphoma (PIOL). It is mostly non-Hodgkin's diffuse large B-cell lymphoma. The major lesions of PVRL, as its name implies, are in the vitreous and the retina, manifesting as diffuse vitreous opacities and multiple retinal or subretinal creamy white lesions. The association between PVRL and PCNSL can be variable as CNS disease can manifest before, simultaneously with, or after ocular presentation. Once CNV is involved, the disease is highly fatal. CNS involvement in patients with PVRL usually appears several years after ocular presentation, affecting 50–80 % of patients (Peterson et al. 1993; Deangelis and Hormigo 2004; Akpek et al. 1999). Conversely, in patients with PCNSL, 15–25 % show ocular involvement at the time of diagnosis and about 25 % without ocular involvement will develop PVRL (Akpek et al. 1999; Rockwood et al. 1984; Chan 2003).

PVRL is one of the most important causes of masquerade syndrome, as it frequently masquerades as posterior uveitis. In a review of 828 consecutive uveitis patients in a referral center, 40 had masquerade syndromes. Of these 40, 13 were diagnosed with intraocular lymphoma, representing approximately 2 % of all uveitis and 33 % of masquerade syndromes (Rothova et al. 2001).

### 4.2.1 Incidence

PCNSL is estimated to represent 3–4 % of newly diagnosed CNS tumors, 1 % of non-Hodgkin's lymphoma, 1 % of intracranial tumors, and far less than 1 % of intraocular tumors (Hoffman et al. 2006; Bardenstein 1998). Age-adjusted incidence of PCNSL in the United States has been reported at 4–5 cases per million person-years or approximately 1,200 cases per year (Hoffman et al. 2006; Villano et al. 2011). In the United States, there has been a significant increase in the incidence of PCNSL from the 1960s, which peaked in the mid-1990s (Schabet 1999; Olson et al. 2002). According to National Cancer Institute Surveillance, Epidemiology, and End Results (SEER) database, the incidence of PCNSL rose from 0.27 per million in 1973 to10.0 per million in early 1990s, indicating a more than 30-fold increase in three decades (Schabet 1999; Corn et al. 1997). After its peak in the mid-1990s, there was a significant decline in the incidence of PCNSL between 1995 and 1999, and then the incidence has been relatively stable in recent years (Fig. 4.1) (Villano et al. 2011). This rise and subsequent leveling off in incidence is generally attributed to the increased incidence of human immunodeficiency virus (HIV)/AIDS and the subsequent development of highly active antiretroviral therapies (HAART) (Villano et al. 2011). PCNSL is one of the four AIDS-defining malignancies and HIV-infected patients carry a 3,600-fold increased risk of developing the disease compared with general population (Cote et al. 1996). The risk increases with duration of the disease, which is about 8, 11, 20, and 57 %

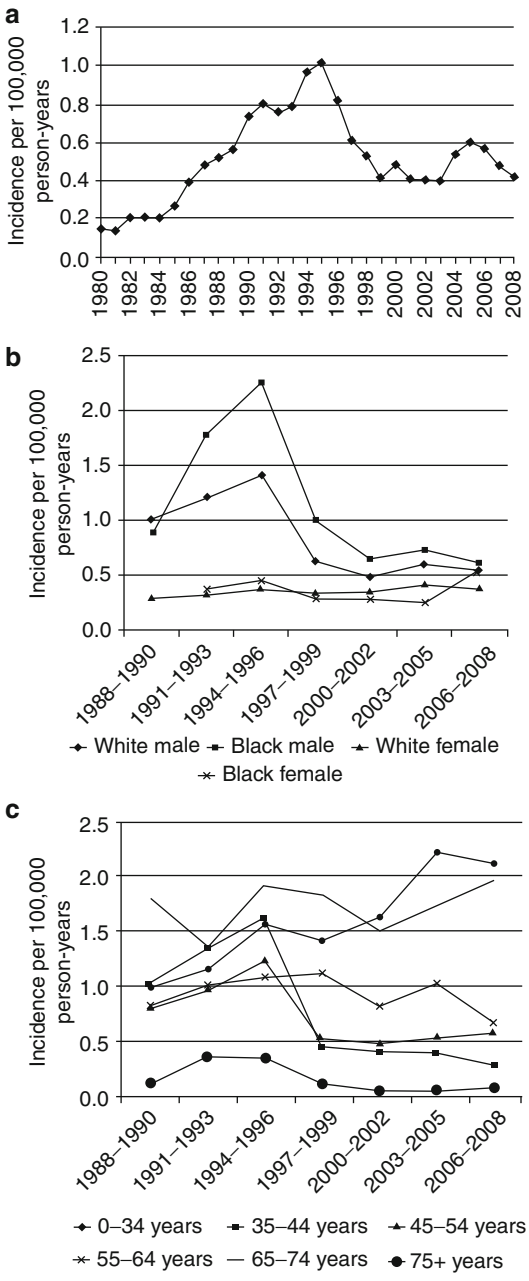


**Fig. 4.1** Incidence rate trends over time for primary CNS lymphoma (**a**), by gender and race (**b**) and age group at diagnosis (**c**) (SEER, 1998–2008). Reproduced with permission from Villano et al. (2011)

within 1, 2, 3, and more years, respectively, following the diagnosis of AIDS (Schabet 1999). An association has been suggested between PCNSL in AIDS patients and infectious agents, especially Epstein-Barr virus (EBV). EBV is usually detected in tumor cells in AIDS-related PCNSL, but not in PCNSL in immunocompetent patients (Bashir et al. 1993). Incidence of PCNSL in AIDS patients has fallen following HAART, declining from 5.33 per 1,000 person-years between 1991 and 1994 (pre-HAART) to 0.32 per 1,000 person-years after 1999 (post-HAART) according to one study (Wolf et al. 2005). There appears to be an increase in incidence of non-HIV/AIDS-related PCNSL as well, which is related to advanced age and improved diagnostic tools (Olson et al. 2002; Norden et al. 2011; Abrey 2009).

The increase in incidence of PCNSL has not been observed in other geographic regions. It reportedly has increased in the UK (Lutz and Coleman 1994), the Netherlands (van der Sanden et al. 2002), Norway (Haldorsen et al. 2007), Korea (Suh et al. 2002), and Japan (Makino et al. 2006) but has remained stable in Canada (Hao et al. 1999), Denmark (Krogh-Jensen et al. 1995), Scotland (Yau et al. 1996), Hong Kong (Au et al. 2000), and India (Sarkar et al. 2005). Whether there exists a true regional difference in PCNSL incidence is unknown.

### 4.2.2 Age

Advancing age is a risk factor for PCNSL, which can be attributed to a possible reduction in immunological surveillance or an increased number of somatic mutations that accrue over a lifetime (Villano et al. 2011). PCNSL typically occurs in elderly individuals of 50–70 years of age (Abrey 2009; Au et al. 2000; Coupland and Damato 2008). The disease can present at a younger age in an immunocompromised population; the AIDS patients are usually diagnosed with PCNSL in their 30s (Schabet 1999).

### 4.2.3 Gender

The incidence of PCNSL seems in general slightly higher in men (Villano et al. 2011; Schabet 1999; Abrey 2009), but some studies show no difference or higher incidence in women (Mochizuki and Singh 2009; Shibamoto et al.

2008; Cassoux et al. 2000). There is a strong male predilection of 7.38:1 in AIDS patients in Western countries, where homosexual intercourse is a major mode of transmission (Fine and Mayer 1993).

#### 4.2.4 Ethnicity

There is no definitive evidence for a racial difference in the incidence of PCNSL (Mochizuki and Singh 2009; Rubenstein et al. 2008; Surawicz et al. 1999). However, some studies suggest a slightly higher incidence in whites, especially in the elderly group (Villano et al. 2011; Abrey 2009). This may be consistent with lower incidence of systemic lymphomas in blacks (Ghafoor et al. 2002). A recent analysis of SEER database showed that in patients younger than 50 years of age, black men had greater than twice the incidence of white men (4.7 versus 2.2 per million person-years, respectively). However, this ratio was reversed in age group of 50 years or more, as white men had twice the incidence of black men (14.3 versus 5.7 per million person-years, respectively) (Villano et al. 2011). This discrepancy may be attributed to the fact that HIV/AIDS usually affects young adults and blacks more than any other race groups in the United States (Morris et al. 2006).

#### 4.2.5 Immunodeficiency and Immunosuppression

A strong risk factor for PCNSL is immunodeficiency, which includes congenital disorders, iatrogenic immunosuppression, and most importantly HIV infection, as described earlier. In organ transplant recipients, PCNSL is the second most common malignancy after skin cancer (Schabet 1999). Approximately 2 % of organ transplant recipients develop PCNSL (Patchell 1988). The risk increases with more intensive immunosuppressive treatment in these patients (Schabet 1999). Patients with congenital immune disorders carry a risk of 4 % to develop PCNSL (Schabet 1999). The median age at diagnosis in these patients is about 10 years (Filipovich et al. 1987).

### 4.3 Primary Uveal Lymphoma

The primary uveal lymphoma can be subdivided into iridal, ciliary body, and choroidal lymphomas. Primary choroidal lymphoma accounts for the most cases. The majority is low-grade B-cell lymphomas, most commonly extranodal marginal zone B-cell lymphoma (Coupland et al. 2002; Cockerham et al. 2000). It is believed that many cases that were diagnosed as reactive lymphoid hyperplasia in the past were in fact low-grade lymphoma, as they share similar clinical features (Cockerham et al. 2000).

#### 4.3.1 Incidence

The incidence of primary iridal and ciliary body lymphoma is unknown, as these tumors are exceptionally rare. Primary iridal lymphoma has been described in about ten cases (Coupland 2007). It appears that only one case of primary ciliary body lymphoma has been reported (Coupland and Damato 2008). Approximately 70–80 cases of primary choroidal lymphoma have been reported in literature (Coupland 2007).

#### 4.3.2 Age and Gender

Primary choroidal lymphoma typically affects patients in the age of 50–60s and men are more frequently affected (Chute et al. 2012).

### 4.4 Secondary Intraocular Manifestations of Systemic Lymphoma

Intraocular lymphoma secondary to disseminated systemic lymphoma most commonly affects the choroid, which can have a similar clinical presentation to primary choroidal lymphoma (Coupland and Damato 2008). Retinal involvement without

choroidal infiltration can occur, but very rarely (Coupland and Damato 2008). The most common systemic lymphoma subtype involving the choroid is diffuse large B-cell lymphoma, followed by multiple myeloma, extramedullary plasmacytoma, lymphoplasmacytic lymphoma/immunocytoma (including Waldenström's macroglobulinemia), and marginal zone B-cell lymphoma (MALT lymphoma) (Coupland and Damato 2008). Secondary iridal lymphoma is more common than primary iridal lymphoma (Coupland 2007). These can either be a secondary manifestation of primary choroidal lymphoma, PVRL/PCNSL, or systemic lymphoma.

## References


Abrey LE (2009) Primary central nervous system lymphoma. Curr Opin Neurol 22(6):675–680

Adamson P, Bray F, Costantini AS et al (2007) Time trends in the registration of Hodgkin and non-Hodgkin lymphomas in Europe. Eur J Cancer 43(2):391–401

Akpek EK, Ahmed I, Hochberg FH et al (1999) Intraocular-central nervous system lymphoma: clinical features, diagnosis, and outcomes. Ophthalmology 106(9):1805–1810

Antle CM, White VA, Horsman DE, Rootman J (1990) Large cell orbital lymphoma in a patient with acquired immune deficiency syndrome. Case report and review. Ophthalmology 97(11):1494–1498

Ascoli V, Lo Coco F, Artini M et al (1998) Extranodal lymphomas associated with hepatitis C virus infection. Am J Clin Pathol 109(5):600–609

Au WY, Chan AC, Srivastava G et al (2000) Incidence and pathology of primary brain lymphoma in Hong Kong Chinese patients. Leuk Lymphoma 37(1–2):175–179

Auw-Haedrich C, Coupland SE, Kapp A et al (2001) Long term outcome of ocular adnexal lymphoma subtyped according to the REAL classification. Revised European and American Lymphoma. Br J Ophthalmol 85(1):63–69

Bairey O, Kremer I, Rakowsky E et al (1994) Orbital and adnexal involvement in systemic non-Hodgkin's lymphoma. Cancer 73(9):2395–2399

Bardenstein DS (1998) Intraocular lymphoma. Cancer Control 5(4):317–325

Bardenstein DS (2005) Ocular adnexal lymphoma: classification, clinical disease, and molecular biology. Ophthalmol Clin North Am 18(1):187–197, x

Bashir R, Luka J, Cheloha K et al (1993) Expression of Epstein-Barr virus proteins in primary CNS lymphoma in AIDS patients. Neurology 43(11):2358–2362

Bernardini FP, Bazzan M (2007) Lymphoproliferative disease of the orbit. Curr Opin Ophthalmol 18(5):398–401

Cartwright R, Brincker H, Carli PM et al (1999) The rise in incidence of lymphomas in Europe 1985-1992. Eur J Cancer 35(4):627–633

Cassoux N, Merle-Beral H, Leblond V et al (2000) Ocular and central nervous system lymphoma: clinical features and diagnosis. Ocul Immunol Inflamm 8(4):243–250

Chan CC (2003) Primary intraocular lymphoma: clinical features, diagnosis, and treatment. Clin Lymphoma 4(1):30–31

Chan CC, Smith JA, Shen DF et al (2004) Helicobacter pylori (H. pylori) molecular signature in conjunctival mucosa-associated lymphoid tissue (MALT) lymphoma. Histol Histopathol 19(4):1219–1226

Chanudet E, Zhou Y, Bacon CM et al (2006) Chlamydia psittaci is variably associated with ocular adnexal MALT lymphoma in different geographical regions. J Pathol 209(3):344–351

Cho EY, Han JJ, Ree HJ et al (2003) Clinicopathologic analysis of ocular adnexal lymphomas: extranodal marginal zone b-cell lymphoma constitutes the vast majority of ocular lymphomas among Koreans and affects younger patients. Am J Hematol 73(2):87–96

Chute DJ, Biscotti CV, Singh AD (2012) Uveal lymphoma. Monogr Clin Cytol 21:31–43

Clarke CA, Glaser SL (2002) Changing incidence of non-Hodgkin lymphomas in the United States. Cancer 94(7):2015–2023

Cockerham GC, Hidayat AA, Bijwaard KE, Sheng ZM (2000) Re-evaluation of "reactive lymphoid hyperplasia of the uvea": an immunohistochemical and molecular analysis of 10 cases. Ophthalmology 107(1):151–158

Corn BW, Marcus SM, Topham A et al (1997) Will primary central nervous system lymphoma be the most frequent brain tumor diagnosed in the year 2000? Cancer 79(12):2409–2413

Cote TR, Manns A, Hardy CR et al (1996) Epidemiology of brain lymphoma among people with or without acquired immunodeficiency syndrome. AIDS/Cancer Study Group. J Natl Cancer Inst 88(10):675–679

Coupland SE (2007) Uveal lymphoproliferative tumors. In: Singh AD, Damato B, Pe'er J et al (eds) Clinical ophthalmic oncology. Elsevier, Inc, Philadelphia

Coupland SE, Damato B (2006) Lymphomas involving the eye and the ocular adnexa. Curr Opin Ophthalmol 17(6):523–531

Coupland SE, Damato B (2008) Understanding intraocular lymphomas. Clin Experiment Ophthalmol 36(6):564–578

Coupland SE, Krause L, Delecluse HJ et al (1998) Lymphoproliferative lesions of the ocular adnexa. Analysis of 112 cases. Ophthalmology 105(8):1430–1441

Coupland SE, Foss HD, Hidayat AA et al (2002) Extranodal marginal zone B cell lymphomas of the uvea: an analysis of 13 cases. J Pathol 197(3):333–340

Coupland SE, Hellmich M, Auw-Haedrich C et al (2004) Prognostic value of cell-cycle markers in ocular

adnexal lymphoma: an assessment of 230 cases. Graefes Arch Clin Exp Ophthalmol 242(2):130–145
Deangelis LM, Hormigo A (2004) Treatment of primary central nervous system lymphoma. Semin Oncol 31(5):684–692
Demirci H, Shields CL, Shields JA et al (2002) Orbital tumors in the older adult population. Ophthalmology 109(2):243–248
Devesa SS, Fears T (1992) Non-Hodgkin's lymphoma time trends: United States and international data. Cancer Res 52(19 Suppl):5432s–5440s
Esmaeli B, Ahmadi MA, Manning J et al (2002) Clinical presentation and treatment of secondary orbital lymphoma. Ophthal Plast Reconstr Surg 18(4):247–253
Ferreri AJ, Guidoboni M, Ponzoni M et al (2004) Evidence for an association between Chlamydia psittaci and ocular adnexal lymphomas. J Natl Cancer Inst 96(8):586–594
Ferreri AJ, Ponzoni M, Guidoboni M et al (2005) Regression of ocular adnexal lymphoma after Chlamydia psittaci-eradicating antibiotic therapy. J Clin Oncol 23(22):5067–5073
Ferreri AJ, Viale E, Guidoboni M et al (2006) Clinical implications of hepatitis C virus infection in MALT-type lymphoma of the ocular adnexa. Ann Oncol 17(5):769–772
Ferry JA, Fung CY, Zukerberg L et al (2007) Lymphoma of the ocular adnexa: a study of 353 cases. Am J Surg Pathol 31(2):170–184
Filipovich AH, Heinitz KJ, Robison LL, Frizzera G (1987) The immunodeficiency cancer registry. A research resource. Am J Pediatr Hematol Oncol 9(2):183–184
Fine HA, Mayer RJ (1993) Primary central nervous system lymphoma. Ann Intern Med 119(11):1093–1104
Fisher SG, Fisher RI (2004) The epidemiology of non-Hodgkin's lymphoma. Oncogene 23(38):6524–6534
Freeman C, Berg JW, Cutler SJ (1972) Occurrence and prognosis of extranodal lymphomas. Cancer 29(1): 252–260
Fung CY, Tarbell NJ, Lucarelli MJ et al (2003) Ocular adnexal lymphoma: clinical behavior of distinct World Health Organization classification subtypes. Int J Radiat Oncol Biol Phys 57(5):1382–1391
Ghafoor A, Jemal A, Cokkinides V et al (2002) Cancer statistics for African Americans. CA Cancer J Clin 52(6):326–341
Greenlee RT, Hill-Harmon MB, Murray T, Thun M (2001) Cancer statistics, 2001. CA Cancer J Clin 51(1):15–36
Groves FD, Linet MS, Travis LB, Devesa SS (2000) Cancer surveillance series: non-Hodgkin's lymphoma incidence by histologic subtype in the United States from 1978 through 1995. J Natl Cancer Inst 92(15):1240–1251
Haldorsen IS, Krossnes BK, Aarseth JH et al (2007) Increasing incidence and continued dismal outcome of primary central nervous system lymphoma in Norway 1989–2003: time trends in a 15-year national survey. Cancer 110(8):1803–1814
Hao D, DiFrancesco LM, Brasher PM et al (1999) Is primary CNS lymphoma really becoming more common? A population-based study of incidence, clinicopathological features and outcomes in Alberta from 1975 to 1996. Ann Oncol 10(1):65–70
Hoffman S, Propp JM, McCarthy BJ (2006) Temporal trends in incidence of primary brain tumors in the United States, 1985–1999. Neuro Oncol 8(1):27–37
Howe HL, Wingo PA, Thun MJ et al (2001) Annual report to the nation on the status of cancer (1973 through 1998), featuring cancers with recent increasing trends. J Natl Cancer Inst 93(11):824–842
Husain A, Roberts D, Pro B et al (2007) Meta-analyses of the association between Chlamydia psittaci and ocular adnexal lymphoma and the response of ocular adnexal lymphoma to antibiotics. Cancer 110(4):809–815
Isaacson PG (1999) Mucosa-associated lymphoid tissue lymphoma. Semin Hematol 36(2):139–147
Jakobiec FA (2008) Ocular adnexal lymphoid tumors: progress in need of clarification. Am J Ophthalmol 145(6):941–950
Jenkins C, Rose GE, Bunce C et al (2000) Histological features of ocular adnexal lymphoma (REAL classification) and their association with patient morbidity and survival. Br J Ophthalmol 84(8):907–913
Johansen S, Heegaard S, Bogeskov L, Prause JU (2000) Orbital space-occupying lesions in Denmark 1974-1997. Acta Ophthalmol Scand 78(5):547–552
Johnson TE, Tse DT, Byrne GE Jr et al (1999) Ocular-adnexal lymphoid tumors: a clinicopathologic and molecular genetic study of 77 patients. Ophthal Plast Reconstr Surg 15(3):171–179
Knowles DM, Jakobiec FA, McNally L, Burke JS (1990) Lymphoid hyperplasia and malignant lymphoma occurring in the ocular adnexa (orbit, conjunctiva, and eyelids): a prospective multiparametric analysis of 108 cases during 1977 to 1987. Hum Pathol 21(9):959–973
Krogh-Jensen M, D'Amore F, Jensen MK et al (1995) Clinicopathological features, survival and prognostic factors of primary central nervous system lymphomas: trends in incidence of primary central nervous system lymphomas and primary malignant brain tumors in a well-defined geographical area. Population-based data from the Danish Lymphoma Registry, LYFO, and the Danish Cancer Registry. Leuk Lymphoma 19(3–4): 223–233
Lauer SA (2000) Ocular adnexal lymphoid tumors. Curr Opin Ophthalmol 11(5):361–366
Levine AM (1992) AIDS-associated malignant lymphoma. Med Clin North Am 76(1):253–268
Lutz JM, Coleman MP (1994) Trends in primary cerebral lymphoma. Br J Cancer 70(4):716–718
Makino K, Nakamura H, Kino T et al (2006) Rising incidence of primary central nervous system lymphoma in Kumamoto, Japan. Surg Neurol 66(5):503–506
Mannami T, Yoshino T, Oshima K et al (2001) Clinical, histopathological, and immunogenetic analysis of ocular adnexal lymphoproliferative disorders: characterization of malt lymphoma and reactive lymphoid hyperplasia. Mod Pathol 14(7):641–649

Margo CE, Mulla ZD (1998) Malignant tumors of the orbit. Analysis of the Florida Cancer Registry. Ophthalmology 105(1):185–190

McKelvie PA, McNab A, Francis IC et al (2001) Ocular adnexal lymphoproliferative disease: a series of 73 cases. Clin Experiment Ophthalmol 29(6):387–393

Meunier J, Lumbroso-Le Rouic L, Vincent-Salomon A et al (2004) Ophthalmologic and intraocular non-Hodgkin's lymphoma: a large single centre study of initial characteristics, natural history, and prognostic factors. Hematol Oncol 22(4):143–158

Mochizuki M, Singh AD (2009) Epidemiology and clinical features of intraocular lymphoma. Ocul Immunol Inflamm 17(2):69–72

Morris M, Handcock MS, Miller WC et al (2006) Prevalence of HIV infection among young adults in the United States: results from the Add Health study. Am J Public Health 96(6):1091–1097

Moslehi R, Devesa SS, Schairer C, Fraumeni JF Jr (2006) Rapidly increasing incidence of ocular non-Hodgkin lymphoma. J Natl Cancer Inst 98(13):936–939

Nadal D, Caduff R, Frey E et al (1994) Non-Hodgkin's lymphoma in four children infected with the human immunodeficiency virus. Association with Epstein-Barr Virus and treatment. Cancer 73(1):224–230

Norden AD, Drappatz J, Wen PY, Claus EB (2011) Survival among patients with primary central nervous system lymphoma, 1973-2004. J Neurooncol 101(3):487–493

Oh DE, Kim YD (2007) Lymphoproliferative diseases of the ocular adnexa in Korea. Arch Ophthalmol 125(12):1668–1673

Olson JE, Janney CA, Rao RD et al (2002) The continuing increase in the incidence of primary central nervous system non-Hodgkin lymphoma: a surveillance, epidemiology, and end results analysis. Cancer 95(7):1504–1510

Patchell RA (1988) Primary central nervous system lymphoma in the transplant patient. Neurol Clin 6(2):297–303

Peterson K, Gordon KB, Heinemann MH, DeAngelis LM (1993) The clinical spectrum of ocular lymphoma. Cancer 72(3):843–849

Reifler DM, Warzynski MJ, Blount WR et al (1994) Orbital lymphoma associated with acquired immune deficiency syndrome (AIDS). Surv Ophthalmol 38(4):371–380

Rockwood EJ, Zakov ZN, Bay JW (1984) Combined malignant lymphoma of the eye and CNS (reticulum-cell sarcoma). Report of three cases. J Neurosurg 61(2):369–374

Rosado MF, Byrne GE Jr, Ding F et al (2006) Ocular adnexal lymphoma: a clinicopathologic study of a large cohort of patients with no evidence for an association with Chlamydia psittaci. Blood 107(2):467–472

Rosenberg SA, Diamond HD, Jaslowitz B, Craver LF (1961) Lymphosarcoma: a review of 1269 cases. Medicine (Baltimore) 40:31–84

Rothova A, Ooijman F, Kerkhoff F et al (2001) Uveitis masquerade syndromes. Ophthalmology 108(2): 386–399

Rubenstein J, Ferreri AJ, Pittaluga S (2008) Primary lymphoma of the central nervous system: epidemiology, pathology and current approaches to diagnosis, prognosis and treatment. Leuk Lymphoma 49(Suppl 1):43–51

Sarkar C, Sharma MC, Deb P et al (2005) Primary central nervous system lymphoma–a hospital based study of incidence and clinicopathological features from India (1980–2003). J Neurooncol 71(2):199–204

Schabet M (1999) Epidemiology of primary CNS lymphoma. J Neurooncol 43(3):199–201

Shibamoto Y, Ogino H, Suzuki G et al (2008) Primary central nervous system lymphoma in Japan: changes in clinical features, treatment, and prognosis during 1985–2004. Neuro Oncol 10(4):560–568

Shields JA, Shields CL, Scartozzi R (2004) Survey of 1264 patients with orbital tumors and simulating lesions: The 2002 Montgomery Lecture, part 1. Ophthalmology 111(5):997–1008

Sjo LD (2009) Ophthalmic lymphoma: epidemiology and pathogenesis. Acta Ophthalmol 87 Thesis 1:1–20

Stefanovic A, Lossos IS (2009) Extranodal marginal zone lymphoma of the ocular adnexa. Blood 114(3):501–510

Suh YL, Koo H, Kim TS et al (2002) Tumors of the central nervous system in Korea: a multicenter study of 3221 cases. J Neurooncol 56(3):251–259

Sullivan TJ, Whitehead K, Williamson R et al (2005) Lymphoproliferative disease of the ocular adnexa: a clinical and pathologic study with statistical analysis of 69 patients. Ophthal Plast Reconstr Surg 21(3):177–188

Surawicz TS, McCarthy BJ, Kupelian V et al (1999) Descriptive epidemiology of primary brain and CNS tumors: results from the Central Brain Tumor Registry of the United States, 1990–1994. Neuro Oncol 1(1):14–25

Tanimoto K, Kaneko A, Suzuki S et al (2007) Primary ocular adnexal MALT lymphoma: a long-term follow-up study of 114 patients. Jpn J Clin Oncol 37(5):337–344

van der Sanden GA, Schouten LJ, van Dijck JA et al (2002) Primary central nervous system lymphomas: incidence and survival in the Southern and Eastern Netherlands. Cancer 94(5):1548–1556

Vargas RL, Fallone E, Felgar RE et al (2006) Is there an association between ocular adnexal lymphoma and infection with Chlamydia psittaci? The University of Rochester experience. Leuk Res 30(5):547–551

Villano JL, Koshy M, Shaikh H et al (2011) Age, gender, and racial differences in incidence and survival in primary CNS lymphoma. Br J Cancer 105(9): 1414–1418

White WL, Ferry JA, Harris NL, Grove AS Jr (1995) Ocular adnexal lymphoma. A clinicopathologic study with identification of lymphomas of mucosa-associated lymphoid tissue type. Ophthalmology 102(12):1994–2006

Wolf T, Brodt HR, Fichtlscherer S et al (2005) Changing incidence and prognostic factors of survival in AIDS-related non-Hodgkin's lymphoma in the era of

highly active antiretroviral therapy (HAART). Leuk Lymphoma 46(2):207–215
Woog JJ, Kim YD, Yeatts RP et al (2006) Natural killer/T--cell lymphoma with ocular and adnexal involvement. Ophthalmology 113(1):140–147
Wotherspoon AC, Ortiz-Hidalgo C, Falzon MR, Isaacson PG (1991) Helicobacter pylori-associated gastritis and primary B-cell gastric lymphoma. Lancet 338(8776):1175–1176
Yau YH, O'Sullivan MG, Signorini D et al (1996) Primary lymphoma of central nervous system in immunocompetent patients in south-east Scotland. Lancet 348(9031):890
Yoo C, Ryu MH, Huh J et al (2007) Chlamydia psittaci infection and clinicopathologic analysis of ocular adnexal lymphomas in Korea. Am J Hematol 82(9):821–823
Yoon JS, Ma KT, Kim SJ et al (2007) Prognosis for patients in a Korean population with ocular adnexal lymphoproliferative lesions. Ophthal Plast Reconstr Surg 23(2):94–99
Zucca E, Roggero E, Maggi-Solca N et al (2000) Prevalence of Helicobacter pylori and hepatitis C virus infections among non-Hodgkin's lymphoma patients in Southern Switzerland. Haematologica 85(2):147–153

# Ocular Adnexal Lymphoma: Clinical Features and Diagnostic Evaluation

5

Mary E. Aronow and Arun D. Singh

## 5.1 Introduction

Ocular and adnexal lymphomas are grouped based upon their site of involvement and whether the disease is primary or secondary. Using this classification scheme, ocular lymphoma can be subdivided into primary vitreoretinal lymphoma (PVRL), primary uveal lymphoma, and secondary intraocular manifestations of systemic lymphoma. By definition, adnexal lymphoma (OAL) affects the eyelids, conjunctiva, lacrimal apparatus, extraocular muscles, and the orbit.

The distinction between the forms of ocular and adnexal lymphoma is important as each varies in its clinical course and response to treatment. Among the ocular lymphomas, PVRL is an aggressive, high-grade malignancy with associated central nervous system (CNS) involvement, and median survival ranging from 1 to 2 years depending on factors such as age and Karnofsky performance status (Abrey et al. 2006). In contrast, uveal lymphoma and OAL are typically low-grade lymphomas that behave in a more indolent manner (Coupland et al. 2004; Jenkins et al. 2000; Fung et al. 2003; McKelvie et al. 2001). The 10-year disease-specific mortality for OAL is approximately 5–10 % (Coupland et al. 2004) The characteristics and clinical behavior of secondary intraocular lymphoma are dependent upon the morphologic and immunophenotypic features of the primary systemic counterpart.

M.E. Aronow, MD (✉) • A.D. Singh, MD
Department of Ophthalmic Oncology,
Cole Eye Institute, Cleveland Clinic Foundation,
9500 Euclid Avenue, Cleveland,
OH 44195, USA
e-mail: turellm2@gmail.com

## 5.2 Clinical Features

### 5.2.1 Primary Vitreoretinal Lymphoma

PVRL is a variant of primary central nervous system lymphoma (PCNSL), which is most frequently a diffuse large B-cell lymphoma (DLBCL) associated with poor prognosis (Pe'er et al. 2009). Overall, PCNSL represents about 1–2 % of all cases of lymphoma and 3–5 % of all primary CNS tumors (Olson et al. 2002; Ahluwalia and Peereboom 2010; Gerstner and Batchelor 2010). The association between PVRL and PCNSL is variable, with ocular disease manifesting prior to, following, or occurring simultaneously with CNS presentation. Approximately 25 % of patients with PCNSL will have concomitant PVRL (Hochberg and Miller 1988). In contrast, 56–85 % of individuals with PVRL ultimately develop central nervous system involvement. (Peterson et al. 1993; Cassoux et al. 2000; Char et al. 1988; Freeman et al. 1987). In immunocompetent individuals, the peak incidence of PVRL is during the fifth to seventh decade, with a mean age of diagnosis of 60 years (Char et al. 1988; Whitcup et al.

A.D. Singh (ed.), *Ocular and Adnexal Lymphoma*, Essentials in Ophthalmology,
DOI 10.1007/978-3-642-38499-8_5, © Springer-Verlag Berlin Heidelberg 2014

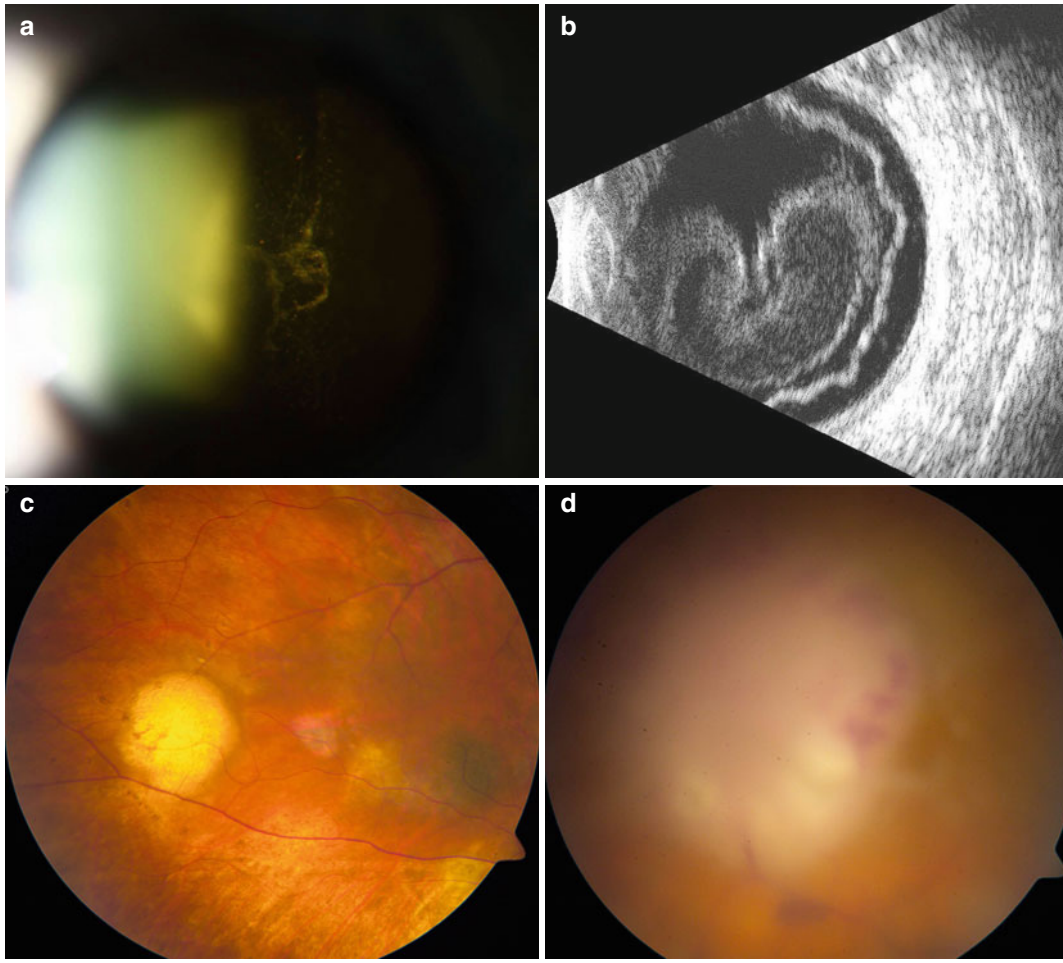


**Fig. 5.1** A 70-year-old female presented with decreased visual acuity and floaters. On dilated fundus examination, vitreous haze and vitreous cellular condensations were observed (**a**). Extensive vitreous cells with associated retinal detachment as documented with B-scan ultrasonography (**b**). Subretinal yellow lesion (**c**) and a larger infiltrative lesion in two different patients with primary vitreoretinal lymphoma (**d**)

1993). Disease may occur at a younger age in the immunocompromised population (Babu et al. 2010). Although PVRL is most frequently DLBCL in origin, rare cases may be secondary to human T-cell lymphotropic virus type 1 (HTLV-1) infection (Hochberg and Miller 1988; Marshall et al. 1998; Coupland et al. 2003) .PVRL arising in T-cells is composed of small-sized lymphocytes (Choi et al. 2003).

The majority of symptomatic individuals present with a painless decrease in vision or floaters (Char et al. 1988). Those who are asymptomatic are often diagnosed when ophthalmic screening is performed in the setting of known PCNSL. The clinical findings are bilateral in 80 % of cases, but are frequently asymmetric (Peterson et al. 1993). The hallmark feature of PVRL on clinical examination is the presence of fine vitreous cells and subretinal pigment epithelium (RPE) deposits (Fig. 5.1). When present, focal, multifocal, or diffuse retinal, choroidal, or chorioretinal infiltrates are considered pathognomonic (Gass et al. 1984). Anterior segment findings are nonspecific and include keratic precipitates, iris nodules, aqueous cells, and flare (Rajagopal and Harbour 2011). Other less commonly reported features include perivasculitis (Char et al. 1988), retinal artery occlusion (Gass and Trattler 1991), exudative retinal detachment (Michelson et al.

1981), multifocal "punched-out" lesions at the level of the RPE (Lang et al. 1985), and optic atrophy (Purvin and Van Dyk 1984). Due to the nonspecific nature of ophthalmic manifestations, a diagnosis of PVRL is difficult to make on clinical grounds alone; therefore, delay in diagnosis is common. A duration of up to 2 years between the initial presentation and histopathological confirmation of PVRL has been reported (Freeman et al. 1987).

When there is CNS disease, personality changes are a common presenting feature because the frontal lobe is the most frequently involved region of the brain. Seizures are rare. Unlike PVRL where delay in diagnosis is common, PCNSL is a rapidly growing tumor. Diagnosis is frequently made within a few months of the onset of symptoms. The brain, spinal cord, and meninges either separately or in various combinations can be involved. Solitary involvement of the spinal cord is rarely observed. The lesions in the CNS tend to be periventricular in location, thus allowing access to cerebrospinal fluid (CSF) and meninges (Fig. 5.2). Brain lesions can be multifocal, particularly in immunocompromised individuals (Table 5.1).

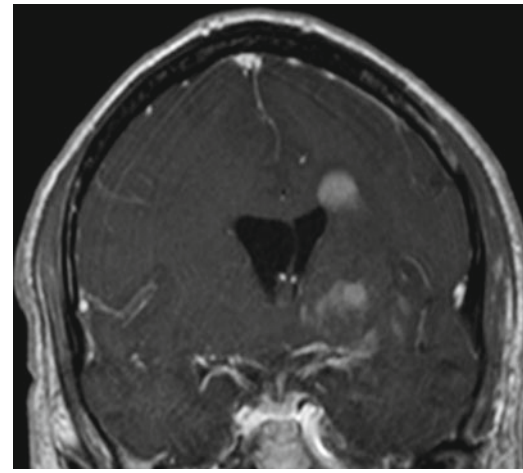

**Fig. 5.2** Magnetic resonance imaging (MRI) of the brain showed multiple periventricular lesions consistent with primary central nervous system lymphoma with vitreoretinal involvement

**Table 5.1** Clinical features of various types of ocular and adnexal lymphoma

| Lymphoma | Epidemiology | Laterality | Symptoms | Clinical features | Subtype | Morphology |
|---|---|---|---|---|---|---|
| Primary vitreoretinal lymphoma | 50–70 years | Frequently bilateral | Decreased vision<br>Floaters | Vitreous cells<br>Retinal/choroidal infiltrates<br>CNS involvement | DLBCL | Large cells<br>Minimal cytoplasm<br>Prominent nucleoli |
| Primary uveal lymphoma | M>F<br>50–70 years | Usually unilateral | Decreased vision<br>Metamorphopsia | Clear vitreous<br>Diffuse choroidal thickening<br>Exudative retinal detachment | EMZL | Small centrocyte-like cells with variable plasmacellular differentiation |
| Secondary intraocular lymphoma | Variable | Unilateral or bilateral | Decreased vision | Variable: choroidal thickening iris infiltrates pseudohypopyon vitreous cells | Dependent on systemic NHL | Similar to systemic NHL |
| Ocular adnexal lymphoma | F>M | Unilateral or bilateral | Variable: decreased vision<br>Proptosis<br>Ptosis | Conjunctival "salmon" patch<br>Ocular adnexal mass<br>Extrascleral extension | EMZL<br>Follicular<br>DLBCL<br>Mantle cell<br>Lymphoplasmacytic | Dependent upon lymphoma subtype |

*M* males, *F* females, *DLBCL* diffuse large B-cell lymphoma, *EMZL* extranodal marginal zone lymphoma, *NHL* non-Hodgkin's lymphoma

### 5.2.2 Primary Uveal Lymphoma

Primary uveal lymphoma can be further subdivided into primary choroidal, iridal, and ciliary body lymphoma. Primary choroidal lymphoma accounts for the majority of cases and is generally a low-grade B-cell lymphoma with a prolonged benign course. Histopathologically, choroidal lymphoma may be one of several subtypes; extranodal marginal zone B-cell lymphoma (EMZL) is the predominant form, comprising 60–80 % of cases (Freeman et al. 1972). Primary iridal lymphomas may be of either B-cell or T-cell origin. The incidence of primarily iridal and ciliary body lymphoma is unknown as these tumors have only been described in a handful of case reports. While also rare, primary choroidal lymphoma has been documented in the literature in approximately 70–80 case reports and small case series (Coupland 2007). The following discussion pertains to primary choroidal lymphoma.

Primary choroidal lymphoma is most commonly a unilateral process that predominantly affects men in the fifth to seventh decade (Coupland et al. 2002). Initial symptoms may include recurrent episodes of painless, blurred vision and metamorphopsia secondary to exudative retinal detachment affecting the fovea. With advanced disease, patients may develop pain and severely reduced vision due to secondary angle-closure glaucoma and extensive retinal detachment respectively. When extraocular extension is present, proptosis and diplopia may occur. A classic feature is the presence of solitary or multiple yellow, creamy subretinal infiltrates (Fig. 5.3). Notably, the vitreous remains clear due to the absence of cellular reaction. Ultimately, diffuse thickening of the uveal tract develops and is often associated with exudative retinal detachment. In some cases, there may be episcleral extension appearing as a nonmobile orange to yellow "salmon" patch.

### 5.2.3 Secondary Ocular Manifestations of Systemic Lymphoma

Intraocular lymphoma secondary to disseminated systemic lymphoma most commonly affects the choroid. For this reason, secondary intraocular lymphoma can have a similar

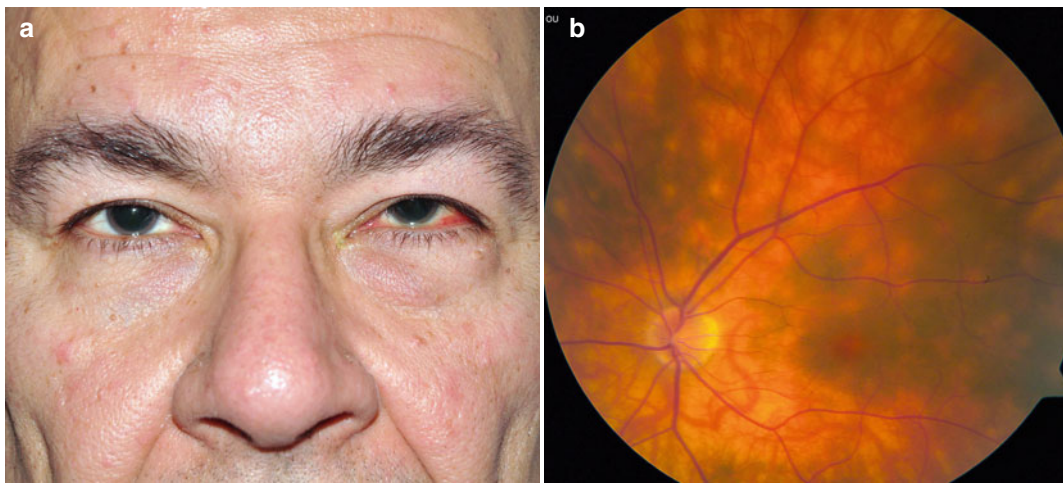


**Fig. 5.3** A 45-year-old male patient with orbital fullness and redness OS (**a**). Fundus examination revealed bilateral diffuse, ill-defined choroidal thickening in the posterior pole extending into the macula of the left eye with yellow choroidal infiltrates (**b**), which appeared hypofluorescent on indocyanine green angiography (**c**). B-scan ultrasonography demonstrated diffuse thickening of the choroid and extrascleral nodular extension (**d**). Subsequent orbital biopsy revealed extranodal marginal zone lymphoma (MALT type)

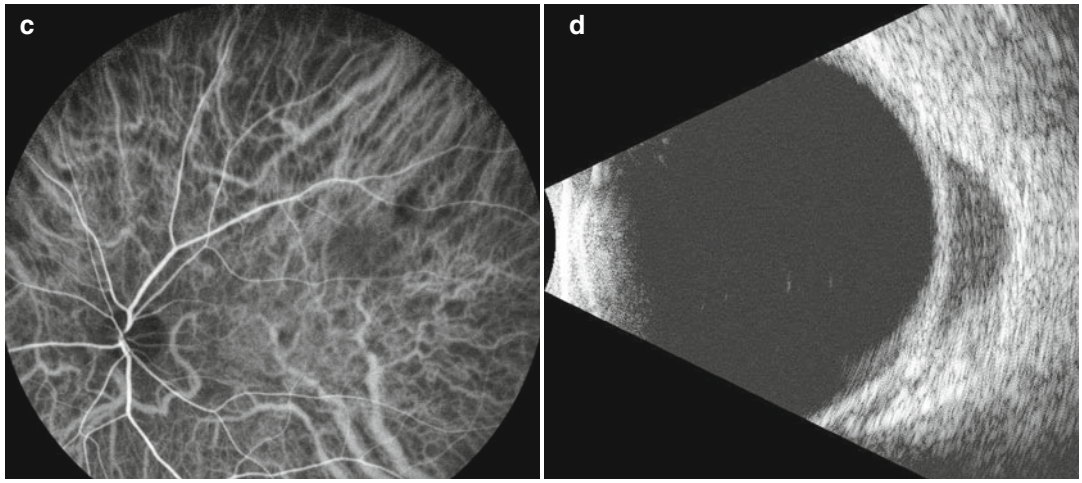


**Fig. 5.3** (continued)

clinical appearance to primary choroidal lymphoma (Coupland and Damato 2008). Spread to the retina without choroidal infiltration can rarely occur (Coupland and Damato 2008). Other atypical presentations of secondary intraocular lymphoma include pseudohypopyon and iris infiltration (Shakin et al. 1988; Tranos et al. 2002). While exceedingly rare, iridal lymphoma secondary to systemic non-Hodgkin's lymphoma is more common than primary iridal lymphoma (Coupland 2007). The most common subtype of systemic lymphoma that affects the eye is DLBCL. This is followed by multiple myeloma, extramedullary plasmacytoma, lymphoplasmacytic lymphoma/immunocytoma, and EMZL (Coupland and Damato 2008). The morphologic and immunophenotypic features of secondary choroidal lymphoma bear resemblance to their primary systemic counterpart.

### 5.2.4 Ocular Adnexal Lymphoma

OAL encompasses a heterogeneous group of malignancies, the vast majority of which are of the non-Hodgkin's type and are primarily of B-cell origin. Rarely, other forms such as T-cell (Ferry et al. 2007) and natural killer (NK)-cell lymphomas (Coupland et al. 1999; Mendenhall et al. 2006) can affect the ocular adnexal structures. The majority of OAL belong to one of five subtypes: EMZL, follicular lymphoma, DLBCL, mantle cell lymphoma, and lymphoplasmacytic lymphoma (Shields et al. 2001). Approximately 80 % are of the EMZL type (Freeman et al. 1972). Among affected sites, the frequencies of involvement are conjunctiva 20–33 %, orbit/lacrimal gland 46–74 %, and eyelid 5–20 % (Coupland et al. 2004; Knowles et al. 1990). Disease may be limited to a single, localized tumor, or it may be multifocal. Overlap with ocular adnexal sites is common (10–20 % of cases), and coexisting uveal involvement has been observed (Fuller et al. 2008). OAL can also affect regional, central, and peripheral lymph nodes as well as other distant extranodal sites.

OAL has a slight female predilection (60 % of cases in most series) (Shields et al. 2001). Individuals with OAL may present with a broad range of symptoms depending upon the site and extent of involvement. Common symptoms include a mass within the ocular adnexal structures, exophthalmos, ptosis, ophthalmoplegia, pain, decreased vision, or diplopia. If the lacrimal gland is involved, dry eye symptoms may occur. On clinical examination, OAL has site-specific presentations that affect how the

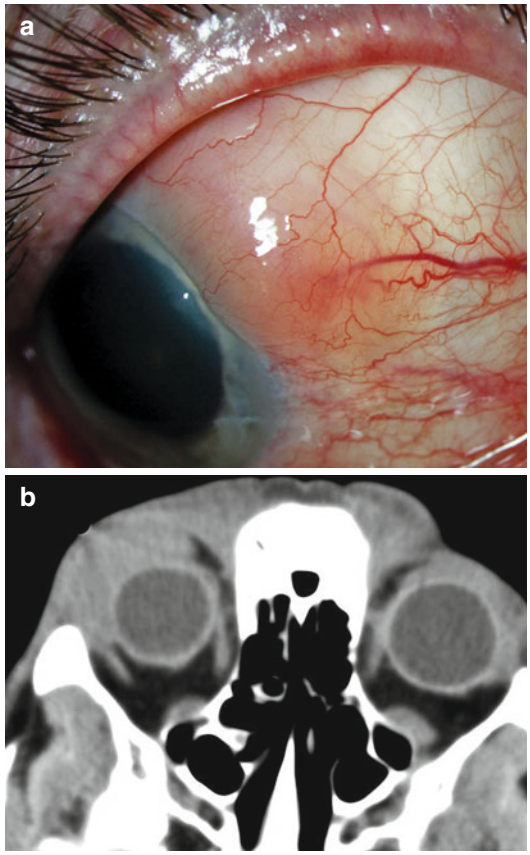


**Fig. 5.4** Ocular adnexal lymphoma. Typical appearance of subconjunctival infiltrate presenting as "salmon" patch (**a**) and diffuse orbital extension without compression of the orbital structures (**b**). Bilateral asymmetric involvement (milder OS)

diagnosis is made. In the conjunctiva, lesions typically present as a mass with a "salmon patch" appearance (Fig. 5.4). In the orbit, lacrimal gland, and eyelid, the lymphoma presents as a mass, which, if palpable, is typically firm. Mobility is variable depending upon attachment to other ocular adnexal structures. Diplopia may occur depending upon how rapidly the mass develops. Exophthalmos and decreased retropulsion of the globe may be the only clinical signs. Involvement of the nasolacrimal drainage system can occur. Compression or invasion of the optic nerve can lead to vision loss. Regardless of the clinical findings, these features do not allow distinction of benign from malignant lymphoproliferative disease.

## 5.3 Diagnostic Evaluation

### 5.3.1 Primary Vitreoretinal Lymphoma

Diagnostic evaluation of PVRL should begin with a thorough history including not only ocular symptoms but also changes in cognitive functioning, neurological decline, and risk factors for immunocompromise. A complete ophthalmic examination of both the anterior and posterior segment is required to assess disease extent and laterality. Due to the high correlation between PVRL and PCNSL, all patients diagnosed with ocular disease should undergo systemic evaluation by an experienced oncologist. Recommended testing includes gadolinium-enhanced MRI of the brain and spinal cord, CSF studies, complete blood count, and HIV testing when appropriate (Chap. 8). Similarly, periodic ophthalmic screening should be part of the diagnostic evaluation and subsequent management of individuals diagnosed with PCNSL.

In the setting of existing PCNSL, the diagnosis of PVRL with classic clinical findings is straightforward, and biopsy of an ophthalmic site is unnecessary. In the absence of PCNSL, diagnosis of PVRL is based upon clinical, histopathological, and cytological features. Biopsy remains the gold standard and should be considered in middle-aged or elderly patients with "idiopathic" unilateral or bilateral recurrent uveitis, particularly cases that are unresponsive to steroids (Table 5.2). Several techniques exist including vitreous biopsy, retinal biopsy, and subretinal biopsy (Chap. 6). Neoplastic cells can be identified by an experienced cytologist, using an array of techniques such as liquid-based cytology, cytospin, cell block preparations stained with modified Papanicolaou, Giemsa, or standard hematoxylin and eosin stains. Proper surgical techniques and handling of the sample are critical as aspirates are generally of low cellularity and fragile lymphoma cells are prone to lysis during collection. Briefly, for vitreous biopsy, an undiluted sample of approximately 1–2 mL is collected prior to the start of the infusion (Coupland 2012). Next, a second diluted vitreous specimen

**Table 5.2** Differential diagnosis of ocular and adnexal lymphomas

| Lymphoma | Differential diagnosis |
|---|---|
| Primary vitreoretinal lymphoma | Sarcoidosis<br>Syphilis<br>Tuberculosis<br>Birdshot retinochoroidopathy<br>Multifocal chorioretinitis<br>Acute posterior multifocal placoid pigmentary epitheliopathy<br>Serpiginous choroiditis<br>Punctate inner choroidopathy<br>Toxoplasmosis |
| Primary uveal lymphoma | Posterior scleritis<br>Uveal metastases<br>Birdshot retinochoroidopathy<br>Diffuse uveal melanoma<br>Uveal effusion syndrome |
| Secondary intraocular lymphoma | Similar to primary choroidal lymphoma |
| Ocular adnexal lymphoma | Extensive including:<br>Benign reactive lymphoid hyperplasia<br>Epithelial tumors<br>Melanocytic tumors<br>Infectious lesions<br>Metastases<br>Dacryoadenitis |

using gentle vitreous cutting is obtained (Margolis et al. 2007). The vitreous cassette may be submitted as a third sample (Yeh et al. 2010). In the presence of chorioretinal lesions, a chorioretinal or retinal biopsy may be required. Specimens should be delivered to the laboratory, without fixative, within 1 h of surgery (Coupland 2012).

Vitreous biopsy is not diagnostic in all cases; therefore, supplemental techniques can be helpful in confirming the diagnosis. Briefly, immunohistochemistry is useful for identifying markers for leukocytes (CD45), B-cells (CD20, CD79a, PAX-5), T-cells (CD45RO), and macrophages (CD68) (Chap. 2) (Coupland 2012). Clonality can be established using antibodies directed against κ and λ light chains (Freeman et al. 1987; Farkas et al. 2004). Flow cytometry provides a means of quantitatively assessing the proportion of cells that demonstrate these markers. Polymerase chain reaction (PCR) gene rearrangement studies can detect monoclonality of the heavy chain variable (V), diversity (D), and joining (J) immunoglobulin gene segments (Chan 2007). Measurement of IL-6 and IL-10 in aqueous or vitreous fluid can facilitate diagnosis, although an elevated IL-10/IL-6 ratio is not specific for PVRL (Akpek et al. 1999). A comprehensive discussion of molecular techniques follows in Chap. 3.

Diagnostic evaluation for CNS disease should include cranio-spinal MRI with gadolinium contrast. Cranial lesions appear as multiple isointense nodules on T1-MRI and demonstrate characteristic dense and diffuse contrast enhancement. Meningeal enhancement with gadolinium is indicative of meningeal involvement. Many centers also perform computed tomography (CT) scans of the chest, abdomen, and pelvis to exclude systemic involvement or systemic origin of the CNS involvement. Visceral involvement is rare at the initial diagnosis but is not uncommon in the terminal stages. CSF studies should be performed in every patient with suspected or confirmed PCNSL. Demonstration of malignant lymphocytes in the CSF is confirmatory of the diagnosis and reveals lymphocytic pleocytosis, raised protein concentration, and normal or low glucose concentration. Testicular ultrasound examination is recommended in elderly patients because of frequent CNS involvement in testicular lymphomas.

While definitive diagnosis of PVRL is based predominantly upon biopsy, ancillary imaging studies can be helpful in some cases. Fundus photography is useful for documenting initial clinical findings and response to treatment. Fluorescein angiography (FA) demonstrates characteristic features including granularity, blockage of fluorescence, and late staining (Velez et al. 2002). These findings have been shown to correlate with histopathologically observed lymphoma cells located between the RPE and Bruch's membrane (Velez et al. 2002). Commonly observed features in uveitic processes such as perivascular staining, leakage, and cystoid macular edema are rarely observed in PVRL (Velez et al. 2002). When the angiographic findings of 53 patients with biopsy confirmed PVRL were compared to 133 patients with simulating conditions (infectious uveitis, immune-mediated uveitis,

and systemic metastases), clusters of small, round, hypofluorescent lesions (50–250 μm in diameter) were visualized in the posterior pole throughout the early to late phase of the angiogram in 45 % of PVRL patients, whereas these findings were present in only 2 % of non-lymphoma cases ($p<0.001$) (Fardeau et al. 2009). In this same series, indocyanine green (ICG) angiography demonstrated distinct fundus features in those with PVRL in comparison to patients with simulating uveitic conditions. Small, round hypofluorescent lesions which disappear in the late phase of the angiogram were observed in 26 % of individuals with PVRL compared to only 9 % of non-lymphoma cases ($p=0.014$) (Fardeau et al. 2009). Optical coherence tomography (OCT) performed for this series demonstrated hyperreflective, nodular RPE lesions in 42 % of patients with PVRL compared to only 15 % of those with non-lymphoma diagnoses ($p=0.076$) (Fardeau et al. 2009). Macular OCT has revealed that foveal thickness is typically near normal in patients with PVRL (mean 231 μm, standard deviation 45 μm), compared to those with simulating uveitic processes in which foveal thickness is more likely to be increased due to inflammatory edema (mean 327 μm, standard deviation 114 μm) ($p<0.001$) (Fardeau et al. 2009). Fundus autofluorescence (FAF) may also highlight several clinical features. In a small series of 5 eyes, sub-RPE infiltrates demonstrated weak autofluorescence by FAF (Ishida et al. 2010). Brown clumps on the surface of these lesions exhibited bright autofluorescence. In contrast, diffuse retinal infiltrates, which appear as retinal whitening, displayed hypofluorescence by FAF. This method is useful in characterizing regions of RPE atrophy, which appear hypofluorescent following treatment and resolution of active disease (Table 5.3) (Ishida et al. 2010).

### 5.3.2 Primary Uveal Lymphoma

Several imaging studies are useful for establishing the diagnosis of primary uveal lymphoma. B-scan ultrasonography typically reveals choroidal thickening with low echogenicity (Fig. 5.3).

**Table 5.3** Ancillary imaging studies for various types of ocular and adnexal lymphoma

| Lymphoma | B-scan ultrasonography | FA | ICG | CT | MRI |
|---|---|---|---|---|---|
| Primary vitreoretinal lymphoma | Vitreous debris | Small, round, hypofluorescent lesions | Small, round, hypofluorescent lesions which disappear in the late phase | CT of the chest, abdomen, and pelvis may detect systemic origin in some cases | Cranial lesions: multiple isointense periventricular lesions on T1-MRI |
| Primary uveal lymphoma | Choroidal thickening<br>Low echogenicity<br>Extrascleral extension | Early hypofluorescence with multiple foci of hyperfluorescence and staining in the late phase | Multiple, round, hypofluorescent lesions | Choroidal thickening<br>Decreased size of vitreous cavity<br>Calcification absent | Choroidal thickening<br>Decreased size of vitreous cavity<br>Calcification absent |
| Secondary intraocular lymphoma | Choroidal thickening<br>Low echogenicity | Similar to primary uveal lymphoma | Similar to primary uveal lymphoma | Similar to primary uveal lymphoma | Similar to primary uveal lymphoma |
| Ocular adnexal lymphoma | Extrascleral extension<br>Orbital mass | Early hyperfluorescence, hypofluorescent spots, choroidal folds, or normal | Multiple, round, hypofluorescent lesions when uveal involvement is present | Enhancing lesions (discrete or diffuse) that mold to the globe or orbit | Enhancing lesions (discrete or diffuse) that mold to the globe or orbit |

*FA* fluorescein angiography, *ICG* indocyanine green angiography, *CT* computed tomography, *MRI* magnetic resonance imaging

B-scan ultrasonography is also useful for detecting occult disease, particularly in the fellow eye, as extrascleral extension in the form of crescentic thickening or a discrete mass is not uncommon. The rate of extrascleral extension has been reported to be as high as 84.6 % in some series (Coupland et al. 2002). CT and MRI of the globes confirm choroidal thickening with a corresponding decrease in the size of the vitreous cavity. Calcification is absent. FA demonstrates early hypofluorescence with multiple foci of hyperfluorescence and staining in the late phase. These angiographic features correlate with choroidal infiltrates observed clinically by ophthalmoscopy. ICG angiography provides superior characterization of the choroidal vasculature in comparison to FA and is therefore preferable in cases of primary uveal lymphoma (Fig. 5.3) (Saatci et al. 2006). Multiple, round, hypofluorescent lesions are typically present and correspond to areas of non-perfusion secondary to space-occupying choroidal infiltrates.

Biopsy of episcleral tumor nodules and choroidal aspirates may also aid in the diagnosis. Histopathologically, the features of primary uveal lymphoma are similar to EMZL located elsewhere in the body. The cells are generally of the centrocyte-like, monocytoid, and plasmacytoid type. Dutcher bodies, or collections of intranuclear immunoglobulin, can often be observed. Immunohistochemistry confirms the expression of B-cell antigens (CD20 and CD-79a). Gene rearrangement studies or flow cytometry supports the clonality and neoplastic nature of these B-cells.

Before initiating localized treatment for primary uveal lymphoma, it is essential to perform systemic studies to exclude the possibility of systemic lymphoma. Recommended testing includes CT of the chest, abdomen, and pelvis, complete blood count, and serum protein electrophoresis (Augsburger and Greatrex 1989). Once disease is confirmed to be limited to the uvea, management consists of low-dose external beam radiotherapy (EBRT) administered over several fractions (Augsburger and Greatrex 1989). Intravenous administration of rituximab is an alternative therapy in cases with bilateral disease. While primary uveal lymphoma typically follows a less aggressive course than primary vitreoretinal lymphoma, staging at the time of diagnosis and continued systemic surveillance are important in disease management.

### 5.3.3 Secondary Ocular Manifestations of Systemic Lymphoma

As intraocular lymphoma secondary to disseminated systemic lymphoma most commonly affects the choroid, the clinical appearance and findings on ancillary imaging studies are similar to those observed in primary choroidal lymphoma. B-scan ultrasonography typically reveals choroidal thickening with low echogenicity. The choroidal thickening can also be visualized on CT and MRI of the globes. Similarly to choroidal lymphoma, calcification is absent. Biopsy demonstrates morphologic and immunophenotypic features of secondary choroidal lymphoma resembling the primary systemic counterpart. Staging evaluation and close collaboration with an experienced oncologist are critical in the management of patients with systemic lymphoma with intraocular involvement.

### 5.3.4 Ocular Adnexal Lymphoma

Diagnostic evaluation of OAL begins with a thorough ocular examination to assess laterality and extent of disease involvement. OAL may be multifocal and can involve contiguous ocular adnexal structures. It is also not uncommon to find coexisting uveal lymphoma (Fuller et al. 2008; Grossniklaus et al. 1998; Baryla et al. 2012; Sarraf et al. 2005; Tagami et al. 2011). Complete ophthalmic examination should therefore be performed and should include external inspection for regional lymph node involvement, bilateral dilated fundus examination, motility testing, and exophthalmometry. Ancillary imaging studies such as B-scan ultrasonography and angiography are useful in characterizing the full extent and laterality of disease. This is particularly important in cases with

subtle extrascleral extension or occult involvement of the fellow eye. B-scan ultrasonography is a sensitive modality for detecting extrascleral extension. The pattern may be crescentic thickening, a discrete mass (often adjacent to the optic nerve), or diffuse choroidal thickening in cases where uveal lymphoma overlaps with OAL. When coexisting uveal lymphoma is present, FA may show early hyperfluorescence, hypofluorescent spots corresponding clinically observed choroidal infiltrates, choroidal folds, or a normal angiogram.

Imaging studies of the orbit play an important role in OAL but are performed at different times depending on the presentation (Fig. 5.4). With conjunctival disease, the lesion is frequently biopsied first and imaging of the orbit follows to assess orbital involvement. With orbital and lid disease, the orbit is usually imaged to optimize the biopsy process. Contrast-enhanced CT and MRI of the orbits will show enhancing lesions, which can be discrete or diffuse. Lymphoid lesions typically mold to structures such as the globe or bony orbit. Neuroimaging will reveal orbital lesions in up to 50 % of clinically unsuspected cases (Stafford et al. 2001). Paranasal sinus involvement is not uncommon.

As malignant OAL cannot be distinguished from benign reactive lymphoid hyperplasia (BRLH) on clinical grounds alone, definitive diagnosis is based upon biopsy (Chap. 9). This should be obtained by open methods to allow sufficient material for multiple studies: pathology, lymphocyte immunophenotypical analysis, and molecular genetic studies to identify gene rearrangements indicative of clonality and/or translocations. Once the diagnosis is confirmed by biopsy, referral to a medical oncologist for systemic screening and staging is important for treatment planning (Chap. 7).

## References


Abrey LE, Ben-Porat L, Panageas KS et al (2006) Primary central nervous system lymphoma: the Memorial Sloan-Kettering Cancer Center prognostic model. J Clin Oncol 24(36):5711–5715

Ahluwalia MS, Peereboom DM (2010) Primary central nervous system lymphoma. Curr Treat Options Neurol 12(4):347–359

Akpek EK, Maca SM, Christen WG, Foster CS (1999) Elevated vitreous interleukin-10 level is not diagnostic of intraocular-central nervous system lymphoma. Ophthalmology 106(12):2291–2295

Augsburger JJ, Greatrex KV (1989) Intraocular lymphoma: clinical presentations, differential diagnosis and treatment. Trans Pa Acad Ophthalmol Otolaryngol 41:796–808

Babu K, Murthy KR, Krishnakumar S (2010) Two successive ocular malignancies in the same eye of a HIV-positive patient: a case report. Ocul Immunol Inflamm 18(2):101–103

Baryla J, Allen LH, Kwan K et al (2012) Choroidal lymphoma with orbital and optic nerve extension: case and review of literature. Can J Ophthalmol 47(1): 79–81

Cassoux N, Merle-Beral H, Leblond V et al (2000) Ocular and central nervous system lymphoma: clinical features and diagnosis. Ocul Immunol Inflamm 8(4): 243–250

Chan CC (2007) Primary intraocular lymphoma, 1st edn. World Scientific Publishing Co. Pte. Ltd, Hackensack

Char DH, Ljung BM, Miller T, Phillips T (1988) Primary intraocular lymphoma (ocular reticulum cell sarcoma) diagnosis and management. Ophthalmology 95(5): 625–630

Choi JS, Nam DH, Ko YH et al (2003) Primary central nervous system lymphoma in Korea: comparison of B- and T-cell lymphomas. Am J Surg Pathol 27(7): 919–928

Coupland SE (2007) Uveal lymphoproliferative tumors. In: Singh AD (ed) Clinical ophthalmic oncology. Elsevier Inc, Philadelphia

Coupland SE (2012) Vitreous biopsy: specimen preparation and interpretation. Monogr Clin Cytol 21:61–71

Coupland SE, Damato B (2008) Understanding intraocular lymphomas. Clin Experiment Ophthalmol 36(6): 564–578

Coupland SE, Foss HD, Assaf C et al (1999) T-cell and T/natural killer-cell lymphomas involving ocular and ocular adnexal tissues: a clinicopathologic, immunohistochemical, and molecular study of seven cases. Ophthalmology 106(11):2109–2120

Coupland SE, Foss HD, Hidayat AA et al (2002) Extranodal marginal zone B cell lymphomas of the uvea: an analysis of 13 cases. J Pathol 197(3): 333–340

Coupland SE, Bechrakis NE, Anastassiou G et al (2003) Evaluation of vitrectomy specimens and chorioretinal biopsies in the diagnosis of primary intraocular lymphoma in patients with Masquerade syndrome. Graefes Arch Clin Exp Ophthalmol 241(10): 860–870

Coupland SE, Hellmich M, Auw-Haedrich C et al (2004) Prognostic value of cell-cycle markers in ocular adnexal lymphoma: an assessment of 230 cases. Graefes Arch Clin Exp Ophthalmol 242(2): 130–145

Fardeau C, Lee CP, Merle-Beral H et al (2009) Retinal fluorescein, indocyanine green angiography, and optic coherence tomography in non-Hodgkin primary

intraocular lymphoma. Am J Ophthalmol 147(5):886–894, 94 e1
Farkas T, Harbour JW, Davila RM (2004) Cytologic diagnosis of intraocular lymphoma in vitreous aspirates. Acta Cytol 48(4):487–491
Ferry JA, Fung CY, Zukerberg L et al (2007) Lymphoma of the ocular adnexa: a study of 353 cases. Am J Surg Pathol 31(2):170–184
Freeman C, Berg JW, Cutler SJ (1972) Occurrence and prognosis of extranodal lymphomas. Cancer 29(1): 252–260
Freeman LN, Schachat AP, Knox DL et al (1987) Clinical features, laboratory investigations, and survival in ocular reticulum cell sarcoma. Ophthalmology 94(12): 1631–1639
Fuller ML, Sweetenham J, Schoenfield L, Singh AD (2008) Uveal lymphoma: a variant of ocular adnexal lymphoma. Leuk Lymphoma 49(12):2393–2397
Fung CY, Tarbell NJ, Lucarelli MJ et al (2003) Ocular adnexal lymphoma: clinical behavior of distinct World Health Organization classification subtypes. Int J Radiat Oncol Biol Phys 57(5):1382–1391
Gass JD, Trattler HL (1991) Retinal artery obstruction and atheromas associated with non-Hodgkin's large cell lymphoma (reticulum cell sarcoma). Arch Ophthalmol 109(8):1134–1139
Gass JD, Sever RJ, Grizzard WS et al (1984) Multifocal pigment epithelial detachments by reticulum cell sarcoma. A characteristic funduscopic picture. Retina 4(3):135–143
Gerstner ER, Batchelor TT (2010) Primary central nervous system lymphoma. Arch Neurol 67(3):291–297
Grossniklaus HE, Martin DF, Avery R et al (1998) Uveal lymphoid infiltration. Report of four cases and clinicopathologic review. Ophthalmology 105(7): 1265–1273
Hochberg FH, Miller DC (1988) Primary central nervous system lymphoma. J Neurosurg 68(6):835–853
Ishida T, Ohno-Matsui K, Kaneko Y et al (2010) Fundus autofluorescence patterns in eyes with primary intraocular lymphoma. Retina 30(1):23–32
Jenkins C, Rose GE, Bunce C et al (2000) Histological features of ocular adnexal lymphoma (REAL classification) and their association with patient morbidity and survival. Br J Ophthalmol 84(8):907–913
Knowles DM, Jakobiec FA, McNally L, Burke JS (1990) Lymphoid hyperplasia and malignant lymphoma occurring in the ocular adnexa (orbit, conjunctiva, and eyelids): a prospective multiparametric analysis of 108 cases during 1977 to 1987. Hum Pathol 21(9): 959–973
Lang GK, Surer JL, Green WR et al (1985) Ocular reticulum cell sarcoma. Clinicopathologic correlation of a case with multifocal lesions. Retina 5(2):79–86
Margolis R, Brasil OF, Lowder CY et al (2007) Vitrectomy for the diagnosis and management of uveitis of unknown cause. Ophthalmology 114(10): 1893–1897
Marshall AG, Pawson R, Thom M et al (1998) HTLV-I associated primary CNS T-cell lymphoma. J Neurol Sci 158(2):226–231
McKelvie PA, McNab A, Francis IC et al (2001) Ocular adnexal lymphoproliferative disease: a series of 73 cases. Clin Experiment Ophthalmol 29(6):387–393
Mendenhall WM, Olivier KR, Lynch JW Jr, Mendenhall NP (2006) Lethal midline granuloma-nasal natural killer/T-cell lymphoma. Am J Clin Oncol 29(2):202–206
Michelson JB, Michelson PE, Bordin GM, Chisari FV (1981) Ocular reticulum cell sarcoma. Presentation as retinal detachment with demonstration of monoclonal immunoglobulin light chains on the vitreous cells. Arch Ophthalmol 99(8):1409–1411
Olson JE, Janney CA, Rao RD et al (2002) The continuing increase in the incidence of primary central nervous system non-Hodgkin lymphoma: a surveillance, epidemiology, and end results analysis. Cancer 95(7):1504–1510
Pe'er J, Hochberg FH, Foster CS (2009) Clinical review: treatment of vitreoretinal lymphoma. Ocul Immunol Inflamm 17(5):299–306
Peterson K, Gordon KB, Heinemann MH, DeAngelis LM (1993) The clinical spectrum of ocular lymphoma. Cancer 72(3):843–849
Purvin V, Van Dyk HJ (1984) Primary reticulum cell sarcoma of the brain presenting as steroid-responsive optic neuropathy. J Clin Neuroophthalmol 4(1):15–23
Rajagopal R, Harbour JW (2011) Diagnostic testing and treatment choices in primary vitreoretinal lymphoma. Retina 31(3):435–440
Saatci AO, Arikan G, Ozcan MA et al (2006) Indocyanine green angiographic features of systemic non-Hodgkin's lymphoma and bilateral choroidal involvement. Ophthalmic Surg Lasers Imaging 37(3):236–239
Sarraf D, Jain A, Dubovy S et al (2005) Mucosa-associated lymphoid tissue lymphoma with intraocular involvement. Retina 25(1):94–98
Shakin EP, Augsburger JJ, Eagle RC Jr et al (1988) Multiple myeloma involving the iris. Arch Ophthalmol 106(4):524–526
Shields CL, Shields JA, Carvalho C et al (2001) Conjunctival lymphoid tumors: clinical analysis of 117 cases and relationship to systemic lymphoma. Ophthalmology 108(5):979–984
Stafford SL, Kozelsky TF, Garrity JA et al (2001) Orbital lymphoma: radiotherapy outcome and complications. Radiother Oncol 59(2):139–144
Tagami M, Nagai T, Sekimukai D et al (2011) Uveal extranodal marginal zone B-cell lymphoma of the mucosa-associated lymphoid tissue type with concomitant extraocular lesions in a Japanese man. Jpn J Ophthalmol 55(5):585–587
Tranos PG, Andreou PS, Wickremasinghe SS, Brazier JD (2002) Pseudohypopyon as a feature of multiple myeloma. Arch Ophthalmol 120(1):87–88
Velez G, Chan CC, Csaky KG (2002) Fluorescein angiographic findings in primary intraocular lymphoma. Retina 22(1):37–43
Whitcup SM, de Smet MD, Rubin BI et al (1993) Intraocular lymphoma. Clinical and histopathologic diagnosis. Ophthalmology 100(9):1399–1406
Yeh S, Weichel ED, Faia LJ et al (2010) 25-Gauge transconjunctival sutureless vitrectomy for the diagnosis of intraocular lymphoma. Br J Ophthalmol 94(5):633–638

# Ocular and Adnexal Lymphoma: Biopsy Techniques

6

Sunil Srivastava

## 6.1 Introduction

The decision to perform biopsy should be made when there is an uncertain clinical diagnosis and access to tissue for sampling that could yield a clear diagnosis. Typically in these cases, noninvasive testing has not provided a diagnosis and/or the clinical scenario has not improved with therapy. There may be situations where the diagnosis is already known, but biopsy is needed to determine staging and treatment regimen.

Prior to performing biopsy, several steps should be performed to ensure correct processing (Gonzales and Chan 2007; Coupland et al. 2002). Discussion of the case with an experienced pathologist who will be analyzing the sample is recommended. Rapid transport of the tissue for processing is also required to reduce the deterioration of viable cells. Specific transport media such as RPMI (Roswell Park Memorial Institute Medium) may be used after discussion with the receiving lab. If the receiving lab is close, a transport medium may not be required; however, if the lab is not in the same facility, the use of medium may be required as lymphoid cells can be very fragile.

S. Srivastava, MD
Cole Eye Institute, Cleveland Clinic,
9500 Euclid Avenue, Cleveland, OH USA
e-mail: srivass2@ccf.org

## 6.2 Conjunctival Biopsy

The conjunctiva offers an easily accessible tissue whose removal usually does not result in vision-threatening complications. Typically, the so-called salmon patch is seen when the conjunctiva is involved (Fig. 6.1). This can occur in cases where there is choroidal or adnexal involvement; thus, the conjunctiva should be carefully examined for possible infiltration when lymphoma is suspected.

The technique for removal of conjunctival lesions involves the use of an operating microscope. After identifying the area of involvement, the whole area of involvement is removed. Scissors are used to make initial incision into the conjunctiva and a peritomy is performed. The area for biopsy is dissected off the sclera and then removed – ideally with an edge of normal conjunctiva. The remaining conjunctiva is dissected in order to allow proper closure with either plain or vicryl suture.

Aspiration of the conjunctival surface (Grossniklaus et al. 2003) has also been described as a technique for sampling the conjunctiva. Using a tuberculin syringe with a needle, the surface of the conjunctival lesion is aspirated. Although the correlation between aspiration technique and biopsy can be high, this technique is not often used to make the diagnosis of lymphoma.

A.D. Singh (ed.), *Ocular and Adnexal Lymphoma*, Essentials in Ophthalmology,
DOI 10.1007/978-3-642-38499-8_6, 

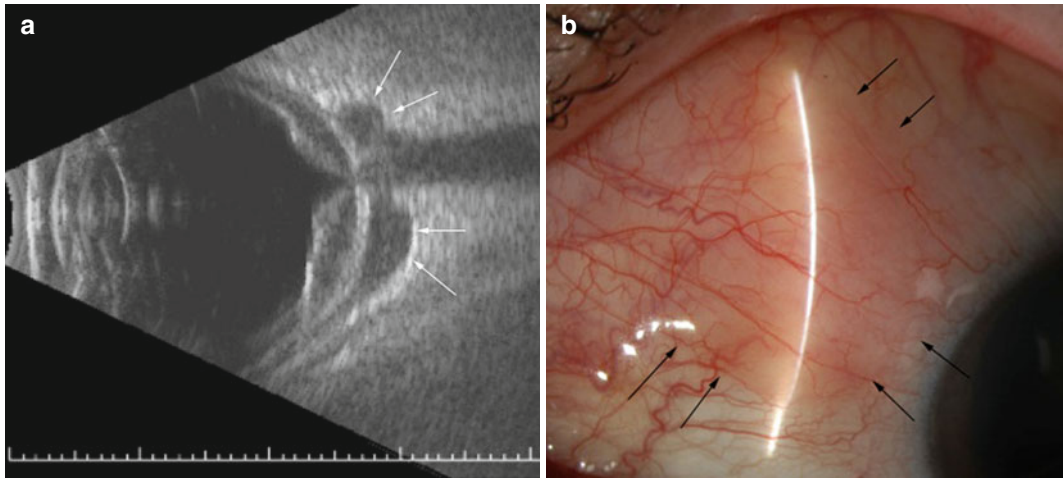


**Fig. 6.1** B-scan ultrasonography reveals peripapillary nodules (*arrows*) suspicious for lymphoma (**a**). Anterior segment examination of the same patient reveals conjunctival lesion with salmon-like color (**b**, *arrows*), which is more accessible to biopsy

## 6.3 Anterior Chamber Biopsy

The anterior chamber (including the iris) is rarely involved in cases of primary lymphoma (Mashayekhi et al. 2013; Finger et al. 2006; Dorrepaal et al. 2010). Both the anterior chamber and iris have been reported as sites for recurrence of systemic lymphoma. Obtaining specimens from the anterior chamber is usually relatively easy with minimal risk of side effects.

An anterior chamber diagnostic paracentesis can be performed in the office or operating room usually a small-bore needle (either 25, 27, or 30 gauge) with the bevel facing up. The needle is pierced at the corneal limbus and fluid is removed with the attached syringe. Typically 1–2 ml can be removed. If performed in the operating room, the anterior chamber can be reformed with balanced salt solution and the sampling repeated again.

An iris tissue biopsy is performed using a fine-needle aspiration technique, whereby a needle is introduced into the body of the lesion and aspiration is performed by the syringe to obtain a sample. Bleeding of the lesion can occur; thus, this is usually performed in the operating room. In previous series of iris lymphoma, often a conjunctival lesion is also found at the time of biopsy and also removed for analysis (Mashayekhi et al. 2013).

## 6.4 Vitreous Biopsy

The vitreous is a common source for biopsy given the relative ease of access with three-port vitrectomy. Vitreous biopsy is often performed in uveitis patients with suspected masquerade syndromes; thus, the clinician must carefully choose the proper tests for the sample of vitreous (Mruthyunjaya et al. 2002; Davis et al. 2005; Margolis et al. 2007). As infectious etiologies can be in the differential, PCR-based identification is often ordered. If lymphoma or any malignancy is high in the differential, it is important to send the undiluted specimen for cytology rather than for PCR or culture. However, if the clinical suspicion for infection is high and low for lymphoma, it may be prudent to forgo cytology and to send the sample for culture and PCR only. A vitreous biopsy can provide up to 1 ml of undiluted sample and often 10 ml or more of a diluted specimen in balanced salt solution. Both undiluted and diluted specimens can be used for testing, thus allowing the clinician to accurately test for both malignancy and infection from one

procedure. Some recommend an unfixed vitreous specimen, which is rapidly delivered to the pathology lab (Coupland et al. 2002). However, others suggest the use of a fixative such as RPMI prior to transport to prevent cell death (Gonzales and Chan 2007).

A standard three-port vitrectomy is set up for the vitreous biopsy. Although 20-gauge vitrectomy has been used for years, smaller gauge (23 or 25 gauge) trocar-based vitrectomy offers several advantages (Yeh et al. 2010; Covert et al. 2012). These include reduction in risk of peripheral retinal tears, self-sealing wounds, and relatively fast post-op recovery. The sample quality from smaller gauge vitrectomy has been shown to be similar to 20-gauge vitrectomy (Yeh et al. 2010). For this author, 25-gauge valved cannulas are used for all diagnostic vitrectomies.

The collection of sample can be performed in a few ways – a sterile syringe can be attached to the vitrector, allowing the syringe to collect the sample. This offers the advantage of direct collection into an easily transported syringe, but requires an assistant to provide suction during the vitrectomy. A sample can be collected without using a separate syringe – however, the sample then needs to be retrieved from the aspiration line of the vitrectomy machine. This process can be somewhat difficult depending on the machine. Recently, a vitreous collection tube has been added to a vitrectomy system, allowing collection into a sterile tube (Fig. 6.2). Most vitrectomy machines require "priming" prior to beginning the vitrectomy – this allows the machine to test if the suction and cutting settings are working properly. During the priming stage, the aspiration line of the vitrector is usually filled with fluid. Thus, in order to obtain a true undiluted sample, either priming should be skipped or a syringe is attached to the aspiration line and used to suction the fluid out of the line. The author usually completes the priming cycle and removes the fluid with a syringe.

After preparing the vitrectomy machine, the sclerotomies are created. Using a 20-gauge system, after opening the conjunctiva, the inferior sclerotomy used for suturing the infusion line is quickly made and the infusion line is placed into the eye. The two superior sclerotomies are then made and plugged. Using a 23- or 25-gauge system – the trocars with cannulas are placed through the conjunctiva. After placing the inferior cannula, the infusion line is attached. The superior cannulas are placed and then plugged. The valved-based cannulas available with the 23- and 25-gauge system eliminate the need for plugs.

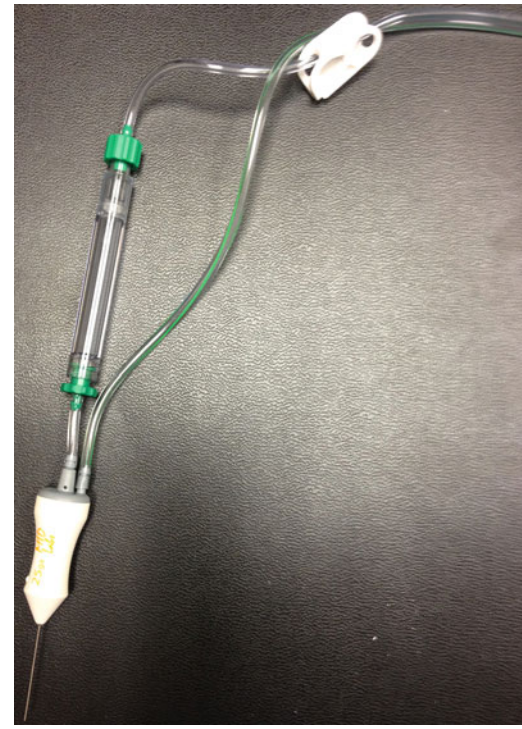

**Fig. 6.2** Biopsy cartridge from the Synergetics Versavit system – allowing for collection and transportation of the specimen

Prior to starting the vitrectomy, the infusion line placement into the vitreous is confirmed. The light pipe and vitrector are placed into the eye and an undiluted specimen is obtained. When using the syringe method, it is important to communicate with the assistant when to begin and to end suction, so as not to pull the vitreous when the vitrector cutting is off. The author uses a 3-ml syringe to collect the undiluted specimen and 10-ml syringe for the diluted specimen. The vitrector is placed over the area with the greatest involvement or as close to the retina as safely as possible, and the vitrectomy

is continued until an adequate sample is obtained or choroidal indentation occurs. Once choroidal indentation appears, the infusion line should be turned on, restoring the pressure in the eye. The vitrector is removed from the eye and the undiluted sample within the syringe is capped and labeled. A 10-ml syringe is then attached to the vitrector and an undiluted specimen is collected. During this stage, it is important for the surgeon to keep the vitrector within the vitreous, as opposed to fluid layer that collects from the infusion line. This allows a higher concentration of vitreous in the collected diluted sample.

After filling the syringe with the diluted sample, the 10-ml syringe is removed and capped. The syringes are passed off the field, properly labeled, and then sent to the lab. Specimen medium may be added at this point if needed. Additionally, the specimens can be aliquoted into smaller tubes, and usually this is performed on the field.

The vitrector is then connected back to the normal aspiration line and a vitrectomy is continued if needed. Other therapeutic maneuvers – including repair of retinal detachment and peeling of preretinal membranes – are then performed. If only a vitreous biopsy was planned, after sample collection surgery can be stopped, however, a fairly complete vitrectomy is recommended (Gonzales and Chan 2007). The peripheral retina should be checked for any retinal breaks. If 20 gauge, the sclerotomies and conjunctiva are sutured closed. If 23 or 25 gauge, the cannulas are removed; sutures are usually not needed (Yeh et al. 2010). The vitrectomy cassette can be passed off as an additional sample for processing if needed.

## 6.5 Chorioretinal/Subretinal Biopsy

The presence of a subretinal or chorioretinal lesions can require biopsy in unknown cases where disease progression occurs despite therapy (Martin et al. 1993; Cole et al. 2008; Lim et al. 2005; Johnston et al. 2004). There are multiple approaches to obtaining a choroidal/subretinal biopsy including transvitreal, fine-needle aspiration, and external choroidal biopsy.

In the transvitreal approach, a standard three-port vitrectomy is performed as described above. The ideal location for biopsy is an area that has low risk of vision loss, retinal detachment, and bleeding. Thus, posterior, nasal, and superior locations are preferred if possible (Fig. 6.3). Farther anterior sites increase the risk of retinal detachments given the risk of vitreous contraction. These biopsy sites usually require endolaser photocoagulation. The closer to the macula – the higher risk of vision loss. However, if macular involvement has already occurred, biopsy of that area may be reasonable. Additionally, the surgeon should choose an area that appears to have active lesions. Many patients will have both inactive and active areas dispersed throughout the retina.

The posterior hyaloid should be elevated and the area over the biopsy site cleared of any residual vitreous. The area for biopsy can be treated with endodiathermy to reduce the risk of bleeding. At this point, multiple different techniques can be employed (Cole et al. 2008; Johnston et al. 2004; Sen et al. 2006; Gonzales and Chan 2007). The small gauge vitreous cutter (23 or 25 gauge) can enter the subretinal space, and with the assistant providing suction via a syringe the tissue is removed with gentle suction and cutting of tissue. The infusion bottle is raised prior to this to reduce the risk of bleeding. Once a sample is obtained, additional fluid can be drawn into the vitrector from the fluid-filled vitreous. Other techniques include placing a soft tip cannula into the subretinal space. Suction of the subretinal material is performed via a connected syringe. Specialized subretinal biopsy forceps can be placed into the tissue to obtain a sample as well (Akgul et al. 2011). Depending on the size and location of the biopsy, laser and gas tamponade are not always needed. It is not uncommon to see hemorrhage over the biopsy site. Bleeding is typically controlled with elevated infusion pressure.

If a larger specimen is needed or a definitive choroidal biopsy is required, a larger area is marked with diathermy. The infusion bottle is

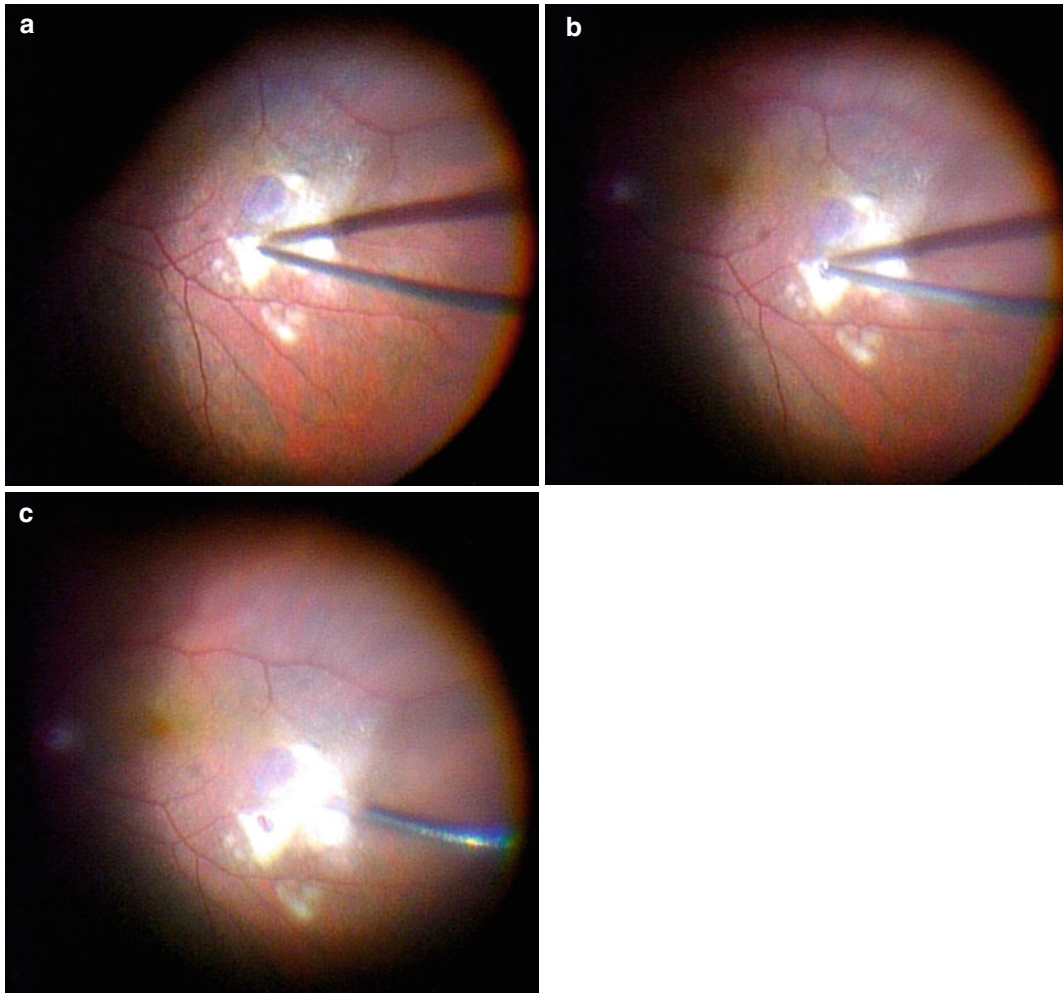


**Fig. 6.3** Intraoperative photos during a pars plana vitrectomy with subretinal biopsy using the vitrector. (**a**) the diathermy probe is used to mark the area for biopsy. (**b**) displays the vitrector probe entering the subretinal space. (**c**) the area of biopsy is seen with no bleeding over the biopsy site

raised, and vertical scissors are then used to cut into the edge of the diathermy and through the retina and choroid. The sclerotomy is then enlarged and the specimen is removed with retinal forceps. Specialized subretinal biopsy forceps can also be used. An air-fluid exchange is performed and endolaser photocoagulation is placed at the edges of normal retina surrounding the biopsy site. A gas tamponade is usually placed.

Fine-needle aspiration biopsy is typically not used for subretinal lesions with possible lymphoma as a vitreous biopsy is usually performed in these cases as well (Gonzales and Chan 2007). Nevertheless, it has been described and used previously (Shields and Shields 1999; Cohen et al. 2001; Eide and Walaas 2009; Sarafzadeh et al. 2010). A 25-gauge to 30-gauge-long needle is attached to a syringe via tubing. Using an indirect ophthalmoscope, the area for biopsy is visualized. The needle is introduced through the pars plana and the lesion is entered. Suction is applied to capture the sample and the needle is then removed from the eye. Postbiopsy hemorrhage over the sight is not uncommon, but usually resolves without complication.

External or transscleral choroidal biopsy can also be performed if the lesion is anterior (Peyman et al. 1981; Gündüz et al. 1999; Johnston et al. 2004; Gonzales and Chan 2007). If the view is clear, laser photocoagulation is placed several days prior to the procedure around the area for biopsy. A standard vitrectomy is performed to obtain vitreous samples. Additional laser can be applied during the vitrectomy. The conjunctiva is opened and the rectus muscles are isolated. The lesion is marked on the sclera and a hinged, nearly full thickness scleral flap is created. The choroid is then visualized and diathermy is placed. Two parallel incisions are then made into the choroid, which is then grabbed once with forceps. Scissors are then used to complete the dissection. Any prolapsed vitreous is removed and the flap is closed with suture (either vicryl or nylon). An air-fluid exchange is performed and gas tamponade is placed.

## 6.6 Adnexal Biopsy

The decision of where to biopsy within in the adnexal structures of the eye is based on orbital imaging which can determine the size, shape, and degree of infiltration. Typically ultrasonography in combination with computed tomography scans and/or magnetic resonance imaging provides the clinician with localization to develop a surgical plan (Garrity and Henderson 2007). If the tumor appears well circumscribed, an excisional biopsy should be performed; if a diffuse tumor is identified, an incisional biopsy is completed (Shields and Shields 1999).

Fine-needle aspiration has been described in the use of the diagnosis of orbital and adnexal tumors (Shields and Shields 1999; Rastogi and Jain 2001; Gupta et al. 2012; Agrawal et al. 2013). Its advantages over traditional incisional biopsy include faster recovery, smaller incisions, and reduction in operative time. However, the amount of tissue obtained is limited in comparison to an incisional biopsy. In cases of suspected lymphoma, some authors recommend FNA only in cases where a known systemic lymphoma exists (Shields and Shields 1999). In those without a known primary, an incisional biopsy can provide more tissue for pathology studies.

After appropriate anesthesia, the area is prepped for needle penetration. For superficial lesions, the needle (typically 22–25 gauge) is placed into the lesion and then suction is applied. For deeper lesions, the lesion is identified with ultrasound or computerized tomography. The ultrasound probe is used to identify and visualize the lesion. The eyeball is fixed in position with the probe and the needle is introduced into lesion. Once the needle is seen within the lesion by ultrasound, suction is applied. The needle is then removed. Bleeding is controlled with direct pressure. The globe can be examined at this point if there is concern about possible injury.

### 6.6.1 Incisional/Excisional Biopsy

In order to directly visualize and biopsy an adnexal lesion, an orbitotomy must be performed. It is beyond the scope of this chapter to describe each possible approach and incision that can be used. The reader is referred to several excellent references that describe the techniques in detail (Shields and Shields 1999; Garrity and Henderson 2007; Meyer and Spoor 2010; Melicher et al. 2010).

In general the location of the orbital tumor determines the specific approach to biopsy. The more anterior the mass, the more likely a conjunctival-based incision can be used. Larger and more posterior lesions usually require skin-based incisions (Shields and Shields 1999).

For access to the medial orbit including structures medical to the optic nerve, a transconjunctival approach is used. This begins either at the medial limbus or just lateral to the caruncle (transcaruncular). The medial rectus is then isolated with muscle hooks. Access to the extraconal space is now possible and retractors can be used for further posterior access. If access to the intraconal space is required, the medial rectus can be removed – a double armed 6-0 vicryl suture is passed through the tendon and then tied and double locked upon itself. The muscle is disinserted and the stump can be sutured to allow further retraction of the globe. Blunt dissection is then continued until the lesion is identified. At this

point the lesion can be carefully incised with scissors. It is important to identify the optic nerve and other vital structures to prevent accidental injury. If the lesion is well encapsulated, blunt dissection around the lesion is performed and the lesion is then removed. Hemostasis is obtained throughout the case with bipolar cautery. Once the biopsy is completed, the rectus muscle is reattached with the 6-0 vicryl suture. The conjunctiva is then closed with vicryl or plain suture.

For lateral lesions including those in the intraconal and extraconal space and within the lacrimal gland fossa, a lateral orbitotomy is used. Prior to beginning the procedure, an injection of lidocaine-epinephrine solution is delivered into the upper lid crease, lateral canthus, lateral orbital rim, and temporalis muscle. A silk traction suture is placed transconjunctivally through the lateral rectus muscle to help identify it during the case. Typically an upper lid crease incision extending into the lateral canthus is created. The incision is carried down to the periosteum covering the lateral orbital rim. Blunt dissection and undermining of the subcutaneous tissue is performed. The periosteum is then incised, and the periosteum and temporalis muscle are dissected off the lateral orbital rim. The area for bone removal is marked and an osteotomy is created with a saw. Holes can be placed in the bone to facilitate closure. Once the bone is removed, the periorbita is incised and soft tissue dissection is performed to isolate and identify the lesion. Traction on the silk suture allows identification of the lateral rectus. Dissection to the tumor is performed and an incisional or excisional biopsy is done. The biopsied tissue is passed off the field and taken to the pathology lab. Once hemostasis is obtained, the bone fragment is replaced and sutured. The periosteum and then subcutaneous tissue are closed. Finally, the skin closed with running nylon or plain suture.

## 6.7 Summary

Ocular and adnexal lymphoma can present in several different ocular and adnexal structures. Correct diagnosis requires proper biopsy technique and careful handling of tissue samples. Multiple methods can be employed to obtain an adequate sample. Discussion with an experienced pathologist is recommended to ensure proper testing is performed on any limited tissue sample.

## References

Agrawal P, Dey P, Lal A (2013) Fine-needle aspiration cytology of orbital and eyelid lesions. Diagn Cytopathol. doi:10.1002/dc.22972, PubMed PMID: 23457033

Akgul H, Otterbach F, Bornfeld N, Jurklies B (2011) Intraocular biopsy using special forceps: a new instrument and refined surgical technique. Br J Ophthalmol 95(1):79–82. doi:10.1136/bjo.2008.148395, PubMed PMID: 21163820

Cohen VM, Dinakaran S, Parsons MA, Rennie IG (2001) Transvitreal fine needle aspiration biopsy: the influence of intraocular lesion size on diagnostic biopsy result. Eye (Lond) 15(Pt 2):143–147, PubMed PMID: 11339578

Cole CJ, Kwan AS, Laidlaw DA, Aylward GW (2008) A new technique of combined retinal and choroidal biopsy. Br J Ophthalmol 92(10):1357–1360. doi:10.1136/bjo.2008.141697, PubMed PMID: 18664500

Coupland SE, Hummel M, Stein H (2002) Ocular adnexal lymphomas: five case presentations and a review of the literature. Surv Ophthalmol 47(5):470–490, Review. PubMed PMID: 12431695

Covert DJ, Henry CR, Bhatia SK, Croskrey J, Sanchez CR, Han DP (2012) Intraoperative retinal tear formation and postoperative rhegmatogenous retinal detachment in transconjunctival cannulated vitrectomy systems compared with the standard 20-gauge system. Arch Ophthalmol 130(2):186–189. doi:10.1001/archopthalmol.2011.1378, PubMed PMID: 22332210

Davis JL, Miller DM, Ruiz P (2005) Diagnostic testing of vitrectomy specimens. Am J Ophthalmol 140(5):822–829, PubMed PMID: 16310459

Dorrepaal SJ, Margolin EA, Wang C (2010) Bilateral pseudohypopyon as a presenting feature of recurrent diffuse large B-cell lymphoma. J Neuroophthalmol 30(1):67–69. doi:10.1097/WNO.0b013e3181ce2c98, PubMed PMID: 20182212

Eide N, Walaas L (2009) Fine-needle aspiration biopsy and other biopsies in suspected intraocular malignant disease: a review. Acta Ophthalmol 87(6):588–601. doi:10.1111/j.1755-3768.2009.01637.x, Review. PubMed PMID: 19719804

Finger PT, Papp C, Latkany P, Kurli M, Iacob CE (2006) Anterior chamber paracentesis cytology (cytospin technique) for the diagnosis of intraocular lymphoma. Br J Ophthalmol 90(6):690–692, PubMed Central PMCID: PMC1860244

Garrity JA, Henderson JW (eds) (2007) Henderson's orbital tumors, 4th edn. Lippincott, Philadelphia

Gonzales JA, Chan CC (2007) Biopsy techniques and yields in diagnosing primary intraocular lymphoma.

Int Ophthalmol 27(4):241–250, Epub 2007 Apr 18. Review. PubMed PMID: 17440686; PubMed Central PMCID: PMC2048742

Grossniklaus HE, Stulting RD, Gansler T, Aaberg TM Jr (2003) Aspiration cytology of the conjunctival surface. Acta Cytol 47(2):239–246, PubMed PMID: 12685195

Gündüz K, Shields JA, Shields CL, Eagle RC Jr, Diniz W, Mercado G, Chang W (1999) Transscleral choroidal biopsy in the diagnosis of choroidal lymphoma. Surv Ophthalmol 43(6):551–555, PubMed PMID: 10416797

Gupta N, Kaur J, Rajwanshi A, Nijhawan R, Srinivasan R, Dey P, Singh U, Gupta P (2012) Spectrum of orbital and ocular adnexal lesions: an analysis of 389 cases diagnosed by fine needle aspiration cytology. Diagn Cytopathol 40(7):582–585. doi:10.1002/dc.21586. Epub 2011 Feb 9, PubMed PMID: 21309008

Johnston RL, Tufail A, Lightman S, Luthert PJ, Pavesio CE, Cooling RJ, Charteris D (2004) Retinal and choroidal biopsies are helpful in unclear uveitis of suspected infectious or malignant origin. Ophthalmology 111(3):522–528, PubMed PMID: 15019330

Lim LL, Suhler EB, Rosenbaum JT, Wilson DJ (2005) The role of choroidal and retinal biopsies in the diagnosis and management of atypical presentations of uveitis. Trans Am Ophthalmol Soc 103:84–91; discussion 91–2. PubMed PMID: 17057791; PubMed Central PMCID: PMC1447562

Margolis R, Brasil OF, Lowder CY, Singh RP, Kaiser PK, Smith SD, Perez VL, Sonnie C, Sears JE (2007) Vitrectomy for the diagnosis and management of uveitis of unknown cause. Ophthalmology 14(10):1893–1897, PubMed PMID: 17509687

Martin DF, Chan CC, de Smet MD, Palestine AG, Davis JL, Whitcup SM, Burnier MN Jr, Nussenblatt RB (1993) The role of chorioretinal biopsy in the management of posterior uveitis. Ophthalmology 100(5):705–714, PubMed PMID: 8493014

Mashayekhi A, Shields CL, Shields JA (2013) Iris involvement by lymphoma: a review of 13 cases. Clin Experiment Ophthalmol 41(1):19–26. doi:10.1111/j.1442-9071.2012.02811.x, PubMed PMID: 22594613

Melicher JS (2010) Lateral orbitotomy. In: Levine M (ed) Manual of oculoplastic surgery, 4th edn. Slack Incorporated, Thorofare, pp 289–296

Meyer DR, Spoor TC (2010) Anterior orbitotomy. In: Levine M (ed) Manual of oculoplastic surgery, 4th edn. Slack Incorporated, Thorofare, pp 289–296

Mruthyunjaya P, Jumper JM, McCallum R, Patel DJ, Cox TA, Jaffe GJ (2002) Diagnostic yield of vitrectomy in eyes with suspected posterior segment infection or malignancy. Ophthalmology 109(6):1123–1129, PubMed PMID: 12045054

Peyman GA, Juarez CP, Raichand M (1981) Full-thickness eye-wall biopsy: long-term results in 9 patients. Br J Ophthalmol 65(10):723–726, PubMed PMID: 7317326; PubMed Central PMCID: PMC1039647

Rastogi A, Jain S (2001) Fine needle aspiration biopsy in orbital lesions. Orbit 20(1):11–23, PubMed PMID: 12045931

Sarafzadeh S, Corrêa ZM, Dhamija A, Augsburger JJ, Trichopoulos N (2010) Intraocular lymphoma diagnosed by fine-needle aspiration biopsy. Acta Ophthalmol 88(6):705–710. doi:10.1111/j.1755-3768.2009.01531.x, PubMed PMID: 19604155

Sen J, Groenewald C, Hiscott PS, Smith PA, Damato BE (2006) Transretinal choroidal tumor biopsy with a 25-gauge vitrector. Ophthalmology 113(6):1028–1031, PubMed PMID: 16751041

Sharara N, Holden JT, Wojno TH, Feinberg AS, Grossniklaus HE (2003) Ocular adnexal lymphoid proliferations: clinical, histologic, flow cytometric, and molecular analysis of forty-three cases. Ophthalmology 110(6):1245–1254, PubMed PMID: 12799255

Shields JA, Shields CL (1999) Atlas of orbital tumors. Lippincott Williams and Wilkins, Philadelphia

Yeh S, Weichel ED, Faia LJ, Albini TA, Wroblewski KK, Stetler-Stevenson M, Ruiz P, Sen HN, Chan CC, Nussenblatt RB (2010) 25-Gauge transconjunctival sutureless vitrectomy for the diagnosis of intraocular lymphoma. Br J Ophthalmol 94(5):633–638. doi:10.1136/bjo.2009.167940, PubMed PMID: 20447965; PubMed Central PMCID: PMC2928256

# Ocular Adnexal Lymphoma: Staging and Treatment

7

Mary E. Aronow, Arun D. Singh,
and John W. Sweetenham

A definitive diagnosis of ocular adnexal lymphoma (OAL) is based upon biopsy as benign conditions including reactive lymphoid hyperplasia cannot be differentiated based on clinical grounds alone. Once the diagnosis has been established, OAL is classified and staging is performed. OAL can coexist with lymphoma in other systemic sites in 15–25 % of cases (Ferry et al. 1996; Fung et al. 2003; Decaudin et al. 2006). For this reason, a multidisciplinary approach is necessary and includes a thorough systemic evaluation and referral to an experienced medical oncologist.

## 7.1 Staging Procedures

### 7.1.1 Ocular Examination

Staging of OAL begins with a thorough ocular examination to assess the extent of disease and laterality. OAL is frequently multifocal and can involve contiguous ocular adnexal structures. A strong correlation has been observed between OAL and coexisting uveal lymphoma (Fuller et al. 2008; Grossniklaus et al. 1998; Baryla et al. 2012; Sarraf et al. 2005; Tagami et al. 2011). For this reason, complete ophthalmic examination should include external inspection for enlarged regional lymph nodes, bilateral dilated fundus examination, motility testing, and exophthalmometry.

### 7.1.2 Ancillary Studies

Ancillary imaging with B-scan ultrasonography may help to further characterize orbital involvement or detect occult orbital disease. Angiography, particularly indocyanine green (ICG) angiography, is useful in suspected cases of overlapping uveal lymphoma. A detailed discussion of the clinical features and diagnostic studies used to characterize OAL is included in Chap. 5.

### 7.1.3 Laboratory Studies

Laboratory evaluation includes a complete blood count (CBC), hepatic enzymes, and serum lactate dehydrogenase (LDH). In view of the plasmacytoid features often seen in extranodal marginal zone lymphomas, measurement of serum immunoglobulins and serum protein electrophoresis may identify a monoclonal protein which can be useful for monitoring disease and response to therapy. Additionally, if treatment with rituximab is anticipated, hepatitis serology should be performed in view of the well-documented risk of reactivation of hepatitis B following treatment with this agent. At most oncology

M.E. Aronow, MD • A.D. Singh, MD
Department of Ophthalmic Oncology,
Cole Eye Institute, Cleveland Clinic Foundation,
9500 Euclid Ave R35, Cleveland,
OH 44195, USA

J.W. Sweetenham, MD (✉)
Huntsman Cancer Institute, University of Utah,
1950 Circle of Hope, Salt Lake City,
UT 84112, USA
e-mail: john.sweetenham@hci.utah.edu

A.D. Singh (ed.), *Ocular and Adnexal Lymphoma*, Essentials in Ophthalmology,
DOI 10.1007/978-3-642-38499-8_7, 

centers, bone marrow aspiration and biopsy is performed at the discretion of the treating oncologist. Bone marrow infiltration is observed in 5–10 % of cases at the time of diagnosis (Ferry et al. 1996; Fung et al. 2003; Decaudin et al. 2006). Bone marrow involvement is very rarely the only site of disseminated disease, and marrow aspiration and biopsy can therefore be omitted for patients with clinical stage IEA disease.

### 7.1.4 Imaging Studies

A series of imaging and laboratory studies are generally performed to fully characterize the extent of ocular disease and to detect systemic involvement. This involves staging through the use of high-resolution contrast-enhanced imaging. Computerized tomographic scan (CT) of the chest, abdomen, and pelvis and magnetic resonance imaging (MRI) of the brain and orbits is routinely performed. Neuroimaging will reveal orbital lesions in up to 50 % of clinically unsuspected cases (Stafford et al. 2001). Paranasal sinus involvement is not uncommon. Imaging of the neck is performed if the cervical lymph nodes can be palpated or noted to be enlarged.

## 7.2 Staging Systems

### 7.2.1 Ann Arbor Classification

While staging is typically performed by the medical oncologist, an understanding of the process is important for multidisciplinary management of OAL. A modified version of the Ann Arbor classification is still commonly used (Table 7.1). Lymphomas are divided into indolent (low grade) or aggressive (high grade) based on their expected clinical behavior. Indolent forms include extranodal marginal zone lymphoma (EMZL), follicular lymphoma (FCL), and lymphoplasmacytic lymphoma (LPCL). Aggressive lymphomas which may (rarely) involve the ocular adnexal structures include mantle cell lymphoma and diffuse large B-cell lymphoma (DLBCL). Although widely used, the Ann Arbor staging system has several limitations for characterizing OAL. In particular, this staging system results in a disproportionate staging distribution. Two-thirds of primary OAL cases present as a localized mass, which under the Ann Arbor system are classified as stage 1E (Hatef et al. 2007; Jenkins et al. 2000; Ferry et al. 2007; Coupland et al. 1999). Using the Ann Arbor system, overall rates for initial staging are 60–80 % for IE, 4–25 % for IIE, and 16–18 % for stages III and IV combined (Coupland et al. 2004; Mannami et al. 2001; McKelvie et al. 2001). Studies using criteria of extraorbital disease result in stage III and IV rates of 22–36 % at diagnosis (Jenkins et al. 2000; Nakata et al. 1999). The limitations of the Ann Arbor system preclude the ability to differentiate the majority of OAL cases from one another based upon disease extent within the ocular adnexal structures which may have important prognostic implications (Knowles et al. 1990; Johnson et al. 1999).

**Table 7.1** Staging of OAL by Ann Arbor system

| | |
|---|---|
| *Indolent lymphomas: EMZL, FCL, LPCL* | |
| Stage I | Localized disease (Ann Arbor [AA] I, IE & II, IIE) |
| Stage II | Disseminated disease (Ann Arbor [AA] III & IV) |
| *Aggressive lymphomas: DLBCL, MCL* | |
| Stage I | Localized or extranodal disease (Ann Arbor [AA] I or IE) |
| Stage II | 2 or more nodal sites; 3 or more extranodal sites |
| Stage III | Stage II with additional poor prognostic features |

*EMZL* extranodal marginal zone lymphoma, *FCL* follicular lymphoma, *LPCL* lymphoplasmacytic lymphoma, *DLBCL* diffuse large B-cell lymphoma, *MCL* mantle cell lymphoma, *E* extranodal disease

### 7.2.2 Tumor-Node-Metastasis (TNM)-Based Staging

More recently, a tumor-node-metastasis (TNM)-based staging system for primary OAL (Table 7.2) has been developed under the guidance of the American Joint Committee on Cancer (AJCC) (Coupland et al. 2009; Delori et al. 1995). This system addresses many of the shortcomings of the Ann Arbor system and more precisely defines

**Table 7.2** Staging of OAL by the tumor-node-metastasis system

| | |
|---|---|
| *T classification* | |
| TX | Lymphoma extent not specified |
| T0 | No evidence of lymphoma |
| T1 | Conjunctival lymphoma alone |
| T2 | Orbital lymphoma with or without conjunctival involvement |
| T3 | Preseptal eyelid lymphoma in addition to conjunctival/orbital disease |
| T4 | Invasion of adjacent structures, such as bone and brain |
| *N classification* | |
| NX | Lymph node involvement not assessed |
| N0 | No evidence of lymph node involvement |
| N1 | Involvement of ipsilateral regional lymph nodes |
| N2 | Involvement of contralateral or bilateral regional lymph nodes |
| N3 | Involvement of peripheral lymph nodes not draining ocular adnexal region |
| N4 | Involvement of central lymph nodes |
| *M classification* | |
| MX | Lymphoma dissemination not assessed |
| M0 | No evidence of involvement of additional extranodal sites |
| M1 | Lymphoma involvement of other organs (at diagnosis or subsequently) |

Modified from the American Joint Committee on Cancer (AJCC) seventh edition TNM-based staging manual for OAL

disease extent. The ultimate goal of the proposed TNM-based system is to facilitate future studies aimed at identifying clinical and histomorphologic features of OAL of prognostic significance and to assess treatment outcomes. To date, the feasibility of this system has only been analyzed in a limited capacity (Lee et al. 2011).

## 7.3 Treatment

The treatment of OAL is an area of controversy, progress, and change. Currently OAL is treated in a manner similar to other malignant lymphomas, most frequently using radiation, immunotherapy, or a combination of therapies. With the recognition that the vast majority of OAL are of the EMZL type and that there may be an infectious basis for this subgroup, the possibility of treatment with antimicrobial therapy instead of cytotoxic modalities has been considered in some cases. This remains an investigational approach at present. Another controversy is whether to treat very indolent OAL or to closely observe these patients, particularly when the disease is widely disseminated, the patient is symptom-free or if there are significant comorbidities which limit treatment options. A discussion of the most commonly utilized treatment modalities for OAL follows. The description of treatment options below is primarily confined to that of indolent histologies, particularly EMZL. Disease with more aggressive histology will require complex multi-agent chemoimmunotherapy regimens not described in detail below.

### 7.3.1 Surgery

The primary role of surgery in the management of OAL is to obtain adequate material for accurate histologic diagnosis. It should generally not be considered as the primary or sole treatment modality. Surgery has been reported to be successful in managing certain cases of highly localized OAL and has been recommended for stage I mucosa-associated lymphoid tissue (MALT) lymphoma in some sites. Its applicability remains very limited in OAL due to the multifocal nature of the disease and frequent juxtaposition of OAL to sensitive ocular tissues. Surgery as a sole therapy is therefore generally reserved for isolated, highly localized lesions of the conjunctiva (Stafford et al. 2001; Coupland et al. 2004). Surgical excision alone is rarely selected as a primary therapy; therefore, limited data exist for meaningful assessment of disease-free survival regarding this treatment modality.

### 7.3.2 Cryotherapy

While cryotherapy is used as primary and adjuvant treatment for many conjunctival malignancies including primary acquired melanosis, malignant melanoma, and squamous cell carcinoma, this modality has limited use in the management of OAL. This therapy has resulted in variable success

due to debulking the tumor without complete elimination of malignant tissue (Shields et al. 2001). Recurrence rates as high as 33 % have been observed in some series of conjunctival lymphoma treat with cryotherapy alone (Eichler and Fraunfelder 1994). For this reason, cryotherapy is generally reserved for patients with highly localized conjunctival OAL who are unable to receive other treatment modalities (Fung et al. 2003).

### 7.3.3 Radiation Therapy

Historically, external beam radiation (EBRT) has been the most frequently used modality for the treatment of OAL. This modality has been effective for many cases with reports of local control rates of conjunctival lymphoma as high as 91–100 % (Tsai and Colby 2005). However, analysis of radiation treatment outcomes is confounded by small patient numbers in most series, the use of early and inaccurate classification schemes, short follow-up times, and apparent lack of ophthalmic follow-up.

Both electron and photon irradiation have been successfully employed in OAL. Dosage is based on the tumor grade or type (Fung et al. 2003; Bhatia et al. 2002). Typical doses are 28–36 Gy for low-grade OAL and 30–40 Gy for high-grade OAL. For the EMZL subtype, an analysis of the radiation dose–response relationship revealed that the 5-year local tumor control rates were 81 % with doses below 30 Gy and 100 % with doses higher than 30 Gy (Fung et al. 2003; Jenkins et al. 2000). Variable sensitivity to radiotherapy based upon lymphoma subtype has been observed, for example, follicular lymphoma has demonstrated a 100 % response rate to both higher and lower doses (Fung et al. 2003). While radiation studies frequently emphasize the ability to obtain local control, the effect of this modality on overall course and prognosis is less clear. Even stage IV-EA disease showed good local control, though survival was significantly lower. Multiple studies revealing higher rates of delayed systemic recurrence suggest that longer follow-up is necessary for accurate assessment of treatment effect (Jenkins et al. 2000).

Complications of radiation to the ocular adnexal structures include dry eye, conjunctivitis, keratitis, cataract, and retinopathy (Tsai and Colby 2005). Mild side effects including temporary injection of the periocular skin or dry eye occur in most patients and have been reported in up to 100 % in some series (Jereb et al. 1984). Several studies have found that dry eye symptoms occur more frequently when radiation is used to treat conjunctival lymphomas compared to radiation for orbital and other OALs (60 % vs. 16 %, respectively) (Liao et al. 2002). This observation may be a direct result of radiation-induced damage to the goblet cells and accessory lacrimal glands within the conjunctiva (Liao et al. 2002; Erickson et al. 1992).

The most frequently encountered long-term complication is cataract formation. Cataracts develop in 17–35 % of patients and are more likely to occur in patients receiving higher doses of radiation (Dunbar et al. 1990). Lens shielding techniques to prevent cataract formation have been developed, including lead blocks or contact lens shields. The role of lens shielding radiation to decrease cataract formation is controversial, with some studies showing no effect on local lymphoma recurrence and others showing high recurrence rates in patients who underwent lens-sparing radiation treatment protocols (Coupland et al. 2004; Le et al. 2002).

### 7.3.4 Chemotherapy

Since OAL frequently presents as localized disease (stage IE), chemotherapy is rarely used, with the exception of aggressive forms such as DLBCL (Stacy et al. 2012). A complete review of chemotherapy used in lymphoma is beyond the scope of this chapter. Briefly, standard chemotherapy for non-Hodgkin's lymphomas, including OAL when it is part of more advanced disease, is that of standard systemic lymphoma regimens using rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP). Chlorambucil and, more recently, purine analogues such as fludarabine, cladarabine, and pentostatin have also been used. While some have used systemic

corticosteroids for tumor suppression of OAL, steroids offer ineffective long-term control. When chemotherapy is used in the treatment of OAL, it can be employed as an adjuvant therapy, for example, following local conjunctival treatment. It may also be used as a sole, primary therapy for patients with localized high-grade OAL, those with diffuse systemic involvement, or in patients with recurrent disease who have failed other treatment regimens.

### 7.3.5 Immunotherapy

#### 7.3.5.1 Interferon

Interferons (IFNs) are proteins produced in response to viral pathogens, bacteria, parasites, and neoplastic cells. IFNs help to modulate the host immune response and in addition have antineoplastic properties. IFN-alpha is a protein that has been rarely used for OAL despite its long-standing use in systemic lymphoma. Its first use for OAL was in a case of conjunctival MALT lymphoma successfully treated with local IFN-alpha injections (Cellini et al. 1996). Following this, several other case reports have described the successful use of IFN-alpha for conjunctival MALT lymphoma (Zinzani et al. 1999; Lachapelle et al. 2000; Lucas et al. 2003). In the majority of series,

IFN-alpha has been used to treat low-grade OAL using a regimen consisting of 1–1.5 million international units (IU) injected intralesionally three times per week over a period of 4 weeks (total of 10–12 doses) (Zinzani et al. 1999; Lachapelle et al. 2000; Lucas et al. 2003; Ross et al. 2004). In one series of 5 cases of conjunctival MALT lymphoma, there was an observed 80 % initial complete response following 8 weeks of intralesional injections with short-term follow-up (Blasi et al. 2001). One patient with stage IIA disease died of systemic lymphoma at 1 year (Blasi et al. 2001). More data regarding local and systemic efficacy are needed prior to acceptance of this modality. Side effects following treatment with IFN-alpha include fevers, chills, myalgias, headaches, nausea, and subconjunctival hemorrhage (Lachapelle et al. 2000).

#### 7.3.5.2 Monoclonal Antibody Therapy

Monoclonal antibodies are a more recent form of lymphoma treatment. The most commonly used has been rituximab, an antibody directed against the B-cell surface antigen CD-20. This interaction leads to destruction of B-cells through several mechanisms of action, including complement activation, antibody-directed cellular cytotoxicity, and direct induction of apoptosis. Rituximab's first reported use in OAL was in two patients with conjunctival EMZL who developed relapse following treatment with radiation therapy (Nuckel et al. 2004). Both patients received 375 mg/m$^2$ of rituximab intravenously once weekly for a course of 4 weeks. One patient had a complete response and the second had a partial response.

Although rituximab is now widely used in multiple subtypes of B-cell lymphoma, very few studies have prospectively evaluated its activity as a single agent in OAL of EMZL subtype. Published data suggest an overall response rate of around 50 %. The risk of subsequent relapse after rituximab appears higher for patients with localized compared with systemic disease, suggesting that involved field radiation should be regarded as the standard of care for disease confined to the OAL structures, reserving the use of rituximab for patients with more extensive disease or to those patients whose disease has relapsed after initial radiation therapy.

Side effects of rituximab include transient flu-like symptoms, such as fatigue, headache, fever, chills, nausea, and vomiting, most of which are immediate infusion-related events (Hainsworth et al. 2002, 2003). Reactivation of hepatitis B infection is an uncommon but well-reported complication of rituximab. Other late infectious complications have also been reported.

#### 7.3.5.3 Antimicrobial Treatment

There is increasing evidence of the role of chronic infection in OAL. Both *C. psittaci* and *H. pylori* have been implicated (Chap. 4) (Ferreri et al. 2004; Chan et al. 2004). Follow-up data from *C. psittaci* detection studies have suggested a therapeutic effect following antibiotic therapy with doxycycline, presumably by eradication of the infection, which underlies lymphomagenesis. Other studies

have shown an effect in small numbers of patients using anti-*H. pylori* triple therapy (Abramson et al. 2005). Overall, antibiotic treatment regimens have shown variable results by study group and geographic location (Husain et al. 2007; Ferreri et al. 2012). Larger studies are needed to clarify the role of antibiotics in treatment of OAL.

#### 7.3.5.4 Observation

There is some evidence pertaining to systemic lymphoma that spontaneous regression occurs at non-ocular sites, in up to 23 % of individuals (Horning and Rosenberg 1984). This observation has raised the possibility of observation for low-grade, highly localized OAL confined to the conjunctiva. In one prospective series, 13 patients with low-grade conjunctival MALT lymphoma were followed after 5 patients chose to undergo radiotherapy and 8 patients chose observation. After a mean follow-up time of 5.4 years, 7 of the 8 patients who chose observation demonstrated spontaneous regression of OAL. One patient in the observation group developed a recurrence of conjunctival OAL in the same eye. Of the five patients who underwent radiotherapy, all showed resolution without recurrence during the follow-up period (Matsuo and Yoshino 2004). Others have demonstrated either a lack of progression or spontaneous resolution of EMZL OAL (Nakata et al. 1999; Chang et al. 2004). Watch and wait policy has also been evaluated in 36 patients with localized OAL in a study reported by Tanimoto et al (2006). With mature follow-up, almost 70 % of patients had not required therapy, although 47 % had disease progression. Our present understanding of the behavior of OAL dictates that caution should be exercised regarding observation as it is possible for low-grade OAL to undergo transformation to high-grade forms (Coupland et al. 2002). In addition, watch and wait approaches should never be considered for aggressive subtypes of NHL, even if localized.

## 7.4 Prognosis

The published data regarding prognosis for OAL is based mostly on single center, predominantly retrospective studies with variable staging criteria and treatment approaches as well as variable follow-up. Consequently, it is difficult to provide accurate prognostic information on this entity. Most studies report high (often in excess of 90 %) progression-free and overall survival rates, especially for patients with disease limited to the OAL (Coupland et al. 2007; Cho et al. 2003; Auw-Haedrich et al. 2001; Rosado et al. 2006; Tanimoto et al. 2007). Reported adverse prognostic factors include primary sites other than the conjunctiva, disseminated disease (to lymph nodes or other extranodal sites), advanced age, and elevated LDH. The risk of widespread disease appears to be lowest for patients with conjunctival disease. Some immunohistochemical markers such as *BCL-6* and *p53* have also been reported to predict for a worse prognosis, although this requires further study.

## References

Abramson DH, Rollins I, Coleman M (2005) Periocular mucosa-associated lymphoid/low grade lymphomas: treatment with antibiotics. Am J Ophthalmol 140(4): 729–730

Auw-Haedrich C, Coupland SE, Kapp A et al (2001) Long term outcome of ocular adnexal lymphoma subtyped according to the REAL classification. Revised European and American Lymphoma. Br J Ophthalmol 85(1):63–69

Baryla J, Allen LH, Kwan K et al (2012) Choroidal lymphoma with orbital and optic nerve extension: case and review of literature. Can J Ophthalmol 47(1): 79–81

Bhatia S, Paulino AC, Buatti JM et al (2002) Curative radiotherapy for primary orbital lymphoma. Int J Radiat Oncol Biol Phys 54(3):818–823

Blasi MA, Gherlinzoni F, Calvisi G et al (2001) Local chemotherapy with interferon-alpha for conjunctival mucosa-associated lymphoid tissue lymphoma: a preliminary report. Ophthalmology 108(3):559–562

Cellini M, Possati GL, Puddu P, Caramazza R (1996) Interferon alpha in the therapy of conjunctival lymphoma in an HIV + patient. Eur J Ophthalmol 6(4): 475–477

Chan CC, Smith JA, Shen DF et al (2004) Helicobacter pylori (H. pylori) molecular signature in conjunctival mucosa-associated lymphoid tissue (MALT) lymphoma. Histol Histopathol 19(4):1219–1226

Chang YC, Chang CH, Liu YT et al (2004) Spontaneous regression of a large-cell lymphoma in the conjunctiva and orbit. Ophthal Plast Reconstr Surg 20(6): 461–463

Cho EY, Han JJ, Ree HJ et al (2003) Clinicopathologic analysis of ocular adnexal lymphomas: extranodal

marginal zone b-cell lymphoma constitutes the vast majority of ocular lymphomas among Koreans and affects younger patients. Am J Hematol 73(2):87–96
Coupland SE, Foss HD, Assaf C et al (1999) T-cell and T/natural killer-cell lymphomas involving ocular and ocular adnexal tissues: a clinicopathologic, immunohistochemical, and molecular study of seven cases. Ophthalmology 106(11):2109–2120
Coupland SE, Hummel M, Stein H (2002) Ocular adnexal lymphomas: five case presentations and a review of the literature. Surv Ophthalmol 47(5):470–490
Coupland SE, Hellmich M, Auw-Haedrich C et al (2004) Prognostic value of cell-cycle markers in ocular adnexal lymphoma: an assessment of 230 cases. Graefes Arch Clin Exp Ophthalmol 24(2):130–145
Coupland SE, Jaehne D, Hummel M, Stein H (2007) The tale and molecular trail of a disseminated ocular adnexal malt lymphoma. Graefes Arch Clin Exp Ophthalmol 245(7):1055–1059
Coupland SE, White VA, Rootman J et al (2009) A TNM-based clinical staging system of ocular adnexal lymphomas. Arch Pathol Lab Med 133(8):1262–1267
Decaudin D, de Cremoux P, Vincent-Salomon A et al (2006) Ocular adnexal lymphoma: a review of clinicopathologic features and treatment options. Blood 108(5):1451–1460
Delori FC, Dorey CK, Staurenghi G et al (1995) In vivo fluorescence of the ocular fundus exhibits retinal pigment epithelium lipofuscin characteristics. Invest Ophthalmol Vis Sci 36(3):718–729
Dunbar SF, Linggood RM, Doppke KP et al (1990) Conjunctival lymphoma: results and treatment with a single anterior electron field. A lens sparing approach. Int J Radiat Oncol Biol Phys 19(2):249–257
Eichler MD, Fraunfelder FT (1994) Cryotherapy for conjunctival lymphoid tumors. Am J Ophthalmol 118(4):463–467
Erickson BA, Harris GJ, Enke CA et al (1992) Periocular lymphoproliferative diseases: natural history, prognostic factors, and treatment. Radiology 185(1):63–70
Ferreri AJ, Guidoboni M, Ponzoni M et al (2004) Evidence for an association between Chlamydia psittaci and ocular adnexal lymphomas. J Natl Cancer Inst 96(8):586–594
Ferreri AJ, Govi S, Pasini E et al (2012) Chlamydophila psittaci eradication with doxycycline as first-line targeted therapy for ocular adnexae lymphoma: final results of an international phase II trial. J Clin Oncol 30(24):2988–2994
Ferry JA, Yang WI, Zukerberg LR et al (1996) CD5+ extranodal marginal zone B-cell (MALT) lymphoma. A low grade neoplasm with a propensity for bone marrow involvement and relapse. Am J Clin Pathol 105(1):31–37
Ferry JA, Fung CY, Zukerberg L et al (2007) Lymphoma of the ocular adnexa: a study of 353 cases. Am J Surg Pathol 31(2):170–184
Fuller ML, Sweetenham J, Schoenfield L, Singh AD (2008) Uveal lymphoma: a variant of ocular adnexal lymphoma. Leuk Lymphoma 49(12):2393–2397
Fung CY, Tarbell NJ, Lucarelli MJ et al (2003) Ocular adnexal lymphoma: clinical behavior of distinct World Health Organization classification subtypes. Int J Radiat Oncol Biol Phys 57(5):1382–1391
Grossniklaus HE, Martin DF, Avery R et al (1998) Uveal lymphoid infiltration. Ophthalmology 105(7):1265–1273
Hainsworth JD, Litchy S, Burris HA 3rd et al (2002) Rituximab as first-line and maintenance therapy for patients with indolent non-hodgkin's lymphoma. J Clin Oncol 20(20):4261–4267
Hainsworth JD, Litchy S, Barton JH et al (2003) Single-agent rituximab as first-line and maintenance treatment for patients with chronic lymphocytic leukemia or small lymphocytic lymphoma: a phase II trial of the Minnie Pearl Cancer Research Network. J Clin Oncol 21(9):1746–1751
Hatef E, Roberts D, McLaughlin P et al (2007) Prevalence and nature of systemic involvement and stage at initial examination in patients with orbital and ocular adnexal lymphoma. Arch Ophthalmol 125(12):1663–1667
Horning SJ, Rosenberg SA (1984) The natural history of initially untreated low-grade non-Hodgkin's lymphomas. N Engl J Med 311(23):1471–1475
Husain A, Roberts D, Pro B et al (2007) Meta-analyses of the association between Chlamydia psittaci and ocular adnexal lymphoma and the response of ocular adnexal lymphoma to antibiotics. Cancer 110(4):809–815
Jenkins C, Rose GE, Bunce C et al (2000) Histological features of ocular adnexal lymphoma (REAL classification) and their association with patient morbidity and survival. Br J Ophthalmol 84(8):907–913
Jereb B, Lee H, Jakobiec FA, Kutcher J (1984) Radiation therapy of conjunctival and orbital lymphoid tumors. Int J Radiat Oncol Biol Phys 10(7):1013–1019
Johnson TE, Tse DT, Byrne GE Jr et al (1999) Ocular-adnexal lymphoid tumors: a clinicopathologic and molecular genetic study of 77 patients. Ophthal Plast Reconstr Surg 15(3):171–179
Knowles DM, Jakobiec FA, McNally L, Burke JS (1990) Lymphoid hyperplasia and malignant lymphoma occurring in the ocular adnexa (orbit, conjunctiva, and eyelids): a prospective multiparametric analysis of 108 cases during 1977 to 1987. Hum Pathol 21(9):959–973
Lachapelle KR, Rathee R, Kratky V, Dexter DF (2000) Treatment of conjunctival mucosa-associated lymphoid tissue lymphoma with intralesional injection of interferon alfa-2b. Arch Ophthalmol 118(2):284–285
Le QT, Eulau SM, George TI et al (2002) Primary radiotherapy for localized orbital MALT lymphoma. Int J Radiat Oncol Biol Phys 52(3):657–663
Lee SE, Paik JS, Cho WK et al (2011) Feasibility of the TNM-based staging system of ocular adnexal extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma). Am J Hematol 86(3):262–266
Liao SL, Kao SC, Hou PK, Chen MS (2002) Results of radiotherapy for orbital and adnexal lymphoma. Orbit 21(2):117–123

Lucas RS, Mortimore R, Sullivan TJ, Waldie M (2003) Interferon treatment of childhood conjunctival lymphoma. Br J Ophthalmol 87(9):1191

Mannami T, Yoshino T, Oshima K et al (2001) Clinical, histopathological, and immunogenetic analysis of ocular adnexal lymphoproliferative disorders: characterization of malt lymphoma and reactive lymphoid hyperplasia. Mod Pathol 14(7):641–649

Matsuo T, Yoshino T (2004) Long-term follow-up results of observation or radiation for conjunctival malignant lymphoma. Ophthalmology 111(6):1233–1237

McKelvie PA, McNab A, Francis IC et al (2001) Ocular adnexal lymphoproliferative disease: a series of 73 cases. Clin Experiment Ophthalmol 29(6):387–393

Nakata M, Matsuno Y, Katsumata N et al (1999) Histology according to the revised European-American lymphoma classification significantly predicts the prognosis of ocular adnexal lymphoma. Leuk Lymphoma 32(5–6):533–543

Nuckel H, Meller D, Steuhl KP, Duhrsen U (2004) Anti-CD20 monoclonal antibody therapy in relapsed MALT lymphoma of the conjunctiva. Eur J Haematol 73(4):258–262

Rosado MF, Byrne GE Jr, Ding F et al (2006) Ocular adnexal lymphoma: a clinicopathologic study of a large cohort of patients with no evidence for an association with Chlamydia psittaci. Blood 107(2): 467–472

Ross JJ, Tu KL, Damato BE (2004) Systemic remission of non-Hodgkin's lymphoma after intralesional interferon alpha-2b to bilateral conjunctival lymphomas. Am J Ophthalmol 138(4):672–673

Sarraf D, Jain A, Dubovy S et al (2005) Mucosa-associated lymphoid tissue lymphoma with intraocular involvement. Retina 25(1):94–98

Shields CL, Shields JA, Carvalho C et al (2001) Conjunctival lymphoid tumors: clinical analysis of 117 cases and relationship to systemic lymphoma. Ophthalmology 108(5):979–984

Stacy RC, Jakobiec FA, Herwig MC et al (2012) Diffuse large B-cell lymphoma of the orbit: clinicopathologic, immunohistochemical, and prognostic features of 20 cases. Am J Ophthalmol 154(1):87–98 e1

Stafford SL, Kozelsky TF, Garrity JA et al (2001) Orbital lymphoma: radiotherapy outcome and complications. Radiother Oncol 59(2):139–144

Tagami M, Nagai T, Sekimukai D et al (2011) Uveal extranodal marginal zone B-cell lymphoma of the mucosa-associated lymphoid tissue type with concomitant extraocular lesions in a Japanese man. Jpn J Ophthalmol 55(5):585–587

Tanimoto K, Kaneko A, Suzuki S et al (2006) Long-term follow-up results of no initial therapy for ocular adnexal MALT lymphoma. Ann Oncol 17(1):135–140

Tanimoto K, Kaneko A, Suzuki S et al (2007) Primary ocular adnexal MALT lymphoma: a long-term follow-up study of 114 patients. Jpn J Clin Oncol 37(5): 337–344

Tsai PS, Colby KA (2005) Treatment of conjunctival lymphomas. Semin Ophthalmol 20(4):239–246

Zinzani PL, Magagnoli M, Galieni P et al (1999) Nongastrointestinal low-grade mucosa-associated lymphoid tissue lymphoma: analysis of 75 patients. J Clin Oncol 17(4):1254

# Vitreoretinal Lymphoma: Staging and Treatment

8

Mary E. Aronow, Arun D. Singh, and David M. Peereboom

## 8.1 Introduction

Approximately 56–85 % of individuals initially diagnosed with PVRL will subsequently develop central nervous system (CNS) involvement over a period of many months to several years (Char et al. 1988; Freeman et al. 1987; Peterson et al. 1993). Conversely, about 25 % of patients with PCNSL will have detectable intraocular involvement at the time of diagnosis (Hochberg and Miller 1988). For this reason, a multidisciplinary team of experts in the fields of ophthalmic oncology, pathology, radiation oncology, and neuro-oncology are recommended for optimal treatment planning and for continued patient surveillance.

## 8.2 Staging Procedures

### 8.2.1 Ophthalmic

Staging of PVRL begins with a complete medical history and ophthalmic examination to assess the extent of laterality of disease. Approximately half of individuals present to an ophthalmologist with complaints of painless blurred vision, floaters, or both (Whitcup et al. 1993; Akpek et al. 1999). The remainder are asymptomatic or diagnosed during ophthalmic screening in the setting of known PCNSL. Ophthalmic examination of both the anterior and posterior segments should be performed. This is particularly important as bilateral involvement occurs in up to 80 % of individuals and is typically asymmetric (Peterson et al. 1993). In suspected cases of PVRL, definitive diagnosis is based upon biopsy (Chap. 6). The exception is in the setting of existing PCNSL and typical ophthalmic findings, in which case biopsy of an ophthalmic site is unnecessary. Cytology, molecular pathology (including immunoglobulin or T-cell receptor gene rearrangement studies), immunohistochemistry, flow cytometry, and cytokine analysis are often required to support the diagnosis. These techniques are discussed elsewhere in this monograph (Chaps. 2 and 3).

### 8.2.2 Central Nervous System

Staging of PCNSL begins with a thorough neuro-oncologic history and examination, imaging studies, and laboratory evaluation. Individuals with PCNSL often present with neurologic symptoms and concomitant magnetic resonance imaging (MRI) findings. Neurologic symptoms are variable and may include generalized signs of increased intracranial pressure (ICP), or more

M.E. Aronow, MD • A.D. Singh, MD
Department of Ophthalmic Oncology, Cole Eye Institute, Cleveland Clinic Foundation, 9500 Euclid Ave R35, Cleveland, OH 44195, USA

D.M. Peereboom, MD (✉)
The Rose Ella Burkhardt Brain Tumor and Neuro-Oncology Center, Neurological Institute, Cleveland Clinic Foundation, 9500 Euclid Ave R35, Cleveland, OH 44195, USA
e-mail: peerebd@ccf.org

A.D. Singh (ed.), *Ocular and Adnexal Lymphoma*, Essentials in Ophthalmology,
DOI 10.1007/978-3-642-38499-8_8, 

focal symptoms such as weakness, sensory deficits, or aphasia (Doucet et al. 2013; Herrlinger et al. 1999). A thorough discussion on the presenting signs and clinical features of PCNSL is included in Chap. 5.

The relationship between PVRL and PCNSL is variable with intraocular involvement preceding, occurring simultaneously, or following CNS manifestations. Therefore, once the diagnosis has been established, it is imperative that all cases of PVRL be thoroughly evaluated by a neuro-oncologist to exclude CNS involvement at the initial diagnosis and periodically thereafter. Conversely, regular ophthalmic screening examinations should be part of the initial diagnostic evaluation and subsequent management of individuals diagnosed with PCNSL.

### 8.2.3 Neuroimaging

Neuroimaging at the time of diagnosis is indicated in all patients with PVRL. Gadolinium-enhanced MRI of the brain is the diagnostic procedure of choice. Cranial lesions appear as multiple isointense nodules on T1-MRI and demonstrate characteristic dense and diffuse contrast enhancement. Meningeal enhancement with gadolinium contrast is indicative of leptomeningeal dissemination (LMD).

### 8.2.4 Cerebrospinal Fluid Sampling

Cerebrospinal fluid sampling can also detect MD and should be performed in every patient with suspected or confirmed PCNSL. In one series of 69 patients, LMD was confirmed in up to 11 % of patients with PCNSL (Kiewe et al. 2010). Demonstration of malignant lymphocytes in the CSF is confirmatory of the diagnosis and typically reveals lymphocytic pleocytosis, raised protein concentration, and normal or low glucose concentration. Many centers additionally perform CT scans of the chest, abdomen, and pelvis to exclude systemic involvement or systemic origin of the CNS involvement. Visceral involvement is rare at the initial diagnosis, but is not uncommon in terminal stages. Testicular ultrasound examination is recommended in elderly patients because of frequent CNS involvement observed in testicular lymphomas.

## 8.3 Treatment

The clinical management of individuals with PVRL and PCNSL remains controversial, and optimal treatment strategies have not yet been clearly defined. At present, treatment planning depends significantly upon local expertise. As PCNSL is very sensitive to corticosteroids, treatment with corticosteroids should be withheld in suspected cases until tissue diagnosis is obtained. Treatment has evolved in the last two decades, and there is a general consensus that regimens containing high-dose methotrexate, with or without whole-brain radiation therapy (WBRT), yield better response rates and outcomes than regimens that do not contain high-dose methotrexate. A schema outlining our current approach of management is shown (Fig. 8.1).

### 8.3.1 Ophthalmic Treatment

Management of PVRL should be undertaken in partnership with a neuro-oncologist who has expertise in lymphoma. As a high percentage of patients with PVRL eventually develop CNS involvement, some experts recommend that the treatment goal for PVRL must be to eradicate the ocular disease and prevent subsequent CNS involvement. Others favor local therapy for disease confined to the eye with close follow-up and systemic therapy if evidence of CNS disease develops.

#### 8.3.1.1 Ophthalmic Radiotherapy

Local therapies for PVRL include ocular radiation and intravitreal chemotherapy. There has been no trial that has compared these therapies head to head. At the present time, some experts prefer intravitreal chemotherapy, while others recommend ocular radiation as first-line therapy. Traditional therapy with ocular radiation

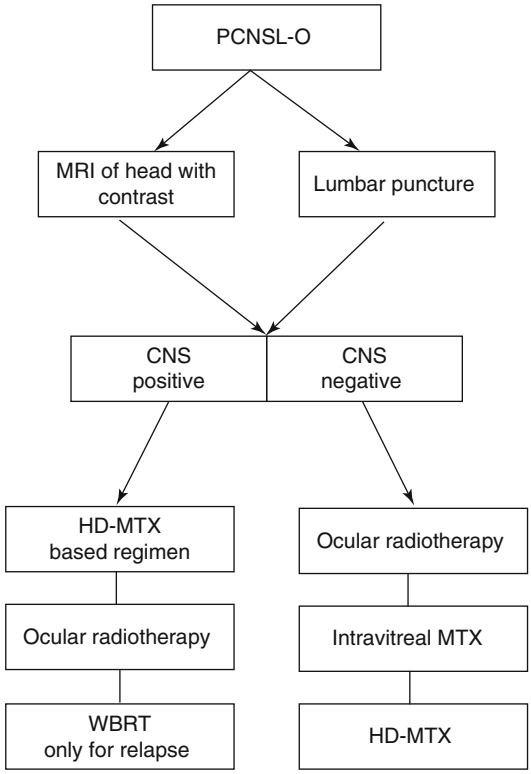


**Fig. 8.1** An approach to management of intraocular lymphoma

(35–40 Gy in approximately 15 divided fractions) controls ocular involvement in the majority of cases (Margolis et al. 1980), but most individuals progress to develop CNS disease (Peterson et al. 1993). Irradiation of both eyes, due to the high incidence of bilaterality, should be strongly considered for patients with proven PVRL. Radiation therapy to the brain may have significant side effects including neurocognitive decline in elderly individuals. Therefore, its use for prophylaxis in patients without proven CNS involvement is not advisable.

#### 8.3.1.2 Intravitreal Chemotherapy

Intravitreal methotrexate as an initial treatment, or for those with recurrence following ocular radiation therapy, has been investigated in a small number of patients with encouraging results (Table 8.1) (Singh et al. 2005). In a study involving 16 patients, intravitreal methotrexate (400 µg/0.1 ml) according to a standard induction-consolidation-maintenance regimen was given over a period of 1 year (Smith et al. 2002). All patients showed initial tumor control after a maximum of 12 methotrexate injections, but three patients relapsed. The median follow-up was 18.5 months (range 6–35 months). Complications included cataract (73 %), corneal epitheliopathy (58 %), maculopathy (42 %), and vitreous hemorrhage (8 %). No patient had irreversible loss of vision. Recently, Frenkel and colleagues reported their experience with intravitreal methotrexate in the largest series to date. They demonstrated clinical remission after a mean of 6.4 ± 3.4 (range, 2–16) injections of methotrexate in 44 eyes of 26 patients with PVRL (Frenkel et al. 2008).

#### 8.3.1.3 Intravitreal Immunomodulatory Therapy

Intravitreal rituximab has been shown to penetrate the entire retina, and in recent times there has been interest in exploring its role in PVRL. Small studies have demonstrated the activity of intravitreal rituximab as monotherapy for PVRL (Singh and Peereboom 2007). There have been early reports of efficacy of combination of intravitreal methotrexate and rituximab, and this combination remains investigational (Itty and Pulido 2009). This combination approach is attractive as it may decrease the need for multiple methotrexate injections and may help in reducing toxicity.

### 8.3.2 Systemic Therapy

#### 8.3.2.1 Systemic Chemotherapy

Disease relapse in the CNS is a major issue, particularly after local treatment with ophthalmic radiotherapy or intravitreal chemotherapy. Systemic chemotherapy offers the advantage of simultaneous treatment of both ocular and microscopic intracranial diseases. High-dose methotrexate forms the backbone of treatment in PCNSL patients (Table 8.1). Batchelor and colleagues reported their experience in nine patients with intraocular involvement of lymphoma treated with methotrexate at a dose of 8 g/m$^2$ (Batchelor et al. 2003). Potentially cytotoxic, micromolar levels of methotrexate were detectable in the aqueous and vitreous humor in the majority of

**Table 8.1** Chemotherapy for treatment of primary vitreoretinal lymphoma

| Author | Year | Cases/eye | Treatment method | | | Response (%) | Side effects |
|---|---|---|---|---|---|---|---|
| | | | Indication | Route | Agent | | |
| Fishburne | 1997 | 47 eyes | Recurrent | Intravitreal with BBB | MTX 400 μg | 100 | Visual loss 15 % |
| Sandor | 1998 | 14 | | Intravenous and intrathecal | MTX, thiotepa, vincristine, cytarabine | 79 | Recurrence 71 % Neurotoxicity 14 % |
| Soussain | 2001 | 22 | Refractory/ recurrent | Intravenous | Multi-agent chemotherapy with stem cell rescue | 75 | Recurrence 10 % Neurotoxicity 35 % |
| Smith | 2002 | 16/26 eyes | Initial | Intravitreal | MTX 400 μg | 100 | Recurrence 12 % Cataract 73 % Epitheliopathy 58 % Maculopathy 42 % Vitreous hem 8 % Optic atrophy 4 % Endophthalmitis 4 % |
| Batchelor | 2003 | 9 | Initial | Intravenous | MTX high dose | 78 | Recurrence 40 % |
| Frenkel | 2008 | 26/44 eyes | Initial/ recurrent | Intravitreal | MTX 400 μg | 91 | Conjunctival hyperemia and some form of keratopathy 100 % |
| Soussain | 2008 | 43 | Refractory/ recurrent | Intravenous | Multi-agent chemotherapy with stem cell rescue | 61 | Treatment-related mortality ~10 % |
| Jahnke | 2009 | 10 | Initial/ recurrent | Intravenous/ oral | Ifosfamide or trofosfamide | 90 | Thrombocytopenia or leukopenia 40 % |

Excluding single-case reports
*BBB* Blood-brain barrier disruption with mannitol, *MTX* Methotrexate

patients. An intraocular response was reported in seven patients, with complete response (CR) in six and a partial response (PR) in one individual. Efficacy of ifosfamide or trofosfamide was assessed in a recent, prospective, single-center study of ten patients with PVRL that were treated with these therapies. There was a 100 % response rate (nine CRs, one PR) observed that resulted in a median overall survival (OS) of 32 months. Of the seven relapses seen in the study, five were ocular and two occurred in the CNS.

Unlike PCNSL, experience with combination chemotherapy in PVRL is extremely limited. Sandor and colleagues reported 100 % response rate (11 CRs, three PRs), in 14 patients (five with intraocular involvement), in patients treated with a complex treatment regimen consisting of intravenous methotrexate, vincristine, and thiotepa as well as intrathecal methotrexate and cytarabine. Although a high initial response was seen, the follow-up duration was limited and additional therapy was required at relapse (Sandor et al. 1998).

High-dose chemotherapy followed by stem cell transplantation has been studied in a limited number of trials that have included small numbers of patients with ocular disease. These studies have included both newly diagnosed patients and patients with refractory or recurrent disease (Abrey et al. 2003; Soussain et al. 2001, 2008).

Although ocular response has been reported with this aggressive approach, high relapse rates along with observed toxicities associated with stem cell transplantation make this approach investigational at the present time.

In a report of 221 immunocompetent patients with PCNSL and/or PVRL, Grimm and colleagues reported no difference in disease progression rates or OS in patients treated with local therapy versus those who received systemic therapies. This study, although the largest series reported to date, was an uncontrolled, multicenter, retrospective study that utilized different treatments depending upon the preference of the treating physician (Grimm et al. 2007). Thus, as noted above, there is no consensus on treatment of PVRL. An individualized, patient-specific approach should be taken.

### 8.3.3 Central Nervous System Treatment

Similar to PVRL, treatment of individuals with CNS involvement remains investigational, and optimal regimens are still being established. Historically, WBRT was the mainstay of treatment; however, newer approaches using chemotherapy alone or in combination with radiotherapy have largely replaced WBRT as a sole therapy.

#### 8.3.3.1 Surgical Management

Surgery, even when radical, does not improve survival due to the multifocal and diffuse infiltrative nature of PCNSL (Chimienti et al. 2009). Additionally, the deep location of most tumors would result in serious and irreversible neurologic damage. Surgical treatment has been investigated in some centers. One large, retrospective study revealed minimal benefit following surgery with a 1-year survival rate of 57 % for complete resection, 32 % for partial resection, and 48 % for stereotactic biopsy (Bataille et al. 2000). In this series, approximately 40 % of patients who underwent surgical resection experienced a major complication; therefore, the benefits of surgical resection have not been shown to outweigh associated risks (DeAngelis et al. 1990).

#### 8.3.3.2 Chemotherapy

WBRT, formerly the mainstay of treatment, improved the median survival to approximately 12–18 months from 4 months in untreated patients (Deangelis and Hormigo 2004). In 1992, trials using a combination of methotrexate-based chemotherapy and radiotherapy first reported an improved median survival of about 40 months (Deangelis and Hormigo 2004). However, the combination of WBRT and chemotherapy is associated with a significant risk of neurotoxicity in older individuals (Correa et al. 2004). A current clinical trial by the Radiation Therapy Oncology Group (ClinicalTrials.gov Identifier: NCT01399372), however, is testing in a randomized fashion the addition of reduced dose WBRT in an effort to harness the activity of radiation for these patients. Outside of a clinical trial, chemotherapy alone is the initial treatment of choice in older individuals (60 years) (Deangelis and Hormigo 2004). As mentioned previously, high-dose methotrexate is the most commonly utilized chemotherapeutic agent and has been shown to be effective at a dose of 8 g/m$^2$.

One Phase II trial using high-dose methotrexate as monotherapy for 25 patients with PCNSL showed a response rate of 74 % (CR 52 % and PR 22 %) when using a dose of 8 g/m$^2$ (Batchelor et al. 2003). Individuals had a progression-free survival (PFS) of 13 months and an OS of 55 months (Gerstner et al. 2008).

#### 8.3.3.3 Blood-brain Barrier Disruption

As the blood-brain barrier is a limiting factor, which restricts drug entry into the CNS, various strategies to circumvent this barrier have been evaluated. These include the use of high doses of chemotherapy, intrathecal drug delivery, intraventricular drug delivery by a reservoir, and temporary disruption of the blood-brain barrier (BBBD) with intra-arterial mannitol infusion (Deangelis and Hormigo 2004). In a large multi-institutional experience of 149 newly diagnosed PCNSL patients (with no prior WBRT) that were treated with osmotic BBBD and intra-arterial (IA) methotrexate, an overall response rate of 82 % (58 % CR; 24 % PR) was reported with a median PFS and OS of 1.8 and 3.1 years, respectively

(Angelov et al. 2009). Maculopathy is an ocular complication associated with BBBD with mannitol (Galor et al. 2007). The characteristic findings include RPE clumping in the macula and hyperpigmentation in the foveal region associated with variable RPE atrophy. Mannitol maculopathy is typically bilateral, but often asymmetric. Unlike age-related wet macular degeneration, there is absence of subretinal fluid or macular edema. The maculopathy may progress, even after completion of treatment.

#### 8.3.3.4 Multi-Agent Regimens

In recent years high-dose methotrexate-containing multi-agent regimens have been commonly adopted as the preferred treatment option for this disease entity. One multicenter trial enrolled 102 patients with meth-based combination therapy of methotrexate, procarbazine, and vincristine along with intrathecal (IT) methotrexate, and WBRT showed a high response rate (58 % CR, 26 % PR) but also had high CNS toxicity (DeAngelis et al. 2002). Another treatment protocol included intravenous (IV) and IT methotrexate along with IV thiotepa and procarbazine with WBRT (Omuro et al. 2005). Although high response rates were observed, the toxicity profile remained high. The timing and dose of whole-brain radiotherapy are still unclear, given the significant risks of late neurotoxic effects, and there is an ongoing large cooperative group study (ClinicalTrials.gov Identifier: NCT01399372) to evaluate the role of radiation in upfront treatment of PCNSL.

## 8.4 Prognosis

Survival after whole-brain radiation therapy ranges from 12 to 18 months. The survival increases to an average of 36–48 months following high-dose methotrexate-based chemotherapy regimen alone or chemotherapy followed by radiation. (Char et al. 1988; Freeman et al. 1987; Margolis et al. 1980) Age less than 60 years at diagnosis and high initial performance status are well-recognized favorable prognostic factors in PCNSL (Blay et al. 1998; Abrey et al. 2006)The International Extranodal Lymphoma Study Group also devised a prognostic scoring system comprising of five variables associated with poor prognosis that include age greater than 60 years, Eastern Cooperative Oncology Group performance status greater than 1, increased CSF protein level, increased serum lactate dehydrogenase level, and tumor involvement of the deep regions within the brain (basal ganglia, periventricular regions, brain stem, or cerebellum) (Ferreri et al. 2003). Involvement of brain stem and meninges implies an unfavorable prognosis (Blay et al. 1998). Expression of p53, c-Myc, or Bcl-6 also suggests a poor prognosis (Chang et al. 2003). The presence or absence of retinal involvement in the setting of existing CNS disease is not a prognostic factor that influences survival (Blay et al. 1998).

## References

Abrey LE, Moskowitz CH, Mason WP et al (2003) Intensive methotrexate and cytarabine followed by high-dose chemotherapy with autologous stem-cell rescue in patients with newly diagnosed primary CNS lymphoma: an intent-to-treat analysis. J Clin Oncol 21(22):4151–4156

Abrey LE, Ben-Porat L, Panageas KS et al (2006) Primary central nervous system lymphoma: the Memorial Sloan-Kettering Cancer Center prognostic model. J Clin Oncol 24(36):5711–5715

Akpek EK, Ahmed I, Hochberg FH et al (1999) Intraocular-central nervous system lymphoma: clinical features, diagnosis, and outcomes. Ophthalmology 106(9):1805–1810

Angelov L, Doolittle ND, Kraemer DF et al (2009) Blood-brain barrier disruption and intra-arterial methotrexate-based therapy for newly diagnosed primary CNS lymphoma: a multi-institutional experience. J Clin Oncol 27(21):3503–3509

Bataille B, Delwail V, Menet E et al (2000) Primary intracerebral malignant lymphoma: report of 248 cases. J Neurosurg 92(2):261–266

Batchelor TT, Kolak G, Ciordia R et al (2003) High-dose methotrexate for intraocular lymphoma. Clin Cancer Res 9(2):711–715

Blay JY, Conroy T, Chevreau C et al (1998) High-dose methotrexate for the treatment of primary cerebral lymphomas: analysis of survival and late neurologic toxicity in a retrospective series. J Clin Oncol 16(3): 864–871

Chang CC, Kampalath B, Schultz C et al (2003) Expression of p53, c-Myc, or Bcl-6 suggests a poor

prognosis in primary central nervous system diffuse large B-cell lymphoma among immunocompetent individuals. Arch Pathol Lab Med 127(2):208–212

Char DH, Ljung BM, Miller T, Phillips T (1988) Primary intraocular lymphoma (ocular reticulum cell sarcoma) diagnosis and management. Ophthalmology 95(5): 625–630

Chimienti E, Spina M, Vaccher E, Tirelli U (2009) Management of immunocompetent patients with primary central nervous system lymphoma. Clin Lymphoma Myeloma 9(5):353–364

Correa DD, DeAngelis LM, Shi W et al (2004) Cognitive functions in survivors of primary central nervous system lymphoma. Neurology 62(4):548–555

Deangelis LM, Hormigo A (2004) Treatment of primary central nervous system lymphoma. Semin Oncol 31(5):684–692

DeAngelis LM, Yahalom J, Heinemann MH et al (1990) Primary CNS lymphoma: combined treatment with chemotherapy and radiotherapy. Neurology 40(1): 80–86

DeAngelis LM, Seiferheld W, Schold SC et al (2002) Combination chemotherapy and radiotherapy for primary central nervous system lymphoma: Radiation Therapy Oncology Group Study 93-10. J Clin Oncol 20(24):4643–4648

Doucet S, Kumthekar P, Raizer J (2013) Primary Central Nervous System Lymphoma. Curr Treat Options Oncol 14(2):185–197

Ferreri AJ, Blay JY, Reni M et al (2003) Prognostic scoring system for primary CNS lymphomas: the International Extranodal Lymphoma Study Group experience. J Clin Oncol 21(2):266–272

Freeman LN, Schachat AP, Knox DL et al (1987) Clinical features, laboratory investigations, and survival in ocular reticulum cell sarcoma. Ophthalmology 94(12): 1631–1639

Frenkel S, Hendler K, Siegal T et al (2008) Intravitreal methotrexate for treating vitreoretinal lymphoma: 10 years of experience. Br J Ophthalmol 92(3):383–388

Galor A, Ference SJ, Singh AD et al (2007) Maculopathy as a complication of blood-brain barrier disruption in patients with central nervous system lymphoma. Am J Ophthalmol 144(1):45–49

Gerstner ER, Carson KA, Grossman SA, Batchelor TT (2008) Long-term outcome in PCNSL patients treated with high-dose methotrexate and deferred radiation. Neurology 70(5):401–402

Grimm SA, Pulido JS, Jahnke K et al (2007) Primary intraocular lymphoma: an International Primary Central Nervous System Lymphoma Collaborative Group Report. Ann Oncol 18(11):1851–1855

Herrlinger U, Schabet M, Bitzer M et al (1999) Primary central nervous system lymphoma: from clinical presentation to diagnosis. J Neurooncol 43(3):219–226

Hochberg FH, Miller DC (1988) Primary central nervous system lymphoma. J Neurosurg 68(6):835–853

Itty S, Pulido JS (2009) Rituximab for intraocular lymphoma. Retina 29(2):129–132

Kiewe P, Fischer L, Martus P et al (2010) Meningeal dissemination in primary CNS lymphoma: diagnosis, treatment, and survival in a large monocenter cohort. Neuro Oncol 12(4):409–417

Margolis L, Fraser R, Lichter A, Char DH (1980) The role of radiation therapy in the management of ocular reticulum cell sarcoma. Cancer 45(4):688–692

Omuro AM, DeAngelis LM, Yahalom J, Abrey LE (2005) Chemoradiotherapy for primary CNS lymphoma: an intent-to-treat analysis with complete follow-up. Neurology 64(1):69–74

Peterson K, Gordon KB, Heinemann MH, DeAngelis LM (1993) The clinical spectrum of ocular lymphoma. Cancer 72(3):843–849

Sandor V, Stark-Vancs V, Pearson D et al (1998) Phase II trial of chemotherapy alone for primary CNS and intraocular lymphoma. J Clin Oncol 16(9): 3000–3006

Singh AD, Peereboom DM (2007) Intraocular rituximab. Eye 21(12):1453–1454

Singh AD, Lewis H, Schachat AP (2005) Primary lymphoma of the central nervous system. Ophthalmol Clin North Am 18(1):199–207, x

Smith JR, Rosenbaum JT, Wilson DJ et al (2002) Role of intravitreal methotrexate in the management of primary central nervous system lymphoma with ocular involvement. Ophthalmology 109(9):1709–1716

Soussain C, Suzan F, Hoang-Xuan K et al (2001) Results of intensive chemotherapy followed by hematopoietic stem-cell rescue in 22 patients with refractory or recurrent primary CNS lymphoma or intraocular lymphoma. J Clin Oncol 19(3):742–749

Soussain C, Hoang-Xuan K, Taillandier L et al (2008) Intensive chemotherapy followed by hematopoietic stem-cell rescue for refractory and recurrent primary CNS and intraocular lymphoma: Societe Francaise de Greffe de Moelle Osseuse-Therapie Cellulaire. J Clin Oncol 26(15):2512–2518

Whitcup SM, de Smet MD, Rubin BI et al (1993) Intraocular lymphoma. Clinical and histopathologic diagnosis. Ophthalmology 100(9):1399–1406

# Ocular and Adnexal Benign Reactive Lymphoid Hyperplasia

9

Eugen C. Minca and Raymond R. Tubbs

## 9.1 Introduction

Oftentimes it is challenging even for the pathologist to distinguish an RLH lesion from various types of lymphoma, which, in this location, are most frequently of the small mature lymphocyte type. Indeed, the histologic architectural abnormalities and cytologic atypia of lymphoid proliferations, as indicators of potential neoplasia, are difficult to reliably identify morphologically. Before the advent of immunohistochemistry (IHC) and molecular methods, OAL were classified solely based on the histomorphologic characteristics into benign or RLH and malignant or lymphoma. An additional category of atypical lymphoid hyperplasia was introduced to accommodate cases with indeterminate histologic features and further complicated the already difficult distinction between benign and malignant lymphoid proliferations. It has now become clear that ancillary studies are essential in classifying the benign or malignant nature of lymphoid proliferations based on clonality and aberrant expression of surface and cytoplasmic antigens.

E.C. Minca • R.R. Tubbs, MD (✉)
Departments of Clinical and Molecular Pathology, Pathology and Laboratory Medicine Institute; Cleveland Clinic Foundation, Cleveland, OH, USA
e-mail: tubbsr@ccf.org

## 9.2 Epidemiology

RLH accounts for 10–20 % of all ocular adnexal lymphoid proliferations in two large series of orbital lesions (Shields et al. 2004; Shinder et al. 2010). The age at presentation for RLH is widespread, varying between 7 and 80 years with a mean of 40–50 years depending on the study, and is not significantly different from the mean age of presentation for ocular lymphomas. RLH does not appear to have consistent gender predominance in various studies, with reported male to female ratios leaning either way (Shinder et al. 2010; Mannami et al. 2001; Stacy et al. 2010). Reports vary also regarding the predominant site of involvement, some describing RLH more frequently in the orbit, including the lacrimal gland and sac, and others in the conjunctiva (Fig. 9.1). Involvement of the eyelid has also been described but appears less common (Stacy et al. 2010; Coupland et al. 1998; Knowles et al. 1990). These differences may stem from different selection criteria for biopsy at various institutions and the resulting bias in tissue sent for examination. RLH is unilateral in the majority of cases, but bilateral orbital lesions or unilateral lesions associated with RLH in other body sites (salivary glands, axilla) are not uncommon, and are most frequently the manifestation of an autoimmune disease such as Sjögren's syndrome.

The clinical manifestations of RLH vary and depend on the site of involvement. Patients with conjunctival lesions often complain of foreign body sensation and show superficial

A.D. Singh (ed.), *Ocular and Adnexal Lymphoma*, Essentials in Ophthalmology,
DOI 10.1007/978-3-642-38499-8_9, © Springer-Verlag Berlin Heidelberg 2014

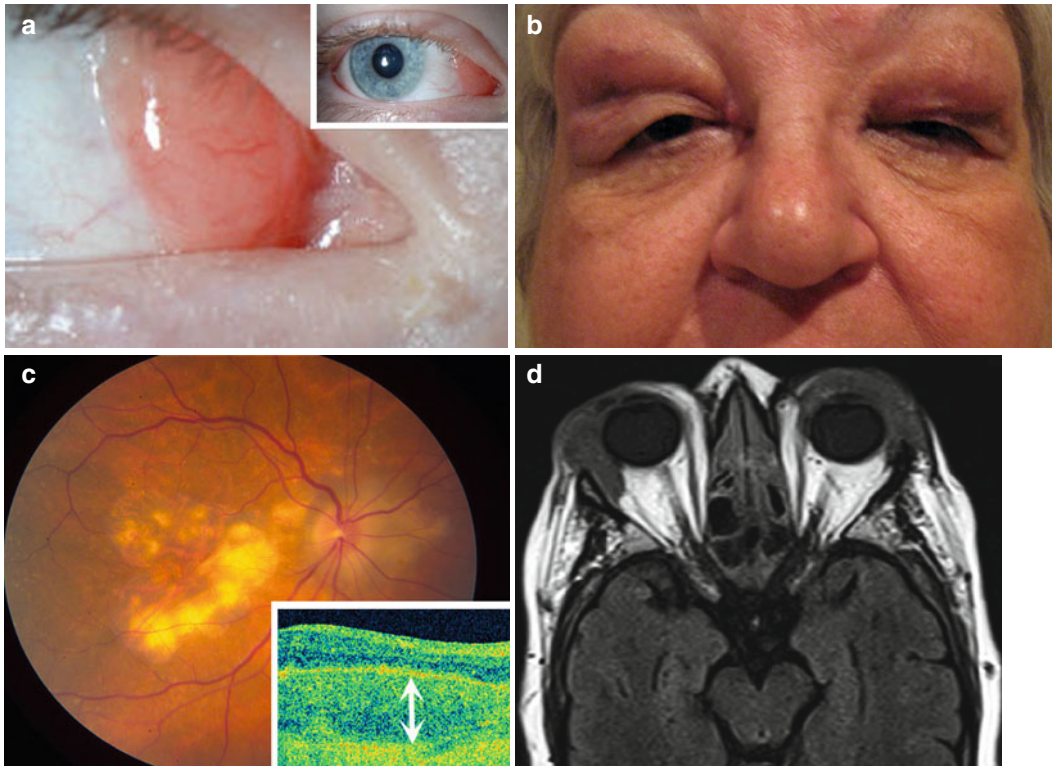


**Fig. 9.1** Clinical presentations of reactive lymphoid hyperplasia. Salmon-patch lesion of the right eye which was biopsy proven RLH (**a**). Inset shows a lesion on the bulbar conjunctiva that was limited to the medial canthal region. Facial photograph of a patient with bilateral RLH of the lacrimal gland demonstrating fullness of both orbits and cheeks (**b**). Fundus photograph of the right eye of the same patient reveals creamy choroidal lesions consistent with uveal reactive hyperplasia (**c**). The inset demonstrates choroidal thickening observed on OCT (*arrow*). The left fundus and OCT revealed similar findings. MRI of same patient demonstrates bilateral lacrimal gland swelling (**d**). Reproduced with permission from: Stacy et al. (2010)

"salmon-patch" lesions. RLH involving the orbit or eyelid manifests with symptoms of swelling, fullness, exophthalmia, pain, and decreased visual acuity and may show palpable rubbery masses beneath the orbital rim on inspection. The size of the lesions is variable. CT imaging studies typically show one or multiple contrast-enhanced infiltrative masses in the eyelids or orbit, with molding to the ocular globe and other adjacent structures and extension along the rectus muscles. Features like mass heterogeneity and lack of bone destruction have been associated with RLH but lack sensitivity or specificity in distinguishing RLH from lymphoma (Westacott et al. 1991). On MRI, RLH lesions are enhanced with gadolinium, with T1-weighted signal that is hypo- or isointense and T2-weighted signal that is hyperintense to muscle. On ultrasound imaging, RLH appears as a variably shaped, regular acoustic structure with low to medium reflectivity.

The clinical and epidemiological characteristics of RLH described above are largely nonspecific and of little value for the differential diagnosis of lymphoma, which relies on histologic examination and molecular studies.

## 9.3 Etiology

The etiology of RLH is unclear. One hypothesis, based on knowledge from other organ sites, proposes that RLH represents a temporary benign precursor lesion with potential to progress to lymphoma. This theory postulates that RLH is initiated from a T-cell immunoregulatory imbalance that drives an exuberant proliferation of

B cells (Jakobiec 2008). The initially polyclonal B-cell proliferation, as hallmark for a benign lesion, may evolve in time to spawn multiple small B-cell clones (oligoclones), of which one may eventually come to predominate and form the basis for a neoplastic proliferation as in the case of marginal zone lymphoma. Whereas in stomach and small bowel chronic inflammatory stimulation produced by infections with *Helicobacter pylori* and *Campylobacter jejuni* has clearly been shown to carry a risk for progression to mucosa-associated lymphoid tissue (MALT) extranodal marginal zone B-cell lymphoma, no such association has been unequivocally proven for ocular and adnexal lymphoid proliferations. Some studies have reported a possible association between infection with *Chlamydia psittaci* and ocular lymphoid proliferations, in particular MALT lymphoma (Ferreri et al. 2005). However, several subsequent studies, including one from our institution, have failed to reproduce these results (Ruiz et al. 2007; Rosado et al. 2006; Vargas et al. 2006). Another infectious agent, proposed as causative in at least a group of RLH, is the Epstein-Barr virus, which in one case report was described as productive of a temporary and clonal B-cell proliferation in the conjunctiva that eventually disappeared without treatment. Such "reactive" EBV-induced B-cell clones complicate the initial histologic interpretation and act as a mimicker for lymphoma (Sharara et al. 2003).

A recent report described that an underlying multisystem inflammatory or autoimmune disease can be identified in approximately 50 % of RLH cases (Kubota and Moritani 2007). Of these underlying conditions, Sjögren's syndrome was the most frequent (4/7 cases), followed by Graves' disease, lupus erythematosus, and bullous pemphigoid (1/7 cases, each). Autoimmune diseases were identified also in patients with ocular MALT lymphoma but in a much lower proportion. The association between RLH and autoimmunity suggests again a possible role that chronic immune stimulation by autoimmune antigens may play into the development of polyclonal RLH which can potentially progress in a subset of cases to monoclonality and lymphoma. A recent case report attempted to illustrate such progression by describing a case of conjunctival MALT lymphoma in a patient with a contralateral lesion that was best classified histologically as RLH but revealed monoclonality on additional molecular studies (Fukuhara et al. 2012).

Other recent reports showed that in a number of RLH cases, subtle infiltration with plasma cells and focal fibrosis can be identified, as well as an increased serum IgG4:IgG ratio (Kubota and Moritani 2007; Matsuo et al. 2010). This group of patients had a different clinical history and diverse systemic associations than those with normal serum IgG4:IgG ratio, and the disease was shown to be occasionally associated with other IgG4-related conditions and even with B-cell clonality.

## 9.4 Histopathology

### 9.4.1 Light Microscopy

Reactive lymphoid hyperplasia (RLH) is regarded as a benign and reversible proliferation of lymphoid tissue, composed of an admixture of predominantly T cells and B cells with a polyclonal immunoglobulin profile. Most commonly, RLH presents as a dense infiltration of small, histologically bland lymphocytes with the formation of reactive lymphoid follicles of varying sizes, in an arrangement similar architecturally to that of a normal lymph node. Most follicles are round to oval but some may coalesce to form irregular-shaped structures. The germinal centers are expanded and composed of a mixture of centrocytes (small B cells with irregular or cleaved nuclei) and centroblasts (larger B cells with round to oval, non-cleaved nuclei and prominent nucleoli) (Fig. 9.2). The interfollicular areas are expanded, with prominent mantle zones. Other histologic features of RLH include prominent interstitial capillaries and the presence of admixed plasma cells, histiocytes, and rare eosinophils.

### 9.4.2 Immunohistochemistry (IHC)

Since the early 1980s IHC has emerged as an invaluable ancillary technique and is now commonplace in the standard diagnostic approach.

Relatively newer methods have been developed for detailed profiling of cell surface antigens like flow cytometry. The immunophenotypic profiling of lymphoid proliferations has greatly reduced the intra- and interobserver variability of morphologic interpretations and is essential for an accurate diagnosis.

Immunophenotypically (Fig. 9.2), the lymphocytes in RLH are a mixture of B cells (CD20-positive), mostly present in the follicles, and immunoregulatory T cells (CD3-positive, predominantly CD4-positive), primarily found in interfollicular areas and scattered in the follicular centers. Within the follicles, a meshwork of antigen-presenting follicular dendritic cells (CD21- and CD23-positive) is present, and tingible-body macrophages (CD68-positive) are also scattered in between B lymphocytes. Cell proliferation, as determined by the presence of mitoses or by IHC for the proliferation marker Ki67, is restricted to the germinal centers in a polarized manner. The reactive germinal centers are also positive for BCL6 (marker of B-cell activation) and CD10 (follicle center marker), and negative for BCL2 (antiapoptotic factor), which may only stain the rare T cells present in the follicles. BCL2 may also stain B cells in the mantle and marginal zone areas of the follicles' periphery. IHC for kappa and lambda immunoglobulin light chains usually shows a polytypic pattern of expression in the mantle zone B cells and the admixed plasma cells in the interfollicular areas.

Detecting the immunoglobulin light chain profile on B lymphocytes, while important diagnostically, is much more difficult by conventional IHC than on plasma cells in routine specimens. This is both because of the significantly lower expression of these proteins in B lymphocytes compared to plasma cells and due to antigen loss following formalin fixation resulting in high levels of background. An alternative and relatively newer method for detecting immunoglobulin light chain expression, chromogenic in situ hybridization (CISH), measures the intracellular levels of mRNA transcripts in formalin-fixed paraffin-embedded samples with significantly reduced background staining compared to IHC (Beck et al. 2003). Recent refinements of CISH methodology involving increased signal amplification boast a level of sensitivity approaching single molecule detection while maintaining a low background (Wang et al. 2012). On platforms like RNAscope (Advanced Cell Diagnostics), CISH for Ig-kappa/lambda mRNA expression is ideal for detecting light chain B-cell clonality patterns in lymphoid proliferations.

### 9.4.3 Flow Cytometry

When enough fresh material is available, flow cytometry analysis is an extremely useful method for analyzing the overall cellular composition (B, T, NK lymphocytes) and the detailed immunophenotype of the lymphoid proliferation, including the pattern of expression for kappa and lambda surface immunoglobulin light chains in B cells. With more recent improvements that allow the analysis of 6, 8, or more concomitant markers in a single tube, flow cytometry can identify very small abnormal cell populations comprising less than 1 % of the total events in the cell suspension, which could otherwise be missed on conventional IHC phenotyping. In one study, flow cytometry showed 94 % sensitivity and 96 % specificity for detecting clonal populations in ocular/orbital lymphoma lesions (Sharara et al. 2003).

Whereas polytypic B- and T-cell populations are the hallmark of benign lymphoid proliferations, caution is required when interpreting the data, as exceptions have been described. Apparent immunoglobulin light chain restriction was reported in a rare case of RLH that demonstrated positivity for EBV antigens, but was only temporary, resolving spontaneously with no therapeutic intervention (Sharara et al. 2003). Conversely, lymphoma is not automatically excluded by an apparent polytypic pattern of a lymphoid proliferation, which must be correlated with the histologic aspects and additional molecular studies (Sharara et al. 2003).

### 9.4.4 Polymerase Chain Reaction

Nucleic acid amplification by polymerase chain reaction (PCR) has replaced the lengthy Southern blotting methodology for detecting

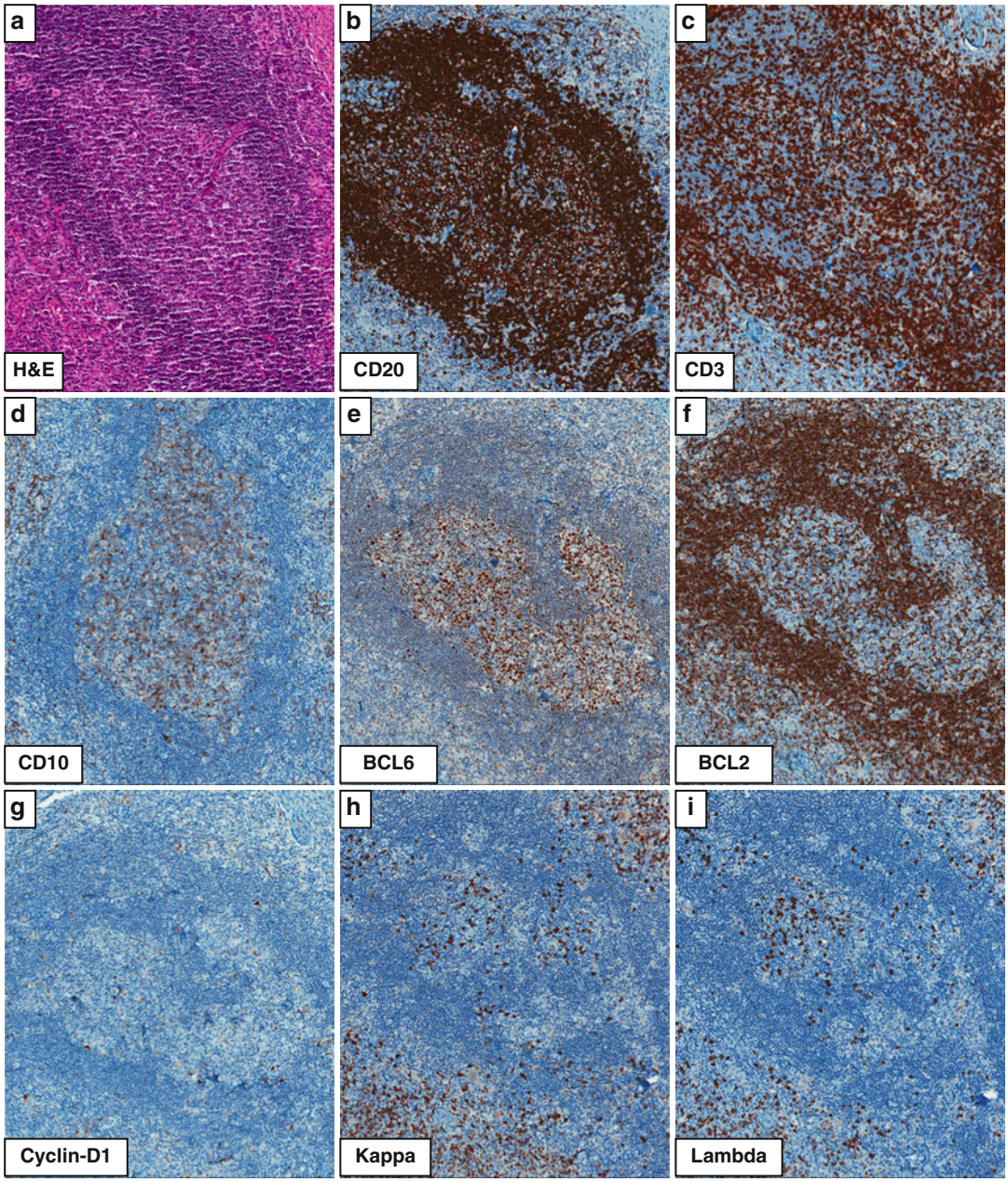


**Fig. 9.2** Histologic and immunophenotypic features of reactive lymphoid hyperplasia. RLH is composed of reactive lymphoid follicles with prominent polarized germinal centers on H&E (**a**). The follicles contain mostly CD20-positive B cells (**b**). Scattered CD3-positive T cells are present in the interfollicular areas, mantle zone, and germinal center (**c**). The B cells in the germinal centers are CD10- and BCL2-positive (**d**, **e**). BCL2-positive B cells are present in the mantle zone but not in the germinal center (**f**). Cyclin-D1 is positive only in rare lymphocytes (**g**). Scattered plasma cells are polytypic for kappa and lambda immunoglobulin light chains (**h**, **i**)

clonal rearrangements of the immunoglobulin heavy chain locus (*IGH*), as well as *BCL2/IGH* rearrangements. Southern blots for T- and B-cell clonality require large quantities of DNA derived from frozen tissue, as the approach depends on predictable gel patterns produced via endonuclease digestion. Current clonality studies by PCR are performed on DNA extracted

from formalin-fixed paraffin-embedded tissue. Most studies have reported good to excellent sensitivities and specificities for this method for both systemic lymphoproliferative disorders and those involving the eye and orbit, including RLH (Mannami et al. 2001; Sharara et al. 2003; McKelvie 2010). However, correlation with histologic, IHC, and flow cytometry data is always required.

## 9.5 Differentiation Between RLH and Lymphoma

In the initial differential diagnosis of an OAL, several histologic features and patterns encountered in RLH lesions may mimic those of lymphoma and may complicate the interpretation and the subsequent ancillary work-up. Some of these features are summarized below.

Primary lymphoid follicles, devoid of germinal centers, and composed only of small monotonous-appearing naïve lymphocytes, with no intervening dendritic cells, may be present in RLH. Although these BCL2-positive follicles may appear malignant, they have reactive interfollicular areas and are negative for CD10 and BCL6, reflecting their lack of germinal center formation. They also lack large centroblasts as typically observed in certain types of lymphoma, follicular lymphoma in particular (see below).

Distinguishing RLH from certain types of lymphoma that can also display a follicular architecture is often difficult on morphology alone (Chap. 2). In follicular lymphoma, the follicles are more tightly packed, monotonous in size, often without a mantle zone, and are composed of monomorphic centrocytes. The follicle centers are characteristically positive for BCL2 and show a more diffuse cell proliferation. It has been described that rare BCL2-positive germinal centers in a background of seemingly RLH usually harbor monoclonal B-cell populations and constitute examples of early or "in situ" follicular lymphoma. In extranodal marginal zone lymphoma, the typical infiltrate consists of small lymphocytes with patchy clusters of plasma cells and monocytoid areas and scattered polykaryocytes (multinucleated cells, resembling follicular dendritic cells). Follicles, when present, can be intact or fragmented, with occasional "colonized" germinal centers best demonstrated by a CD21 or CD23 immunostain that highlights the follicular meshwork of antigen-presenting follicular dendritic cells. Infiltration of epithelial structures with resulting lymphoepithelial lesions is characteristic and permits distinction from other types of low-grade lymphoma. The neoplastic lymphocytes are negative for CD5, CD10, or cyclin-D1 in the majority of cases. Mantle cell lymphoma, a rare entity in the orbit, shows follicles with expanded mantle zones and diffuse infiltrates or colonization of normal follicles by small atypical lymphocytes that are characteristically positive for cyclin-D1.

## 9.6 Clinical and Pathologic Variants

### 9.6.1 Atypical Lymphoid Hyperplasia

The ambiguous category of atypical lymphoid hyperplasia (ALH) has been initially used to describe lymphoid proliferations with scattered large atypical cells, often with immunoblast morphology, increased interfollicular mitotic activity, or presence of small cells with irregular nuclear contours (Jakobiec et al. 1979). It has subsequently been used for lymphoid lesions with generally indeterminate histologic features such as diffuse proliferations of small mature lymphocytes without atypical features suggestive of neoplasia but also lacking germinal centers or mixed leukocyte composition indicative of a reactive process. The development of immunohistologic markers of molecular clonality has lessened the need for classifying lymphoid proliferations in the indefinite atypical category, as many lesions with "atypical" features can now be reliably classified directly as lymphoma on the basis of phenotypic or genotypic lymphocyte clonality. However, this category is still used by some authors to describe certain lymphoid lesions lacking B-cell clonality but with proliferating

behavior suggesting lymphoma. At our institution, this category is not used, and OAL with indeterminate histologic features are ultimately classified as RLH or lymphoma on the basis of Ig-kappa/lambda expression pattern and/or the presence or absence of clonal molecular rearrangements.

### 9.6.2 Progressive Transformation of Germinal Centers

A reactive process commonly associated with nodal follicular hyperplasia, progressive transformation of germinal centers (PTGC), is recently described also within the spectrum of ocular RLH (Amin and Ramsay 2012). It has been proposed that nodal PTGC is part of a progressive sequence starting with follicular hyperplasia, follicular lysis, and eventual PTGC caused by inward migration of T cells followed by mantle B cells. PTGC can be focal and limited to a small number of follicles or can be florid, involving numerous germinal centers. The inward migration of T cells and mantle B cells results in fragmentation of the germinal center into islands of centrocytes and centroblasts with obscuring of the mantle zone that gives the germinal center a "moth-eaten" edge. The pathogenesis of this process is unknown. PTGC has been associated with an increased incidence in nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL). NLPHL is extremely rare in the orbit and is diagnosed by identifying the "lymphocyte-predominant" (LP) cells that are large, often surrounded by a collarette of CD3-, CD57-, and/or PD1-positive T cells, and are themselves positive for BCL6, occasionally positive for CD30 and negative for CD15 and CD20. PTGC must be differentiated from follicular colonization by small cell B-cell lymphomas, usually marginal zone lymphoma or rarely mantle cell lymphoma, leading to expansion of existing reactive germinal centers by small B cells. A helpful feature to distinguish between PTGC and B-cell lymphomas colonizing germinal centers is the clustering of T cells around the germinal center remnants in cases of PTGC. PTGC is usually associated with a background of RLH. Immunohistochemical staining for CD10 may help in distinguishing from follicular lymphoma, as only a few remnants of CD10 follicle center B cells are present in PTGC (Amin and Ramsay 2012).

### 9.6.3 Castleman's Disease

A benign inflammatory condition, Castleman's disease, has rarely been described affecting the orbit (Venizelos et al. 2010) and could in principle be considered a subtype of RLH. The disease is characterized by atrophied follicles with hyaline-vascular structures and expanded mantle zone in a characteristic "onion-skin" fashion. Mantle cell lymphoma, with prominent mantle zones, is in the differential diagnosis of this condition but is distinguished by the lack of hyaline-vascular lesions and cyclin-D1 positivity. Sclerotic follicles can be seen in follicular lymphoma, but in this neoplasm the mantle zones are not prominent, and the immunophenotype is characteristic with BCL2-positive germinal centers. The rare plasma cell variant of Castleman's disease has an increased association with several types of neoplasms including lymphoma.

### 9.6.4 Benign Inflammatory Conditions

Orbital "pseudotumor" is an inflammatory lesion of the orbit that, unlike RLH, is characterized by acute onset of ocular symptoms (pain, erythema, edema, loss of visual acuity) and a mixed inflammatory infiltrate composed of T and B cells, neutrophils, eosinophils, and prominent progressive vasculocentric fibrosis. The disorder is associated with systemic autoimmune diseases such as rheumatoid arthritis, inflammatory bowel disease, or Wegener granulomatosis and may be related to the IgG4-associated systemic disease (Origuchi et al. 2012).

Other systemic benign inflammatory conditions such as lupus erythematosus or Kikuchi-Fujimoto disease can also rarely affect the orbit, but the diagnosis should be prompted by the clinical presentation and the systemic involvement.

## 9.7 Treatment and Prognosis

The current treatment options for RLH lesions include a wait-and-watch approach or specific therapy. The latter option has no established definitive recommendations or guidelines for management but is most often adopted because of the location and symptomatology. An important aspect of management is monitoring for the risk of RLH to evolve to systemic lymphoma. Such risk has been estimated in older reports to be 27–29 % (Knowles et al. 1990; Knowles and Jakobiec 1980; Polito and Leccisotti 1996). Recent studies with more accurate diagnosis based on improved ancillary methods indicated the risk to be lower, less than 10 % (Mannami et al. 2001), and managed to more precisely distinguish RLH from neoplastic proliferations, showing a clear dichotomy in the clinical course of the two disease categories (Coupland et al. 1998; Sharara et al. 2003). Regardless of the treatment option and of the actual real chance of progression to lymphoma, careful clinical follow-up is still preferred and recommended in all cases of RLH. Specific therapy includes systemic corticosteroids, to which patients show an initial but often unsustained response. Surgical excision or cryotherapy may also be considered but carry the risk of scar formation and cosmetic alterations. Local radiotherapy, usually limited to 15–20 Gy, may also be effective for localized lesions. The recurrence rate for RLH after low-dose external beam radiotherapy has been reported at 5 % after 5 years follow-up in one study (Kennerdell et al. 1999). For lesions that are diffuse, refractory, or recurrent, the use of low-dose chemotherapy such as chlorambucil may be necessary for control.

Recent reports have described the successful use of biological response modifiers such as rituximab of bevacizumab in the treatment of RLH. Rituximab, a chimeric monoclonal antibody, targets the CD20 surface B-cell antigen. Rituximab is thought to promote the immune-mediated depletion of CD20-positive B cells and is approved for the treatment of several types of B-cell lymphomas, rheumatoid arthritis, and other autoimmune diseases. In the case of orbital RLH, rituximab therapy has been reported to result in 91 % responsiveness (Witzig et al. 2007), reduce the infiltrate size, and allow for consolidative radiotherapy (Talaulikar et al. 2010). Bevacizumab is an anti-vascular endothelial growth factor (VEGF)-A antibody approved for the treatment of metastatic colorectal cancer and studied as a treatment option for conditions that induce neovascularization of the ocular surface. Bevacizumab has been used successfully as an alternative therapy in a case of RLH with hypervascular infiltrates and medial limbal neovascularization that was not a candidate for surgical excision or radiation (Oh et al. 2011).

## 9.8 Summary

Representing the benign side of the OAL spectrum, RLH demonstrates clinical and radiologic features that are similar or indistinguishable from most lymphomas but require very different management and are associated with different clinical outcomes. The diagnosis of RLH and its imperative differentiation from lymphoma is ultimately accomplished by the pathologist based on histologic examination, supplemented with ancillary and invaluable immunophenotypic and molecular tools. Early detection, accurate diagnosis with improved methods, and new therapeutic options are promising factors for further improving the outcome of RLH and decreasing its risk of progression to lymphoma.

## References

Amin S, Ramsay A (2012) Orbital and lacrimal gland progressive transformation of germinal centres–an underdiagnosed entity? Br J Ophthalmol 96(9):1242–1245

Beck RC, Tubbs RR, Hussein M, Pettay J, Hsi ED (2003) Automated colorimetric in situ hybridization (CISH) detection of immunoglobulin (Ig) light chain mRNA expression in plasma cell (PC) dyscrasias and non-Hodgkin lymphoma. Diagn Mol Pathol 12(1):14–20

Coupland SE, Krause L, Delecluse HJ et al (1998) Lymphoproliferative lesions of the ocular adnexa. Analysis of 112 cases. Ophthalmology 105(8): 1430–1441

Ferreri AJ, Ponzoni M, Guidoboni M et al (2005) Regression of ocular adnexal lymphoma after

Chlamydia psittaci-eradicating antibiotic therapy. J Clin Oncol 23(22):5067–5073
Fukuhara J, Kase S, Noda M et al (2012) Conjunctival lymphoma arising from reactive lymphoid hyperplasia. World J Surg Oncol 10:194
Jakobiec FA (2008) Ocular adnexal lymphoid tumors: progress in need of clarification. Am Journal Ophthalmol 145(6):941–950
Jakobiec FA, McLean I, Font RL (1979) Clinicopathologic characteristics of orbital lymphoid hyperplasia. Ophthalmology 86(5):948–966
Kennerdell JS, Flores NE, Hartsock RJ (1999) Low-dose radiotherapy for lymphoid lesions of the orbit and ocular adnexa. Ophthal Plast Reconstr Surg 15(2): 129–133
Knowles DM 2nd, Jakobiec FA (1980) Orbital lymphoid neoplasms: a clinicopathologic study of 60 patients. Cancer 46(3):576–589
Knowles DM, Jakobiec FA, McNally L, Burke JS (1990) Lymphoid hyperplasia and malignant lymphoma occurring in the ocular adnexa (orbit, conjunctiva, and eyelids): a prospective multiparametric analysis of 108 cases during 1977 to 1987. Hum Pathol 21(9): 959–973
Kubota T, Moritani S (2007) High incidence of autoimmune disease in Japanese patients with ocular adnexal reactive lymphoid hyperplasia. Am J Ophthalmol 144(1):148–149
Mannami T, Yoshino T, Oshima K et al (2001) Clinical, histopathological, and immunogenetic analysis of ocular adnexal lymphoproliferative disorders: characterization of malt lymphoma and reactive lymphoid hyperplasia. Mod Pathol 14(7):641–649
Matsuo T, Ichimura K, Sato Y et al (2010) Immunoglobulin G4 (IgG4)-positive or -negative ocular adnexal benign lymphoid lesions in relation to systemic involvement. J Clin Exp Hematop 50(2):129–142
McKelvie PA (2010) Ocular adnexal lymphomas: a review. Adv Anat Pathol 17(4):251–261
Oh DH, Chun YS, Kim JC (2011) A case of ocular benign lymphoid hyperplasia treated with bevacizumab injection. Korean J Ophthalmol 25(1):57–59
Origuchi T, Yano H, Nakamura H, Hirano A, Kawakami A (2012) Three cases of IgG4-related orbital inflammation presented as unilateral pseudotumor and review of the literature. Rheumatol Int (in press)
Polito E, Leccisotti A (1996) Prognosis of orbital lymphoid hyperplasia. Graefes Arch Clin Exp Ophthalmol 234(3):150–154
Rosado MF, Byrne GE Jr, Ding F et al (2006) Ocular adnexal lymphoma: a clinicopathologic study of a large cohort of patients with no evidence for an association with Chlamydia psittaci. Blood 107(2):467–472
Ruiz A, Reischl U, Swerdlow SH et al (2007) Extranodal marginal zone B-cell lymphomas of the ocular adnexa: multiparameter analysis of 34 cases including interphase molecular cytogenetics and PCR for Chlamydia psittaci. Am J Surg Pathol 31(5):792–802
Sharara N, Holden JT, Wojno TH, Feinberg AS, Grossniklaus HE (2003) Ocular adnexal lymphoid proliferations: clinical, histologic, flow cytometric, and molecular analysis of forty-three cases. Ophthalmology 110(6):1245–1254
Shields JA, Shields CL, Scartozzi R (2004) Survey of 1264 patients with orbital tumors and simulating lesions: The 2002 Montgomery Lecture, part 1. Ophthalmology 111(5):997–1008
Shinder R, Al-Zubidi N, Esmaeli B (2010) Survey of orbital tumors at a comprehensive cancer center in the United States. Head Neck 33(5):610–614
Stacy RC, Jakobiec FA, Schoenfield L, Singh AD (2010) Unifocal and multifocal reactive lymphoid hyperplasia vs follicular lymphoma of the ocular adnexa. Am J Ophthalmol 150(3):412–26 e1
Talaulikar D, Tridgell D, Leong D et al (2010) Novel therapeutic option for orbital atypical lymphoid hyperplasia. Clin Experiment Ophthalmol 38(9): 892–894
Vargas RL, Fallone E, Felgar RE et al (2006) Is there an association between ocular adnexal lymphoma and infection with Chlamydia psittaci? The University of Rochester experience. Leuk Res 30(5):547–551
Venizelos I, Papathomas TG, Papathanasiou M et al (2010) Orbital involvement in Castleman disease. Surv Ophthalmol 55(3):247–255
Wang F, Flanagan J, Su N et al (2012) RNAscope: a novel in situ RNA analysis platform for formalin-fixed, paraffin-embedded tissues. J Mol Diagn 14(1): 22–29
Westacott S, Garner A, Moseley IF, Wright JE (1991) Orbital lymphoma versus reactive lymphoid hyperplasia: an analysis of the use of computed tomography in differential diagnosis. Br J Ophthalmol 75(12): 722–725
Witzig TE, Inwards DJ, Habermann TM et al (2007) Treatment of benign orbital pseudolymphomas with the monoclonal anti-CD20 antibody rituximab. Mayo Clin Proc 82(6):692–699

# Ocular and Adnexal T-Cell Lymphoma

# 10

Yujuan Wang and Chi-Chao Chan

## 10.1 Introduction

T-cell lymphomas represent the smaller subsets of non-Hodgkin's lymphoma (NHL) and are much less common than their B-cell counterpart. Ocular and adnexal T-cell lymphomas are particularly rare and mostly secondary to systemic disease (Jakobiec and Knowles 1989; Cook et al. 1999; Ferry et al. 2007; Woolf et al. 2012). Compared with its B-cell counterpart, T-cell lymphoma is more rapidly aggressive and carries a poorer prognosis (Gisselbrecht et al. 1998). In patients with a low international prognostic index (IPI) score of 1–2 (good prognosis), 5-year overall survival is 55 % in those with T-cell lymphomas and 71 % in those with B-cell lymphomas; the overall survival difference is also reflected in patients with an IPI greater than 2 (poor prognosis), 11 and 35 %, respectively (Melnyk et al. 1997). T-cell lymphomas, however, are heterogeneous and show varying clinical characteristics, prognosis, and therapeutic responses.

### 10.1.1 Classification of T-Cell Lymphoma

In the 1960s, Henry Rappaport proposed the first clinically relevant classification of lymphoma. However, lymphoma of T-cell origin was first differentiated from its B counterpart by the system developed by Robert Lukes and Robert Collins using a camera lucida approach (Lukes and Collins 1974). In this classification, there were five subtypes of T-cell lymphoma. Subsequently, Kiel classification and its updated form also categorized lymphomas as T- or B-cell subtype based on ultrastructure and immunophenotype (Lukes and Collins 1974, 1975; Stansfeld et al. 1988).

In 1994, the Revised European-American Classification of Lymphoid Neoplasms (REAL) was published by the International Lymphoma Study Group (Harris et al. 1994). The REAL classification not only had a distinction between lymphomas derived from B and T lymphocytes, it also differentiated neoplasms stemming from precursor and mature peripheral components. The REAL classification influenced a new round of lymphoma classification initiated by the World Health Organization (WHO).

In 2001, WHO classification became the leading system for lymphoma classification. All lymphomas

Y. Wang, MD, PhD
Immunopathology Section,
Laboratory of Immunology, National Eye Institute,
National Institutes of Health,
Bethesda, MD, USA

Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou, China
e-mail: yujuan.wang@nih.gov

C.-C. Chan, MD (✉)
Immunopathology Section,
Laboratory of Immunology, National Eye Institute,
National Institutes of Health,
Bethesda, MD, USA
e-mail: chanc@nei.nih.gov

A.D. Singh (ed.), *Ocular and Adnexal Lymphoma*, Essentials in Ophthalmology,
DOI 10.1007/978-3-642-38499-8_10, 

were classified into three basic types: B-cell, T-cell and natural killer (NK)-cell neoplasms, and Hodgkin lymphoma. The fourth edition of the WHO classification published in 2008 reveals a better understanding based on clinical and clinicopathological entities (World Health Organization 2008). Like B-cell lymphoma, T-cell lymphoma is further classified into two categories: precursor T-cell lymphoblastic neoplasms derived from maturing thymocytes and peripheral T-cell lymphomas (PTCL) from mature post-thymic T cells (Harris et al. 1994; World Health Organization 2008; De Leval et al. 2009). Precursor T-cell lymphoblastic lymphoma includes acute T-cell lymphoblastic leukemia (T-ALL) and T-cell lymphoblastic lymphoma (T-LBL), the T-lineage counterparts of B-ALL/LBL, respectively. These T-cell lymphomas are most aggressive and commonly affect adolescents and young adults. The mature PTCL encompasses less than 15 % of all NHL. It is further divided based on clinical features such as leukemic or disseminated, extranodal, cutaneous, and nodal subtypes (World Health Organization 2008). Cutaneous T-cell lymphomas or primary cutaneous T-cell lymphoma (PCTCL) belongs to the lymphomas of mature T cells, is generally less aggressive, and has a different prognosis and different treatment approach than other PTCLs. Mycosis fungoides (MF) is the most common PCTCL and is characterized by a slow growing cancer in the skin appearing as a scaly red rash on the body with no unusual exposure to the sun (Beyer et al. 2011). PTCL unspecified (PTCL-US), accounting for 25.9 % of PTCL, is the most common and heterogeneous category (Vose et al. 2008; Savage et al. 2011). The other subtypes of PTCL include angioimmunoblastic type (18.5 %), NK/T-cell lymphoma, nasal type (10.4 %), and adult T-cell leukemia/lymphoma (ATLL; 9.6 %) (Vose et al. 2008). All PTCLs have been reported to affect the eye and/or ocular adnexal tissues.

### 10.1.2 Primary and Metastatic T-Cell Lymphoma

PTCL is the major type affecting ocular and adnexal tissues. Ferry et al. studied 353 cases of ocular adnexal lymphoma and found only two cases of T-cell origin (Ferry et al. 2007). Another study reported that only 5 of 192 (2.6 %) consecutive lymphoma patients with orbital involvement are secondary to T-cell lymphoma (Woolf et al. 2012).

Although rare, T-cell neoplasm can affect all the ocular and adnexal tissues that B-cell lymphoma does, including intraocular tissues and ocular adnexa (the eyelids, conjunctiva, orbit, extraocular muscles, or lacrimal gland). Primary presentation of both intraocular T-cell lymphoma (known as primary vitreoretinal lymphoma [PVRL], a subset of primary central nervous system lymphoma [PCNSL]), and adnexal T-cell tumor is extremely rare (Coupland et al. 1999). To date, very few cases of T-cell PVRL without associated MF have been reported (Goldey et al. 1989; Jensen et al. 1994; Lobo et al. 2003; Coupland et al. 2005; Mihaljevic et al. 2010). Coupland et al. studied seven cases of ocular and adnexal lymphoma with T-cell and NK/T-cell origin, finding only one case to be primary PTCL adnexal lymphoma (Coupland et al. 1999). In addition to this case, only a few other cases of primary adnexal T-cell lymphoma have been reported (Hirasaka et al. 1992; Leidenix et al. 1993; Lee et al. 2006; Janatpour et al. 2007; Chen et al. 2009; Amit et al. 2012), with two of the cases being diagnosed in young children (Leidenix et al. 1993; Amit et al. 2012).

Contrastingly, most cases of intraocular and adnexal T-cell lymphoma are metastatic from systemic malignancy (Coupland et al. 1998; Hoffman et al. 2003; Levy-Clarke et al. 2008). In intraocular T-cell lymphoma, more than 50 cases confirmed by cytology/pathology have been reported (Qualman et al. 1983; Reim et al. 1990; Ridley et al. 1992; Kohno et al. 1993; Brown et al. 1994; Cochereau et al. 1996; Merle et al. 2005; Levy-Clarke et al. 2008; Cimino et al. 2009; Lee et al. 2010; Liu et al. 2010; Mihaljevic et al. 2010; Wickremasinghe et al. 2010; Reddy and Kim 2011; Shunmugam et al. 2011; Mudhar et al. 2012; Yoo et al. 2012). Approximately 80 % of them had a primary neoplasm outside the eye (Coupland et al. 2005).

### 10.1.3 Epidemiology

T-cell lymphomas are uncommon malignancies accounting for 10–15 % of all NHL (The Non-Hodgkin's Lymphoma Classification Project

1997). Although there are many different subtypes of T-cell lymphoma, most of them are extremely rare and diagnosed in only a few patients per year worldwide. Some T-cell lymphoma subsets appear to have distinguished geographical predilections (Anderson et al. 1998). PTCL has a higher incidence in Asia than in Europe and North America (Kwong et al. 2009); PTCL-US is more common in North America than in European and Asian countries, while angioimmunoblastic T-cell lymphoma (AITL) is found more often in Europe (Foss et al. 2011). NK/T-cell lymphoma occurs most commonly in East Asia, frequently in Native Americans in Mexico and South/Central America, but rare in Europe (Aozasa et al. 2008; Kwong et al. 2009; Aozasa and Zaki 2011). ATLL commonly occurs in southwestern Japan, Central Africa, and the Caribbean (Jang et al. 2012). In addition to race-linked factors in various subsets, the differences in the geographic regions are considered due to the prevalence of particular viral infections, such as Epstein-Barr virus (EBV) associated with NK/T-cell lymphoma and human T-cell leukemia virus type 1 (HTLV-1) with ATLL in specific areas (Harabuchi et al. 2009; Bellei et al. 2012).

Although intraocular T-cell lymphoma is rare compared with its B-cell counterpart, two older studies have reported that T-cell lymphoma composes roughly 21 % of total intraocular lymphomas (Brown et al. 1994; Hoffman et al. 2003). Ocular and adnexal T-cell lymphomas typically occur in elderly patients with an average age between 45 and 63 years old (Coupland et al. 1999, 2005; Woog et al. 2006); however, primary orbital T-cell lymphoma has been diagnosed in children younger than 10 years old (Leidenix et al. 1993; Amit et al. 2012). Although early studies indicate a slight male predominance (Hoffman et al. 2003), later reports do not support this gender difference in intraocular T-cell lymphoma (Levy-Clarke et al. 2008). MF is commonly involved in ocular and adnexal tissues; nearly 28–55 % of ocular and adnexal T-cell lymphomas are originated from MF lesions (Jensen et al. 1994; Cook et al. 1999; Read et al. 2002; Hoffman et al. 2003; Levy-Clarke et al. 2008).

## 10.2 Etiology

### 10.2.1 Genetic Predisposition

T-cell lymphoma encompasses a wide range of clinical, biological, and pathological heterogeneity. Monoclonal rearrangements of *T-cell receptor* (*TCR*) genes, most commonly *TCRγ* gene rearrangement, serve as a reliable biomarker in T-cell lymphoma (Wang et al. 2011; Beaufils et al. 2012). Over the past decade, high-throughput genome-wide analytical methods, including gene expression profiling and array-based comparative genomic hybridization (aCGH), have emerged as powerful tools in exploring the genetic and molecular alterations of T-cell lymphoma.

In T-ALL/LBL, translocation, deletion, duplication, and mutation are reported as major types of genetic abnormalities. Translocations juxtapose several genes in *TCR* loci, including *TCRβ* at 7q35, *TCRα* and *TCRδ* at 14q11.2, and/or *TCRγ* at 7p14–15 (De Leval and Gaulard 2011). Oligoa CGH helps identify the deletion of *cyclin-dependent kinase inhibitor 2A*(*CDKN2A*) locus at 9p21.3 occurring in 46 % of T-ALL/LBL cases, which results in the loss of this tumor suppressor gene (Usvasalo et al. 2008). Duplications of *MYB* gene at 6q23 as well as *Notch homolog 1* (*NOTCH1*), *translocation-associated* (*Drosophila*), *MRLP41*, *Sjögren's syndrome nuclear autoantigen1* (*SSNA1*), and *phosphohistidine phosphatase 1* (*PHPT1*) genes at 9q34 are common findings (De Leval et al. 2009). More than 50 % of human T-ALLs have activating mutations that involve the extracellular heterodimerization domain and/or the C-terminal PEST (polypeptide enriched in proline, glutamate, serine, and threonine) domain of the *NOTCH1* gene, which results in a dysregulation of NOTCH1 protein and tumorigenesis (Weng et al. 2004).

Several recurrent chromosomal translocations have been identified in PTCL. *Anaplastic lymphoma kinase* (*ALK*) gene translocations at chromosome 2 are the most well established. Among them, several translocations, such as t(2;5)(p23;p35), t(1;2)(q25;p23), and t(2;3)(p23;q11), fuse the *ALK* gene to different partner genes, which subsequently generates chimeric fusion proteins and induces constitutive

activation of tyrosine kinase ALK (Mason et al. 1990; Morris et al. 1994). The t(5;9)(q33;q22) translocation fusing *interleukin-2* (*IL-2) inducible T-cell kinase* (*ITK*) gene at chromosome 5 with the *spleen tyrosine kinase* (*SYK*) gene at chromosome 9 represents the first recurrent chromosomal translocation in the PTCL-US subset; this is also associated with tumors of peculiar morphology with so-called follicular features (Streubel et al. 2006).

Some other chromosomal abnormalities are reported in nasal NK/T-cell lymphoma. Deletion at 6q21–25 is the most recurrent chromosomal aberration (Sun et al. 2003; Yoon and Ko 2003). Isochromosome 7q is encountered in both nasal NK/T-cell lymphoma and ALK-negative anaplastic large cell lymphoma (Feldman et al. 2008).

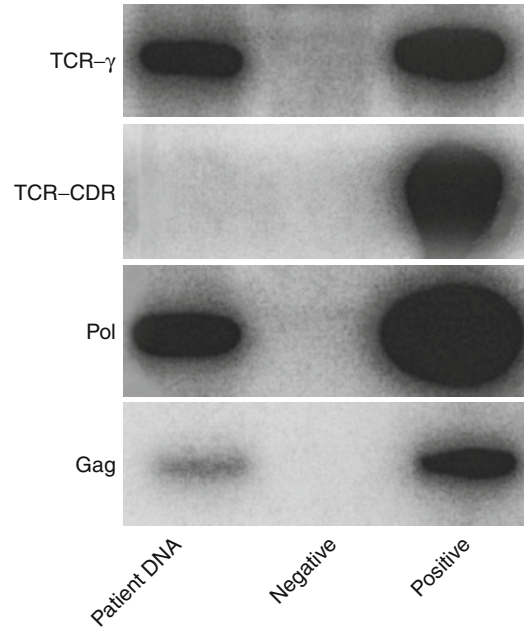


**Fig. 10.1** Polymerase chain reaction of *T-cell receptor* (*TCR*) gene rearrangements and presence of HTLV-1 in a patient with ATLL. *TCRγ* gene rearrangement of ATLL was detected using a primer pair of TCR**γ** but not TCR-CDR; HTLV-1 *pol* and *gag* genes in the lymphoma cells were also detected in the specimen of the same patient (*lane 1* patient DNA, *lane 2* negative control, *lane 3* positive control)

## 10.2.2 Infectious Agents

### 10.2.2.1 HTLV-1 Infection

HTLV-1 infection is the well-established cause of ATLL. The HTLV-1 virus was first identified from fresh peripheral blood lymphocytes obtained from a patient with MF (Poiesz et al. 1980). HTLV-1 infection is highly endemic in southwestern Japan, sub-Saharan Africa and South America, the Caribbean, and focal regions in Middle East and Australo-Melanesia (Gessain and Cassar 2012). The targets of HTLV-1 are predominantly CD4+ T cells and rarely CD8+ T cells. A proportion of 1–3 % of HTLV-1-infected individuals develop ATLL after prolonged viral persistence, usually two decades after the infection (Grassmann et al. 2005). Transactivation is the major mechanism involved in HTLV-1-mediated T-cell leukemogenesis (Jarrett 2006). Tax protein is the primary HTLV-1-encoded factor used by the virus to stimulate T-cellular proliferation. HTLV-1 genome contains *gag*, *pol*, and *env* structural genes that encode important proteins to facilitate replication and infection. Additionally, several open reading frames are identified in the pX region at its 3' end, which encodes important regulatory proteins (Tax and Rax) and accessory proteins (Rof and Tof) essential for viral infection and replication (Grassmann et al. 2005). Expression of HTLV-1 *gag* and *pol* genes has been detected in specimens of T-cell ocular lymphoma (Fig. 10.1) (Buggage et al. 2001; Levy-Clarke et al. 2002; Liu et al. 2010).

### 10.2.2.2 EBV Infection

In addition to infecting B-cell NHL, EBV, a member of the human herpes virus family, is highly associated with nasal NK/T-cell lymphoma (Harabuchi et al. 1990; Hjalgrim and Melbye 2007). EBV infection is common in Asia and Central/South America. EBV genomic DNA and EBV-encoded small RNA (EBER) have been identified in lymphoma cell nuclei (Harabuchi et al. 2009). Most EBV-associated extranodal T-cell lymphomas have a cytotoxic phenotype with expression of T-cell intracellular antigen-1 (TIA-1) and granzyme B, supporting the hypothesis that cytotoxic T cells are one

of the targets EBV tends to infect (Brink et al. 2000). In ocular and adnexal involved tissues from NK/T-cell lymphoma patients, there is a high percentage of EBER expression in the nuclei of atypical T-lymphoma cells by in situ hybridization (Cochereau et al. 1996; Coupland et al. 1999; Kase et al. 2006; Woog et al. 2006; Jakobiec et al. 2012). EBV DNA has also been detected in the aqueous and vitreous of nasal NK/T-cell lymphoma patients with intraocular involvement (Kase et al. 2006; Cimino et al. 2009).

#### 10.2.2.3 Others

Studies also have shown that EBV and human herpes virus 6 (HHV6) infections are associated with AITL (Chan et al. 1999; Zhou et al. 2007). Zhou et al. demonstrated EBV and HHV6B infection in 36/42 cases and 19/42 cases of AITL, respectively; both viral infections were found in 15/42 cases (Zhou et al. 2007). The results indicate that these viruses may contribute to pathogenesis of AITL.

## 10.3 Clinical Presentation

Metastatic ocular and adnexal manifestation may occur either before or after systemic involvement. Some metastatic T-cell lymphomas can simultaneously involve intraocular and adnexal regions (Coupland et al. 1999; Hon et al. 2002).

### 10.3.1 Intraocular T-Cell Lymphoma

Like B-cell PVRL, T-cell lymphoma often masquerades as uveitis/vitritis, accompanied by yellowish retinal/subretinal infiltrates, hemorrhages, retinal vasculitis, optic nerve infiltrates, and/or serous retinal detachment (Cochereau et al. 1996; Coupland et al. 2005). Approximately 30 % of them have concurrent CNS involvement. A few patients initially develop anterior segment manifestations, including corneal edema, hypopyon, distorted pupil, iritis, and/or iris infiltration (Goldey et al. 1989; Lobo et al. 2003; Mihaljevic et al. 2010). An elevated intraocular pressure is also found in T-cell PVRL (Lobo et al. 2003). Unlike metastatic systemic B-cell lymphoma with uveal involvement, metastatic T-cell lymphoma may present lesions in the vitreoretinal site (Levy-Clarke et al. 2008); thus, it closely mirrors the clinical features of B-cell PVRL, presenting mainly with vitreal and retinal infiltrations (Levy-Clarke et al. 2008; Cao et al. 2011). Other intraocular manifestations, such as corneal edema, hypopyon, iritis, iris nodules, ciliary body mass, papillitis, retinal hemorrhages, retinitis, and/or subretinal infiltration, can occur independently or concurrently with vitreous/retinal lesions (Brown et al. 1994; Kumar et al. 1994; Shibata et al. 1997; Coupland et al. 1999; Williams et al. 2000; Yahalom et al. 2002; Hoffman et al. 2003). Secondary glaucoma is a common finding with uveal involvement, probably due to tumor infiltration or neovascularization in the anterior chamber angle (Jensen et al. 1994). A rare case of T-cell lymphoma with granulomatous iritis mimicking a ring melanoma has been reported (Jensen et al. 1994). Fluorescein angiography often shows hypofluorescence due to subretinal/RPE infiltrates and hyperfluorescent window defects. Ultrasonography helps to identify the flat retinal tumor or choroid thickening in some intraocular T-cell lymphoma cases (Coupland et al. 2005).

### 10.3.2 Ocular Adnexal T-Cell Lymphoma

Ocular adnexal T-cell lymphoma often presents as gradually progressive, painless masses. The tumor usually molds to the surrounding ocular tissues, most of which do not have bone destruction. Eyelid and orbit are the most frequently located sites reported in cases of adnexal lymphoma (Coupland et al. 1999; Woog et al. 2006; Janatpour et al. 2007). Both primary and metastatic adnexal T-cell lymphomas can cause orbital and/or periorbital pain, visual loss, and/or diplopia (Coupland et al. 1999; Woog et al. 2006). Clinical presentations can be varied depending on different locations, such as the eyelids, conjunctiva, lacrimal system, extraocular muscles, and orbit. Other accompanying ocular symptoms

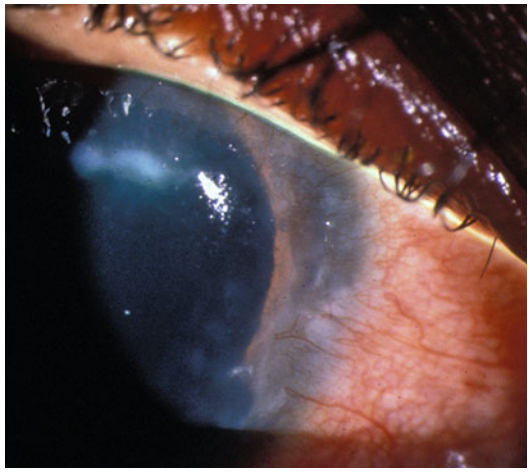

**Fig. 10.2** Slit-lamp photo of a patient with HTLV-1 associated with ATLL. There were conjunctival injection, whitish corneal stromal infiltration, and neovascularization near the limbus

include elevated intraocular pressure, proptosis, ptosis, entropion or ectropion, eyelid mass, eyelid edema, conjunctival injection/edema, subconjunctival mass, corneal edema, orbital mass, retro-orbital mass, periorbital edema, and limited extraocular motility (Fig. 10.2) (Hirasaka et al. 1992; Coupland et al. 1999; Buggage et al. 2001; Lee et al. 2006; Woog et al. 2006; Janatpour et al. 2007; Kirn et al. 2007; Chen et al. 2009).

Nasal NK/T-cell lymphoma with ophthalmic involvement is uncommon. Hon et al. reviewed 35 cases with NK/T-cell lymphoma (Hon et al. 2002). Six of 24 (25 %) patients with primary nasal/nasopharyngeal lymphoma had intraocular and/or orbital involvement. NK/T-cell lymphoma is usually highly malignant, with rapid progression and vast tissue destruction (Yang et al. 2007).

Computed tomography (CT) can detect orbital soft tissue mass with indistinct borders and moderate enhancement of the ocular adnexal tumors (Politi et al. 2010; Woog et al. 2006). Typical magnetic resonance imaging (MRI) features consist of well- or ill-defined masses, enlargement/extension of the soft tissue mass, and/or abnormal enhancing soft tissue (Coupland et al. 1999; Woog et al. 2006; Janatpour et al. 2007; Politi et al. 2010; Amit et al. 2012). After gadolinium-based contrast material administration, ocular adnexal tumors show homogeneous contrast enhancement with signal intensity similar to that of normal lacrimal glands and extraocular muscles (Politi et al. 2010).

## 10.4 Diagnosis

Prompt diagnosis of T lymphoblastic lymphoma is required for speedy treatment of this aggressive malignancy. Early and accurate diagnosis is important for appropriate intervention in both primary and metastatic T-cell lymphomas. T-cell lymphoma should be ruled out in patients with an index of suspicious clinical presentations, systemic lymphoma history, painless intense intraocular inflammation, and resistance to anti-inflammatory therapy. Apart from clinical history and presentations, imaging techniques such as fluorescein angiography and ocular ultrasonography are helpful for diagnosis. High-resolution neuroimaging of the CNS is often valuable for the diagnosis of PVRL, a subset of PCNSL. Similarly, in adnexal lymphoma, clinical presentations with gradually progressive painless mass and systemic lymphoma history can hint at the diagnosis; orbital imaging as X-ray, CT, and MRI is also helpful in diagnosing of ocular adnexal tumors.

### 10.4.1 Histopathology

The gold standard for ocular or adnexal T-cell lymphoma is detection of atypical lymphoid cells in the tissues. In intraocular T-cell lymphoma, aqueous humor, vitreous/subretinal fluids, iris, retina/subretinal tissue, and/or choroid can be sites of biopsy. Vitrectomy is the most common procedure for diagnosis. For adnexal lymphomas, conjunctival biopsy is easy and safe to perform. However, an open-sky biopsy of the eyelids, lacrimal sac, or orbit is often needed to establish an accurate diagnosis. Intraocular and orbital fine needle aspiration (FNA) biopsy is a useful diagnostic adjuvant (Char et al. 2012). In intraocular malignancy, FNA biopsy yields less

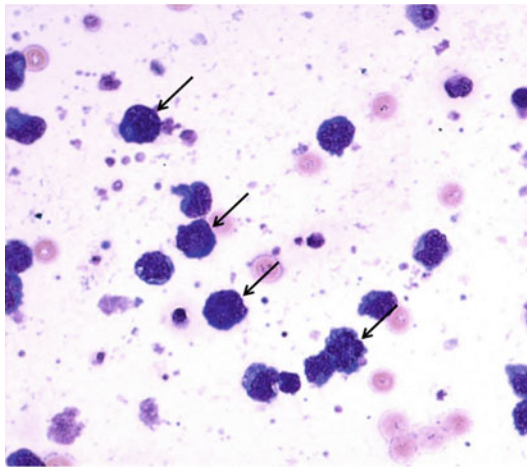

**Fig. 10.3** Cytology of a patient with T-cell lymphoma. The specimen from the diagnostic vitrectomy showed large atypical lymphoid cells (*arrows*) with irregular and large nuclei, prominent nucleoli, and scanty basophilic cytoplasm (Giemsa, original magnification, ×400)

distortion but a smaller number of cells compared with vitrectomy; multiple biopsies may be required for an accurate diagnosis (Char et al. 1988). FNA may eliminate more invasive surgical intervention in the diagnosis of adnexal lymphoma (Spitz et al. 2000). However, reported risks of FNA include false-negative diagnosis and tumor seeding (Char et al. 2012; Kung et al. 2012).

The malignant T-lymphoma cells are pleomorphic, hyperchromatic, and atypical lymphoid cells, with scant basophilic cytoplasm as well as irregular and prominent nuclei (Fig. 10.3) (Kohno et al. 1993; Lobo et al. 2003; Coupland et al. 2005; Janatpour et al. 2007; Levy-Clarke et al. 2008; Liu et al. 2010; Cao et al. 2011). Some malignant cells display folded or multilobed nuclei (Shields et al. 2002). Nuclear convolutions and mitotic figures are also observed in some cases (Meekins et al. 1985; Jensen et al. 1994; Shields et al. 2002; Yang et al. 2007). Due to the scarcity and fragility of the tumor cells, atypical lymphoid cells in the biopsied specimens, especially the vitreous fluid, can often be difficult to identify. Moreover, a considerable variation in the size of the atypical T-lymphoid cells makes the diagnosis even more challenging than that of B-cell lymphoma (Coupland et al. 2005).

### 10.4.2 Immunophenotyping

Immunohistochemistry (IHC) staining of the T-cell marker provides a convenient and valuable way to differentiate the monoclonality of tumor cells. Many case series and case report studies have shown that T-cell lymphomas are positive for CD3 (pan-T-cell marker), CD4 or CD8, and CD45 (Buggage et al. 2001; Coupland et al. 1999; Woog et al. 2006). T-cell receptor β-chain (βF1) is highly expressed in PTCL specimens (Went et al. 2006). Most NK/T-cell lymphoma also expresses CD56, TIA-1, and granzyme B (Coupland et al. 1999; Woog et al. 2006).

Flow cytometric immunophenotyping (FCI) is becoming a useful adjunct to conventional IHC procedures, particularly for FNA, intraocular fluid, or CSF specimens (Zaldivar et al. 2004; Rajagopal and Harbour 2011). It can effectively differentiate T-lymphoma cells from normal T lymphocytes and B lymphocytes; it is also useful in detecting the immunophenotype subsets of T-cell lymphoma (Yokote et al. 2005; Muzzafar et al. 2009). However, FCI needs more cells than conventional IHC; multiple biopsies may be required to achieve a final diagnosis.

### 10.4.3 Molecular Pathology

Advancement in the molecular demonstration of *TCR* gene rearrangement in T-cell lymphoma provides insights on the diagnosis and classification of this malignancy. The *TCR* gene is composed of segments encoding constant and variable regions. Among the variable regions of *TCR*, genetic rearrangements associated with T-cell lymphoma are most commonly observed in the *TCRγ* region (Fig. 10.1). Moreover, polymerase chain reaction (PCR) amplification of the *TCRγ* gene has proven to be a good choice for monoclonality analysis in paraffin-embedded tissues due to its lower false-negative rate compared with *TCRβ* PCR (Diss et al. 1995). Our study, comparing cytology and molecular diagnosis, has found that microdissection-based molecular diagnosis for *TCR* gene rearrangement has a higher test efficiency value (0.995 vs. 0.890) (Wang et al. 2011). It is even

more critical for tiny amounts of specimens on which immunophenotyping cannot be performed. Sharara et al. have shown that conventional histology and immunophenotyping overlook some small clonal populations, which could be detected by molecular techniques (Sharara et al. 2003). Based on the above, molecular analysis of *TCR* gene rearrangements is a reliable marker of T-cell lymphoma (Chan and Sen 2013). It serves as a promising adjunct to cytopathological diagnosis and classification (Allam and Kabelitz 2006).

Considering the close association between NK/T-cell lymphoma and EBV infection, several studies use in situ hybridization to detect EBER in the atypical lymphoid cells. They have independently found consistent association between EBV infection and presence of NK/T-cell lymphoma (Coupland et al. 1999; Woog et al. 2006). The detection of HTLV-1 *gag* and *pol* genes in the ocular specimens has also been reported in several patients with ATLL (Fig. 10.1) (Buggage et al. 2001; Levy-Clarke et al. 2002; Liu et al. 2010).

## 10.5 Treatment

Therapeutic modalities for ocular and adnexal T-cell lymphoma have not been well standardized. According to published case reports, globe irradiation, intravitreal/intrathecal chemotherapy, and systemic chemotherapy are commonly applied in both intraocular and adnexal T-cell lymphomas (Coupland et al. 1998, 1999; Hoffman et al. 2003; Woog et al. 2006; Levy-Clarke et al. 2008; Chan et al. 2011). The treatment strategy largely depends on the individual patient and local expertise. Although the prognosis is dismal in some rapidly growing subtypes of T-cell lymphoma, aggressive combination of therapies is effective in some cases of ocular and adnexal T-cell lymphoma.

Treatment of T-cell PVRL follows guidelines recommended by the International PCNSL Collaborative Group conference. Local therapy (intravitreal methotrexate or external-beam radiotherapy [EBRT]) is applied in unilateral or bilateral PVRL without CNS or systemic involvement, while systemic therapy is not excluded in the bilateral lesions (Chan et al. 2011). Moreover, in PVRL cases with CNS involvement, high-dose methotrexate-based therapy is recommended in conjunction with local therapy. Local EBRT might be preferable in bilateral involvement, whereas intravitreal chemotherapy may be preferred in unilateral cases (Chan et al. 2011).

Treatment of adnexal T-cell lymphoma mainly incorporates local radiotherapy combined with systemic chemotherapy, which is reported to be effective for both T- and NK/T-cell lymphomas (Woog et al. 2006). Surgical excision of adnexal lesions is especially useful in cases with encapsulated lesions that can be entirely removed (Decaudin et al. 2006).

### 10.5.1 Chemotherapy

#### 10.5.1.1 Methotrexate

Methotrexate is an antimetabolite that inhibits the cellular enzyme dihydrofolate reductase. High-dose methotrexate has been shown to significantly increase event-free survival and reduce cumulative incidence of CNS relapse for T-ALL patients (Asselin et al. 2011). Because methotrexate can penetrate the blood-brain barrier when given in high doses intravenously, it can be used to treat PCNSL. High-dose methotrexate has greatly increased median survival rates, with significantly fewer treatment-associated toxic adverse effects in patients with PCNSL (Plotkin and Batchelor 2001; Deangelis and Hormigo 2004; Yamanaka and Tanaka 2004). Thus, it is becoming the single most important agent in PIOL/PCNSL treatment (Levy-Clarke et al. 2005; Chan et al. 2011). Recent studies using intravitreal methotrexate injection as an adjunctive therapy have reported impressive results in intraocular T-cell lymphoma (Frenkel et al. 2008; Wickremasinghe et al. 2010; Reddy and Kim 2011). Methotrexate maintains its therapeutic dosage for 48–72 h in the nonvitrectomized rabbit eyes and appears to be safe in combination with fluorouracil and dexamethasone (Velez et al. 2001). In addition, toxic effects of methotrexate have been modest and manageable (Asselin et al. 2011).

#### 10.5.1.2 Systemic Chemotherapy Regimen

For systemic chemotherapy regimens, the most common for T-cell lymphoma is a combination of cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP). Other CHOP-like combinations include a combination of etoposide + CHOP (EPOCH) and a combination of cyclophosphamide, vincristine, Adriamycin, and dexamethasone (CVAD). All these are common systemic combinations used in T-cell lymphoma with extraocular involvement (Coupland et al. 1999; Woog et al. 2006). However, some cases have shown that once T-cell lymphoma dissemination occurs, the prognosis is poor and long-term remissions are rare even with aggressive systemic chemotherapy (Coupland et al. 1999).

#### 10.5.1.3 Corticosteroids

Corticosteroids are one of the traditional immunosuppressants used in combination with other chemotherapeutic agents. Common corticosteroids used in lymphoma are prednisolone and dexamethasone. Because corticosteroids can only cause temporary tumor regression and decrease of peritumoral edema, prolonged efficacy requires combination with chemotherapy for T-cell lymphoma. On the other hand, corticosteroids have a direct lymphocytolytic effect that has the potential to disrupt cellular morphology and result in pathological diagnostic inaccuracy. Thus, their use should be withheld or avoided prior to the diagnostic biopsy. In cases with concurrent systemic disease, both systemic and local corticosteroids are recommended (Levy-Clarke et al. 2008).

### 10.5.2 Radiation

Local globe/orbit irradiation is commonly used in intraocular and adnexal T-cell lymphoma (Coupland et al. 1999, 2005; Woog et al. 2006; Levy-Clarke et al. 2008). Localized orbital T-cell lymphoma should be planned carefully with local radiation and also combined systemic chemotherapy (Coupland et al. 1999; Woog et al. 2006). Even localized nasal NK/T-cell lymphoma featuring more rapid progression is generally responsive to radiotherapy (Coupland et al. 1999). EBRT is preferable in patients with bilateral intraocular involvement (Chan et al. 2011). Whole brain radiation therapy (WBRT) in conjunction with ocular radiotherapy should be considered for patients who fail systemic therapy and those who cannot have autologous stem cell transplantation (ASCT) (Chan et al. 2011). Although WBRT results in responses of more than 90 %, radiation therapy is associated with high relapse rates and delayed neurotoxicity (Correa et al. 2003; Chan and Sen 2013).

### 10.5.3 Surgery

Surgery rarely serves as a treatment in intraocular lymphomas. Conversely, in ocular adnexal lymphoma, resection of malignant mass has been recommended as both diagnostic and therapeutic procedures in qualified patients; this is especially important in cases of lacrimal gland tumors, in which the encapsulated lesions can be entirely removed (Decaudin et al. 2006).

In patients with systemic T-cell lymphoma, ASCT usually follows high-dose chemotherapy (HDC). Some studies support the use of HDC/ASCT, showing a multiple-year disease-free survival in a small proportion of patients (Visani et al. 2012). The Nordic Lymphoma Group conducted a large prospective phase II study to evaluate the efficacy of HDC/ASCT in patients with untreated systemic PTCL (D'amore et al. 2012). Ninety of 115 patients are in complete remission 3 months after HDC/ASCT. Eighty-three patients are alive at 60.5 months follow-up. The consolidated 5-year overall and progression-free survival rates are 51 % (95 % confidence interval, 43–59 %) and 44 % (95 % confidence interval, 36–52 %), respectively. Therefore, HDC/ASCT is a rational up-front strategy in transplantation-eligible patients with systemic PTCL. In the future, ASCT may play an increasing role for T-cell lymphoma patients, including high-risk groups needing first-line therapy (Visani et al. 2012).

## Abbreviations

| | |
|---|---|
| aCGH | Array-based comparative genomic hybridization |
| AITL | Angioimmunoblastic T-cell lymphoma |
| ALK | Anaplastic lymphoma kinase |
| ASCT | Autologous stem cell transplantation |
| ATLL | Adult T-cell leukemia/lymphoma |
| CDKN2A | Cyclin-dependent kinase inhibitor 2A |
| CHOP | Cyclophosphamide, doxorubicin, vincristine, and prednisone |
| CT | Computed tomography |
| CVAD | Cyclophosphamide, vincristine, Adriamycin, and dexamethasone |
| EBER | EBV-encoded small RNA |
| EBNA3 | EBV-encoded nuclear antigen 3 |
| EBRT | External-beam radiotherapy |
| EBV | Epstein-Barr virus |
| EPOCH | Etoposide+CHOP |
| FCI | Flow cytometric immunophenotyping |
| FNA | Fine needle aspiration |
| HDC | High-dose chemotherapy |
| HHV6 | Human herpes virus 6 |
| HTLV-1 | Human T-cell leukemia virus type 1 |
| IHC | Immunohistochemistry |
| IPI | International prognostic index |
| ITK | Interleukin-2 (IL-2) inducible T-cell kinase |
| MF | Mycosis fungoides |
| MRI | Magnetic resonance imaging |
| NHL | Non-Hodgkin's lymphoma |
| NK | Natural killer |
| NOTCH1 | Notch homolog 1, translocation-associated (Drosophila) |
| PCNSL | Primary central nervous system lymphoma |
| PCR | Polymerase chain reaction |
| PCTCL | Primary cutaneous T-cell lymphoma |
| PHPT1 | Phosphohistidine phosphatase 1 |
| PTCL | Peripheral T-cell lymphoma |
| PTCL-US | PTCL unspecified |
| PVRL | Primary vitreoretinal lymphoma |
| REAL | Revised European-American Classification of Lymphoid Neoplasms |
| SSNA1 | Sjögren's syndrome nuclear autoantigen 1 |
| SYK | Spleen tyrosine kinase |
| T-ALL | T-cell lymphoblastic leukemia |
| TCR | T-cell receptor |
| TIA-1 | T-cell intracellular antigen-1 |
| T-LBL | T-cell lymphoblastic lymphoma |
| WHO | World Health Organization |
| βF1 | T-cell receptor β-chain |

## References


Allam A, Kabelitz D (2006) TCR trans-rearrangements: biological significance in antigen recognition vs the role as lymphoma biomarker. J Immunol 176:5707–5712

Amit S, Purwar N, Agarwal A et al (2012) Primary orbital non-Hodgkin's lymphoma. BMJ Case Rep. doi:10.1136/bcr-2012-006847

Anderson JR, Armitage JO, Weisenburger DD (1998) Epidemiology of the non-Hodgkin's lymphomas: distributions of the major subtypes differ by geographic locations. Non-Hodgkin's Lymphoma Classification Project. Ann Oncol 9:717–720

Aozasa K, Zaki MA (2011) Epidemiology and pathogenesis of nasal NK/T-cell lymphoma: a mini-review. ScientificWorldJournal 11:422–428

Aozasa K, Takakuwa T, Hongyo T et al (2008) Nasal NK/T-cell lymphoma: epidemiology and pathogenesis. Int J Hematol 87:110–117

Asselin BL, Devidas M, Wang C et al (2011) Effectiveness of high-dose methotrexate in T-cell lymphoblastic leukemia and advanced-stage lymphoblastic lymphoma: a randomized study by the Children's Oncology Group (POG 9404). Blood 118:874–883

Beaufils N, Lassoued AB, Essaydi A et al (2012) Analysis of T-cell receptor-gamma gene rearrangements using heteroduplex analysis by high-resolution microcapillary electrophoresis. Leuk Res 36:1119–1123

Bellei M, Chiattone CS, Luminari S et al (2012) T-cell lymphomas in South America and Europe. Rev Bras Hematol Hemoter 34:42–47

Beyer M, Mobs M, Humme D et al (2011) Pathogenesis of Mycosis fungoides. J Dtsch Dermatol Ges 9:594–598

Brink AA, Ten Berge RL, Van Den Brule AJ et al (2000) Epstein-Barr virus is present in neoplastic cytotoxic T cells in extranodal, and predominantly in B cells in nodal T non-Hodgkin lymphomas. J Pathol 191:400–406

Brown SM, Jampol LM, Cantrill HL (1994) Intraocular lymphoma presenting as retinal vasculitis. Surv Ophthalmol 39:133–140

Buggage RR, Smith JA, Shen D et al (2001) Conjunctival T-cell lymphoma caused by human T-cell lymphotrophic virus infection. Am J Ophthalmol 131: 381–383

Cao X, Shen D, Callanan DG et al (2011) Diagnosis of systemic metastatic retinal lymphoma. Acta Ophthalmol 89:e149–e154

Chan AC, Ho JW, Chiang AK et al (1999) Phenotypic and cytotoxic characteristics of peripheral T-cell and NK-cell lymphomas in relation to Epstein-Barr virus association. Histopathology 34:16–24

Chan CC, Rubenstein JL, Coupland SE et al (2011) Primary vitreoretinal lymphoma: a report from an International Primary Central Nervous System Lymphoma Collaborative Group symposium. Oncologist 16:1589–1599

Chan CC, Sen HN (2013) Current concepts in diagnosing and managing primary vitreoretinal (intraocular) lymphoma. Discov Med 15:93–100

Char DH, Ljung BM, Miller T et al (1988) Primary intraocular lymphoma (ocular reticulum cell sarcoma) diagnosis and management. Ophthalmology 95:625–630

Char DH, Cole TB, Miller TR (2012) False negative orbital fine needle biopsy. Orbit 31:194–196

Chen YJ, Chen JT, Lu DW et al (2009) Primary peripheral T-cell lymphoma of the orbit. Arch Ophthalmol 127:1070–1072

Cimino L, Chan CC, Shen D et al (2009) Ocular involvement in nasal natural killer T-cell lymphoma. Int Ophthalmol 29:275–279

Cochereau I, Hannouche D, Geoffray C et al (1996) Ocular involvement in Epstein-Barr virus-associated T-cell lymphoma. Am J Ophthalmol 121:322–324

Cook BE Jr, Bartley GB, Pittelkow MR (1999) Ophthalmic abnormalities in patients with cutaneous T-cell lymphoma. Ophthalmology 106:1339–1344

Correa D, Anderson ND, Glass A et al (2003) Cognitive functions in primary central nervous system lymphoma patients treated with chemotherapy and stem cell transplantation: preliminary findings. Clin Adv Hematol Oncol 1:490

Coupland SE, Krause L, Delecluse HJ et al (1998) Lymphoproliferative lesions of the ocular adnexa. Analysis of 112 cases. Ophthalmology 105:1430–1441

Coupland SE, Foss HD, Assaf C et al (1999) T-cell and T/natural killer-cell lymphomas involving ocular and ocular adnexal tissues: a clinicopathologic, immunohistochemical, and molecular study of seven cases. Ophthalmology 106:2109–2120

Coupland SE, Anastassiou G, Bornfeld N et al (2005) Primary intraocular lymphoma of T-cell type: report of a case and review of the literature. Graefes Arch Clin Exp Ophthalmol 243:189–197

D'amore F, Relander T, Lauritzsen GF et al (2012) Up-front autologous stem-cell transplantation in peripheral T-cell lymphoma: NLG-T-01. J Clin Oncol 30:3093–3099

De Leval L, Gaulard P (2011) Tricky and terrible T-cell tumors: these are thrilling times for testing: molecular pathology of peripheral T-cell lymphomas. Hematology Am Soc Hematol Educ Program 2011:336–343

De Leval L, Bisig B, Thielen C et al (2009) Molecular classification of T-cell lymphomas. Crit Rev Oncol Hematol 72:125–143

Deangelis LM, Hormigo A (2004) Treatment of primary central nervous system lymphoma. Semin Oncol 31:684–692

Decaudin D, De Cremoux P, Vincent-Salomon A et al (2006) Ocular adnexal lymphoma: a review of clinicopathologic features and treatment options. Blood 108:1451–1460

Diss TC, Watts M, Pan LX et al (1995) The polymerase chain reaction in the demonstration of monoclonality in T cell lymphomas. J Clin Pathol 48:1045–1050

Feldman AL, Law M, Grogg KL et al (2008) Incidence of TCR and TCL1 gene translocations and isochromosome 7q in peripheral T-cell lymphomas using fluorescence in situ hybridization. Am J Clin Pathol 130:178–185

Ferry JA, Fung CY, Zukerberg L et al (2007) Lymphoma of the ocular adnexa: a study of 353 cases. Am J Surg Pathol 31:170–184

Foss FM, Zinzani PL, Vose JM et al (2011) Peripheral T-cell lymphoma. Blood 117:6756–6767

Frenkel S, Hendler K, Siegal T et al (2008) Intravitreal methotrexate for treating vitreoretinal lymphoma: 10 years of experience. Br J Ophthalmol 92:383–388

Gessain A, Cassar O (2012) Epidemiological aspects and world distribution of HTLV-1 infection. Front Microbiol 3:388

Gisselbrecht C, Gaulard P, Lepage E et al (1998) Prognostic significance of T-cell phenotype in aggressive non-Hodgkin's lymphomas. Groupe d'Etudes des Lymphomes de l'Adulte (GELA). Blood 92:76–82

Goldey SH, Stern GA, Oblon DJ et al (1989) Immunophenotypic characterization of an unusual T-cell lymphoma presenting as anterior uveitis. A clinicopathologic case report. Arch Ophthalmol 107:1349–1353

Grassmann R, Aboud M, Jeang KT (2005) Molecular mechanisms of cellular transformation by HTLV-1 Tax. Oncogene 24:5976–5985

Harabuchi Y, Yamanaka N, Kataura A et al (1990) Epstein-Barr virus in nasal T-cell lymphomas in patients with lethal midline granuloma. Lancet 335:128–130

Harabuchi Y, Takahara M, Kishibe K et al (2009) Nasal natural killer (NK)/T-cell lymphoma: clinical, histological, virological, and genetic features. Int J Clin Oncol 14:181–190

Harris NL, Jaffe ES, Stein H et al (1994) A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. Blood 84:1361–1392

Hirasaka T, Sahori A, Inoue M et al (1992) A case of primary orbital T-cell lymphoma. Nihon Ganka Gakkai Zasshi 96:1487–1493

Hjalgrim HFJ, Melbye M (2007) Chapter 53 The epidemiology of EBV and its association with malignant disease. In: GC-F Arvin A, Mocarski E, Moore PS, Roizman B, Whitley R, Yamanishi K (eds) Human herpesviruses biology, therapy, and immunoprophylaxis. Cambridge University Press, Cambridge

Hoffman PM, Mckelvie P, Hall AJ et al (2003) Intraocular lymphoma: a series of 14 patients with clinicopathological features and treatment outcomes. Eye (Lond) 17:513–521

Hon C, Kwok AK, Shek TW et al (2002) Vision-threatening complications of nasal T/NK lymphoma. Am J Ophthalmol 134:406–410

Jakobiec FA, Knowles DM (1989) An overview of ocular adnexal lymphoid tumors. Trans Am Ophthalmol Soc 87:420–442; discussion 442–424

Jakobiec FA, Zakka FR, Kirzhner M et al (2012) Epstein-Barr virus-positive T-cell lymphoma involving the lacrimal gland of an adult. Arch Ophthalmol 130:523–525

Janatpour KA, Choo PH, Lloyd WC 3rd (2007) Primary orbital peripheral T-cell lymphoma: histologic, immunophenotypic, and genotypic features. Arch Ophthalmol 125:1289–1292

Jang MS, Kang DY, Park JB et al (2012) Cutaneous T-cell lymphoma in asians. ISRN Dermatol 2012:575120

Jarrett RF (2006) Viruses and lymphoma/leukaemia. J Pathol 208:176–186

Jensen OA, Johansen S, Kiss K (1994) Intraocular T-cell lymphoma mimicking a ring melanoma. First manifestation of systemic disease. Report of a case and survey of the literature. Graefes Arch Clin Exp Ophthalmol 232:148–152

Kase S, Namba K, Kitaichi N et al (2006) Epstein-Barr virus infected cells in the aqueous humour originated from nasal NK/T cell lymphoma. Br J Ophthalmol 90:244–245

Kirn TJ, Levy NB, Gosselin JJ et al (2007) Peripheral T-cell lymphoma presenting as sclerouveitis. Cornea 26:1147–1149

Kohno T, Uchida H, Inomata H et al (1993) Ocular manifestations of adult T-cell leukemia/lymphoma. A clinicopathologic study. Ophthalmology 100:1794–1799

Kumar SR, Gill PS, Wagner DG et al (1994) Human T-cell lymphotropic virus type I-associated retinal lymphoma. A clinicopathologic report. Arch Ophthalmol 112:954–959

Kung YH, Wu TT, Lin CS (2012) Tumor seeding after diagnostic vitrectomy for choroidal metastasis in breast cancer. J Chin Med Assoc 75:483–486

Kwong YL, Anderson BO, Advani R et al (2009) Management of T-cell and natural-killer-cell neoplasms in Asia: consensus statement from the Asian Oncology Summit 2009. Lancet Oncol 10:1093–1101

Lee DS, Woo KI, Chang HR (2006) T-cell lymphoma presenting as painful ophthalmoplegia. Korean J Ophthalmol 20:192–194

Lee EJ, Kim TW, Heo JW et al (2010) Natural killer/T-cell lymphoma of nasal type with intraocular involvement: case report. Eur J Ophthalmol 20:215–217

Leidenix MJ, Mamalis N, Olson RJ et al (1993) Primary T-cell immunoblastic lymphoma of the orbit in a pediatric patient. Ophthalmology 100:998–1002

Levy-Clarke GA, Buggage RR, Shen D et al (2002) Human T-cell lymphotropic virus type-1 associated t-cell leukemia/lymphoma masquerading as necrotizing retinal vasculitis. Ophthalmology 109: 1717–1722

Levy-Clarke GA, Chan CC, Nussenblatt RB (2005) Diagnosis and management of primary intraocular lymphoma. Hematol Oncol Clin North Am 19:739–749, viii

Levy-Clarke GA, Greenman D, Sieving PC et al (2008) Ophthalmic manifestations, cytology, immunohistochemistry, and molecular analysis of intraocular metastatic T-cell lymphoma: report of a case and review of the literature. Surv Ophthalmol 53:285–295

Liu MM, Furusato E, Cao X et al (2010) Ocular manifestations and pathology of adult T-cell leukemia/lymphoma associated with human T-lymphotropic virus type 1. Rare Tumors 2:e63

Lobo A, Larkin G, Clark BJ et al (2003) Pseudo-hypopyon as the presenting feature in B-cell and T-cell intraocular lymphoma. Clin Experiment Ophthalmol 31: 155–158

Lukes RJ, Collins RD (1974) Immunologic characterization of human malignant lymphomas. Cancer 34:suppl:1488–1503

Lukes RJ, Collins RD (1975) New approaches to the classification of the lymphomata. Br J Cancer Suppl 2:1–28

Mason DY, Bastard C, Rimokh R et al (1990) CD30-positive large cell lymphomas ('Ki-1 lymphoma') are associated with a chromosomal translocation involving 5q35. Br J Haematol 74:161–168

Meekins B, Proia AD, Klintworth GK (1985) Cutaneous T-cell lymphoma presenting as a rapidly enlarging ocular adnexal tumor. Ophthalmology 92: 1288–1293

Melnyk A, Rodriguez A, Pugh WC et al (1997) Evaluation of the Revised European-American Lymphoma classification confirms the clinical relevance of immunophenotype in 560 cases of aggressive non-Hodgkin's lymphoma. Blood 89:4514–4520

Merle H, Donnio A, Gonin C et al (2005) Retinal vasculitis caused by adult T-cell leukemia/lymphoma. Jpn J Ophthalmol 49:41–45

Mihaljevic B, Sretenovic A, Jakovic L et al (2010) A case of primary peripheral T-cell type non-Hodgkin lymphoma originating in the iris–clinicopathological findings. Vojnosanit Pregl 67:1025–1028

Morris SW, Kirstein MN, Valentine MB et al (1994) Fusion of a kinase gene, ALK, to a nucleolar protein gene, NPM, in non-Hodgkin's lymphoma. Science 263:1281–1284

Mudhar HS, Fernando M, Rennie IG et al (2012) Enteropathy-associated T-cell lymphoma, lacking MHC class II, with immune-privileged site recurrence, presenting as bilateral ocular vitreous humour involvement – a case report. Histopathology 61:1227–1230

Muzzafar T, Wei EX, Lin P et al (2009) Flow cytometric immunophenotyping of anaplastic large cell lymphoma. Arch Pathol Lab Med 133:49–56

Plotkin SR, Batchelor TT (2001) Primary nervous-system lymphoma. Lancet Oncol 2:354–365

Poiesz BJ, Ruscetti FW, Gazdar AF et al (1980) Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. Proc Natl Acad Sci USA 77: 7415–7419

Politi LS, Forghani R, Godi C et al (2010) Ocular adnexal lymphoma: diffusion-weighted mr imaging for differential diagnosis and therapeutic monitoring. Radiology 256:565–574

Qualman SJ, Mendelsohn G, Mann RB et al (1983) Intraocular lymphomas. Natural history based on a clinicopathologic study of eight cases and review of the literature. Cancer 52:878–886

Rajagopal R, Harbour JW (2011) Diagnostic testing and treatment choices in primary vitreoretinal lymphoma. Retina 31:435–440

Read RW, Zamir E, Rao NA (2002) Neoplastic masquerade syndromes. Surv Ophthalmol 47:81–124

Reddy R, Kim SJ (2011) Intraocular T-cell lymphoma due to mycosis fungoides and response to intravitreal methotrexate. Ocul Immunol Inflamm 19:234–236

Reim H, Dieler R, Wessing A (1990) Non-Hodgkin's lymphoma simulating chorioretinitis. Fortschr Ophthalmol 87:557–559

Ridley ME, Mcdonald HR, Sternberg P Jr et al (1992) Retinal manifestations of ocular lymphoma (reticulum cell sarcoma). Ophthalmology 99:1153–1160; discussion 1160–1151

Savage KJ, Ferreri AJ, Zinzani PL et al (2011) Peripheral T-cell lymphoma–not otherwise specified. Crit Rev Oncol Hematol 79:321–329

Sharara N, Holden JT, Wojno TH et al (2003) Ocular adnexal lymphoid proliferations: clinical, histologic, flow cytometric, and molecular analysis of forty-three cases. Ophthalmology 110:1245–1254

Shibata K, Shimamoto Y, Nishimura T et al (1997) Ocular manifestations in adult T-cell leukemia/lymphoma. Ann Hematol 74:163–168

Shields CL, Shields JA, Eagle RC (2002) Clinicopathologic reports, case reports, and small case series: rapidly progressive T-cell lymphoma of the conjunctiva. Arch Ophthalmol 120:508–509

Shunmugam M, Chan E, O'brart D et al (2011) Cutaneous gammadelta T-cell lymphoma with bilateral ocular and adnexal involvement. Arch Ophthalmol 129:1379–1381

Spitz DJ, Reddy V, Kluskens L et al (2000) Fine-needle aspiration of intra- and extraocular masses. Diagn Cytopathol 22:199–200

Stansfeld AG, Diebold J, Noel H et al (1988) Updated Kiel classification for lymphomas. Lancet 1:292–293

Streubel B, Vinatzer U, Willheim M et al (2006) Novel t(5;9)(q33;q22) fuses ITK to SYK in unspecified peripheral T-cell lymphoma. Leukemia 20:313–318

Sun HS, Su IJ, Lin YC et al (2003) A 2.6 Mb interval on chromosome 6q25.2-q25.3 is commonly deleted in human nasal natural killer/T-cell lymphoma. Br J Haematol 122:590–599

The Non-Hodgkin's Lymphoma Classification Project (1997) A clinical evaluation of the International Lymphoma Study Group classification of non-Hodgkin's lymphoma. The Non-Hodgkin's Lymphoma Classification Project. Blood 89:3909–3918

Usvasalo A, Savola S, Raty R et al (2008) CDKN2A deletions in acute lymphoblastic leukemia of adolescents and young adults: an array CGH study. Leuk Res 32:1228–1235

Velez G, Yuan P, Sung C et al (2001) Pharmacokinetics and toxicity of intravitreal chemotherapy for primary intraocular lymphoma. Arch Ophthalmol 119:1518–1524

Visani G, Picardi P, Tosi P et al (2012) Autologous stem cell transplantation for aggressive lymphomas. Mediterr J Hematol Infect Dis 4:e2012075

Vose J, Armitage J, Weisenburger D (2008) International peripheral T-cell and natural killer/T-cell lymphoma study: pathology findings and clinical outcomes. J Clin Oncol 26:4124–4130

Wang Y, Shen D, Wang VM et al (2011) Molecular biomarkers for the diagnosis of primary vitreoretinal lymphoma. Int J Mol Sci 12:5684–5697

Weng AP, Ferrando AA, Lee W et al (2004) Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. Science 306:269–271

Went P, Agostinelli C, Gallamini A et al (2006) Marker expression in peripheral T-cell lymphoma: a proposed clinical-pathologic prognostic score. J Clin Oncol 24:2472–2479

Wickremasinghe SS, Ojaimi E, Lim L et al (2010) Intravitreal methotrexate as adjunctive, palliative therapy in intraocular T-cell lymphoma. Ocul Immunol Inflamm 18:184–186

Williams GC, Holz E, Lee AG et al (2000) T-cell lymphoproliferative disorder of vitreous associated with mycosis fungoides. Arch Ophthalmol 118:278–280

Woog JJ, Kim YD, Yeatts RP et al (2006) Natural killer/T--cell lymphoma with ocular and adnexal involvement. Ophthalmology 113:140–147

Woolf DK, Ahmed M, Plowman PN (2012) Primary lymphoma of the ocular adnexa (orbital lymphoma) and primary intraocular lymphoma. Clin Oncol (R Coll Radiol) 24:339–344

World Health Organization (2008) WHO classification of tumours of haematopoietic and lymphoid tissues. IARC Press, Lyon

Yahalom C, Cohen Y, Averbukh E et al (2002) Bilateral iridociliary T-cell lymphoma. Arch Ophthalmol 120: 204–207

Yamanaka R, Tanaka R (2004) Advances for the treatment of primary central nervous system lymphoma (review). Oncol Rep 12:563–568

Yang Y, Luo Q, He W et al (2007) Primary ocular natural killer/T-cell lymphomas: clinicopathologic features and diagnosis. Ophthalmologica 221:173–179

Yokote T, Akioka T, Oka S et al (2005) Flow cytometric immunophenotyping of adult T-cell leukemia/lymphoma using CD3 gating. Am J Clin Pathol 124: 199–204

Yoo JH, Kim SY, Jung KB et al (2012) Intraocular involvement of a nasal natural killer T-cell lymphoma: a case report. Korean J Ophthalmol 26:54–57

Yoon J, Ko YH (2003) Deletion mapping of the long arm of chromosome 6 in peripheral T and NK cell lymphomas. Leuk Lymphoma 44:2077–2082

Zaldivar RA, Martin DF, Holden JT et al (2004) Primary intraocular lymphoma: clinical, cytologic, and flow cytometric analysis. Ophthalmology 111: 1762–1767

Zhou Y, Attygalle AD, Chuang SS et al (2007) Angioimmunoblastic T-cell lymphoma: histological progression associates with EBV and HHV6B viral load. Br J Haematol 138:44–53

# Index



A.D. Singh (ed.), *Ocular and Adnexal Lymphoma*, Essentials in Ophthalmology,
DOI 10.1007/978-3-642-38499-8, © Springer-Verlag Berlin Heidelberg 2014